Title:

A comparison of males and females' temporal patterning to short and long term heat acclimation

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Sex comparison of temporal patterning to HA

Abstract

The current study assessed the sex differences in thermoregulatory and physiological adaptation to short term (STHA) and long term heat acclimation (LTHA). Sixteen (8M; 8F) participants performed three running heat tolerance tests (RHTT), preceding HA (RHTT1), following 5 days HA (RHTT2) and 10 days HA (RHTT3). The RHTT involved 30 minutes running (9km.hr^{-1}, 2% gradient) in 40°C, 40% relative humidity. Following STHA, resting rectal temperature (Tr_{rest}) (Males: -0.24±0.16°C, p≤0.001; Females: -0.02±0.08°C, p=0.597), peak rectal temperature (Tr_{peak}) (Males: -0.39±0.36°C, p≤0.001; Females -0.07±0.18°C, p=0.504), and peak heart rate (HR_{peak}) (Males: -14±12 beats.min^{-1}, p≤0.001; Females: -5±3 beats.min^{-1}, p=0.164) reduced in males, but not females. Following STHA, sweat rate relative to body surface area (SR_{BSA}) increased (428±269 g.hr^{-1}.m^2, p=0.029) in females, but not males (-11±286 g.hr^{-1}.m^2, p=0.029). Following LTHA, Tr_{rest} (Males: -0.04±0.15°C, p=0.459; Females: -0.22±0.12°C, p≤0.01) and Tr_{peak} (Males: -0.05±0.26°C, p=0.590; Females: -0.41±0.24°C, p≤0.01) reduced in females, but not males. Following LTHA, SR_{BSA} increased in males (308±346 g.hr^{-1}.m^2, p=0.029), but not females (44±373 g.hr^{-1}.m^2, p=0.733). Males and females responded to STHA; however females required LTHA to establish thermoregulatory and cardiovascular stability. HA protocols should be designed to target sex differences in thermoregulation for optimal adaptation.

Keywords:

Controlled hyperthermia, heat illness, males, females, acclimatisation

Introduction

Increasing ambient temperature is known to have a detrimental effect on endurance performance (Galloway & Maughan 1997). During prolonged submaximal exercise in high ambient conditions, there is a greater requirement for heat loss due to either a rate of heat gain from the environment or a lower gradient for dry heat loss, typically resulting in a greater change in body heat content compared to temperate conditions. Many athletes, soldiers and manual operatives exposed to high ambient conditions are susceptible to heat illnesses; including heat cramps, heat syncope, heat exhaustion and heat stroke. Prior to a heat illness, individuals vary in their ability to tolerate exercise heat stress, some demonstrating a decreased capability to dissipate heat under the same exercise heat stress (Epstein 1990). These individuals are characterized by an earlier and greater rise in body temperature, greater storage of metabolic heat, greater physiological strain, and reduced sweating sensitivity when exercising in the heat (Epstein et al. 1983; Moran et al. 2004).

Males and females differ in their thermoregulatory responses to exercise-heat stress, largely due to females having a reduced sudomotor function (Gagnon & Kenny 2011) thus, decreasing evaporative heat loss capacity, with the resultant increase in physiological strain (Moran et al. 1999). It has been shown that males and females display similar rates of heat dissipation at low requirements for heat loss. However, sex differences in sudomotor function have been demonstrated beyond a certain requirement for heat loss (Gagnon & Kenny 2012). On the other hand, when males and females display similar heat loss for a given heat production, females may display a higher change in body temperature due to physical characteristics (Gagnon et al. 2009; Havenith 2001). These results suggest that females may reach hyperthermic levels in a shorter time period than males, consequently females have been more frequently diagnosed as heat intolerant compared with males (Druyan et al. 2012); potentially putting them at greater risk of obtaining a heat related illness. The observed sex differences in thermoregulation are not always evident, but the difference may become more evident as the heat stress increases (Gagnon & Kenny 2012). Furthermore, hormonal fluctuations associated with the menstrual cycle are suggested to modify central regulatory mechanisms for thermoregulation (Inoue et al. 2005). Elevated progesterone concentrations during the luteal phase of the menstrual cycle, have been reported to increase resting body temperature by ~0.34°C, the onset threshold for sweating by 0.29°C, and the body temperature threshold for cutaneous vasodilation by 0.23-0.30°C (Inoue et al. 2005).

Heat Acclimation (HA) improves heat transfer from the body's core to the skin and ultimately to the external environment, serving to attenuate physiological strain and improve exercise capacity (Sunderland et al. 2008; Lorenzo et al. 2010). HA reduces heat storage, partially as a result of adaptations to the sudomotor function causing an increase in whole body evaporative heat loss (Poirier et al. 2014). Additionally, HA increases blood volume preserving stroke volume and reducing heart rate (HR) at a given workload (Frank et al. 2001; Lorenzo & Minson 2010). For a more comprehensive review of adaptation to exercise heat stress, the reader is referred to a recent review article on human heat adaptation (Taylor 2014).

There is a dearth of literature assessing female's responses to HA. Previously, the physiological responses of males and females to 10 days fixed intensity HA were assessed with females initially exhibiting lower rectal temperature (Tr) and HR, despite a lower sweat rate (SR) compared with males (Avellini et al. 1980). Following HA, the physiological strain was similar between males and females, although males maintained a greater SR. This study adopted a traditional HA protocol which results in a progressive decline in the adaptation stimulus over the duration of HA. Controlled hyperthermia ensures consistent potentiating stimuli for adaptation throughout the HA period, eliciting reductions in thermal strain and increases in work capacity during both short term HA (STHA) (Garrett et al. 2009; Garrett et al. 2012) and long term HA (LTHA) (Patterson et al. 2004), potentially promoting more complete adaptation (Taylor & Cotter 2006). It remains unknown the extent to which males and female's adapt to the controlled hyperthermia model of HA.

HA is often separated into STHA (< 8 days) and LTHA (> 10 days) (Garrett et al. 2011). STHA is a preferred regime, as it provides less disruption of quality training prior to competition. Approximately 70% of adaptations have been demonstrated to occur following STHA, evidenced by reductions in thermoregulatory and cardiovascular strain combined with an improved sudomotor function (Poirier et al. 2014). Acknowledging previous observations that males typically have a superior sudomotor function compared with females (Inoue et al. 2005; Gagnon & Kenny 2011), we may expect females to achieve superior sudomotor adaptation following STHA compared with males. However, following STHA, Sunderland and colleagues (2008) only achieved partial HA in trained females with a 33% increase in intermittent sprint performance in the heat, despite no alterations in classic indicators of HA including HR, Tr and SR. These typical adaptive responses have been previously observed in trained males following STHA (Garrett et al. 2011; Buono et al. 1998; Fujii et al. 2012; Racinais et al. 2012;

Poirier et al. 2014); suggesting females may require LTHA to achieve adaptation. Due to the self-paced exercise administered pre and post HA, participants were exercising at a higher absolute intensity following HA, suggesting an increase in metabolic heat production; potentially negating any improvements in thermoregulation achieved through HA. Research is required to determine the extent to which females adapt to STHA when using a fixed intensity heat tolerance test to monitor adaptations.

A paucity of data exists on best practise for HA in females with practitioners relying upon HA literature obtained from male participants. Therefore the primary aim of the current study was to compare males and females' thermoregulatory and physiological adaptation to STHA and LTHA using the controlled hyperthermia model of HA. We hypothesised that males and females will differ in their temporal patterning to heat acclimation.

Materials and Methods

Participants

Sixteen (8 males; 8 females) physically active volunteers provided written informed consent to participate in the current study (table 1), which was approved by the institution's ethics committee and conducted in accordance with the Declaration of Helsinki (2013). All experimental trials were performed between 0800 hrs and 1200 hrs to control for time of day effects (Winget et al. 1985). Experimentation occurred during the UK winter (mean ambient temperature of 5°C), therefore participants had been absent from repeated external heat exposure for the previous 3 months. Participant characteristics for age, body mass, height, sum of 4 skin folds, absolute $\dot{V}O_2$ peak, reported as mean and standard deviation are shown in table 1. To control for hormonal fluctuations associated with the menstrual cycle, female participants (n = 5) began testing during the early-follicular phase (3-5 days after the onset of menstruation) of their self-reported menstrual cycle. Female participants (n = 3) taking oral contraceptive began the experimental sessions on day 2 of the pill phase of oral contraceptive use.

Preliminary Testing

Forty-eight hours prior to conducting all trials participants were instructed to maintain normal hydration and refrain from the consumption of alcohol, caffeine, and exhaustive exercise. Two hours prior to arrival participants were instructed to consume 3-5 $ml.kg^{-1}$ of water. On arrival to the laboratory, participants voided

their bladder to provide a urine sample. When two out of the following three criteria were achieved, adequate hydrated to perform the trial was assumed; an osmolality value of ≤ 700 mOsm.kg-1, a urine specific gravity value of ≤ 1.020 or body mass within 1% of daily average (Sawka et al. 2007). These experimental controls were not violated for any participant for any of the preliminary or experimental procedures.

During the first visit to the laboratory, data was collected for height recorded to 1cm using a fixed stadiometer (Detecto Physicians Scales; Cranlea & Co., Birmingham, UK), and nude body mass recorded to 0.01kg from digital scales (ADAM GFK 150, USA). Sum of skin folds was determined from four sites; the bicep, triceps, subscapular and supra-iliac area using Harpenden skin fold callipers (Harpenden, UK). A graded exercise test was performed in temperate laboratory conditions (20°C, 40% RH) to determine $\dot{V}O_2$ peak using a cycle ergometer (Monark e724, Vansbro, Sweden). Following a 5 minute warm up, participants were informed to maintain a constant cadence of 80 rpm. The cycling intensity was set to 80W and resistance applied to the flywheel to elicit an increase of 24 $W.min^{-1}$ and 20 $W.min^{-1}$ for males and females, respectively. The test was terminated when participants reached volitional exhaustion and/or the cadence could no longer be maintained at 80 ± 5 rpm despite strong verbal encouragement. Expired air was measured using online gas analysis (Metalyzer Sport, Cortex, Germany). Peak $\dot{V}O_2$ was determined as the highest $\dot{V}O_2$ averaged over 10 seconds. A regression equation was computed from the data obtained to calculate the required intensity (65% $\dot{V}O_2$ peak) for the experimental exercise bouts. HR using a HR monitor (Polar Electro Oyo, Temple, Finland) was recorded in the final minute of each stage.

Experimental Design

Testing was completed over a 17 day period. Volunteers performed 10 HA sessions separated by three running heat tolerance tests (RHTT). The first RHTT was performed 48 hours prior to beginning HA (RHTT1), the second 48 hours following five days HA (RHTT2) and the third 48 hours following ten HA sessions (RHTT3). Towel-dried nude body mass was measured and recorded (Adam GFK 150, Adam Equipment Inc., USA) to the nearest gram before and immediately after all trials as a measure of whole body SR. Between these two measures of nude body mass fluid intake was restricted. Values were corrected for urine output (zero incidences); however values were uncorrected for respiratory and metabolic weight losses, since these were assumed as similar between trials due to the matched exercise intensity and environmental conditions. Participants inserted a rectal

thermometer (Henley, Reading, UK) 10 cm past the anal sphincter to measure Tr. Exercise was terminated if Tr ≥ 39.7°C (zero incidences), or the participant withdrew due to volitional exhaustion, or the participants could no longer maintain exercise intensity despite strong verbal encouragement. After a 20 minute seated stabilisation period, resting measures were recorded and participants entered the environmental chamber (TISS, Hampshire, UK). HR, Tr, were recorded at 5 minute intervals and ratings of perceived exertion (RPE) (Borg 1962) and thermal sensation (TS) (Toner et al. 1986) every 10 minutes during all trials.

Running Heat Tolerance Test

The RHTT involved 30 minutes exposure to 39.8 ± 0.8°C and 39.5 ± 1.3% RH whilst running at 9km.hr^{-1} and 2% gradient (Mee et al., 2015). The RHTT procedure was adopted due to its fixed absolute intensity that enabled the accurate quantification of adaptations following HA. Previous data collected within our laboratory demonstrates the RHTT to be a repeatable protocol that is sensitive to monitor adjustments in classic markers of heat tolerance associated with chronic heat alleviating interventions. Tr (Henleys Medical Supplies Ltd, Hertfordshire, UK), HR and T_{skin} were recorded at 5 minutes intervals throughout the RHTT. Skin temperature (T_{Skin}) was recorded using skin thermistors (Eltek Ltd, Cambridge, UK) attached to four sites; the midpoint of the right pectoralis major (T_{chest}), midpoint of the triceps brachii lateral head (T_{arm}), right rectus femoris ($T_{upper\ leg}$) and right gastrocnemius lateral head ($T_{lower\ leg}$) and connected to a Squirrel temperature logger (Squirrel 1000 series, Eltek Ltd., UK).

Skin temperature (Tskin) was calculated as follows (Ramanathan 1964);

$$Tskin = 0.3 \cdot (T_{chest} + T_{arm}) + 0.2 \cdot (T_{upper\ leg} + T_{lower\ leg})$$

Heat Acclimation

Heat acclimation involved two, five consecutive day blocks separated by 48 hours. The daily sessions consisted of a 90 minute exposure to 40°C, 40% RH. Exercise intensity was set at 65% $\dot{V}O_2$ peak from the outset and adjusted with work rest intervals to maintain a Tr ~ 38.5°C (Garrett et al. 2012; Patterson et al. 2004), or if participants were unable to maintain a cadence of 80 rpm (zero incidences). A cycling mode of exercise was

selected due to the consecutive nature of the heat acclimation sessions, thus reducing muscle damage and subsequently reducing the chance of participants incurring an injury.

Statistical Analysis

All data were first checked for normality using Shapiro-Wilk and sphericity using the Greenhouse Geisser method. An independent sample t-test was used to identify differences between male and female characteristics. A two-way mixed design analysis of variance (ANOVA) was performed to identify differences between the performance and physiological characteristics during STHA and LTHA, the physiological responses on day 1, 5 and 10 of HA and the physiological responses during RHTT1, RHTT2 and RHTT3. When a main effect or interaction effect was found, results were followed up using a Bonferroni corrected post hoc comparison. Effect sizes (partial Eta squared (np^2)) were calculated to analyse the magnitude and trends of the interventions. All data was analysed using a standard statistical package (SPSS version 20.0), and reported as mean ± standard deviation. Statistical significance was accepted at the level of $p \leq 0.05$.

Results

Performance Responses during Heat Acclimation (days 1-5 and 5-10)

Table 2 presents the mean ± SD data for the performance and physiological responses during STHA and LTHA. All participants completed ten, 90 minute HA sessions. ANOVA revealed a main effect of HA phase on exercise duration ($F_{(1, 14)} = 7.728$, $p = 0.015$, $np^2 = 0.356$). Exercise duration was lower in STHA (70 ± 8 minutes) compared with LTHA (75 ± 7 minutes). There was no interaction effect of HA phase and sex for exercise duration ($F_{(1, 14)} = 0.340$, $p = 0.569$, $np^2 = 0.024$).

There was a main effect of HA phase on exercise intensity ($F_{(1, 14)} = 4.710$, $p = 0.048$, $np^2 = 0.252$). Exercise intensity was lower in STHA (57 ± 6 %$\dot{V}O_2$ max) compared with LTHA (59 ± 5 %$\dot{V}O_2$ max). There was no interaction effect of HA phase and sex for exercise intensity ($F_{(1, 14)} = 0.587$, $p = 0.456$, $np^2 = 0.04$).

There was a main effect of HA phase on total work ($F_{(1, 14)} = 16.272$, $p < 0.001$, $np^2 = 0.538$). Total work was lower in STHA (484 ± 105 kJ) compared with LTHA (570 ± 124 kJ). There was no interaction effect of HA phase and sex for total work ($F_{(1, 14)} = 0.186$, $p = 0.673$, $np^2 = 0.013$).

ANOVA revealed a main effect of HA phase on duration Tr ≥ 38.5°C ($F_{(1, 14)}$ = 4.982, p = 0.042, np^2 = 0.262). The duration Tr ≥ 38.5°C was higher in STHA (49 ± 8 minutes) compared with LTHA (46 ± 8 minutes). There was no interaction effect of HA phase and sex for duration Tr ≥ 38.5°C ($F_{(1, 14)}$ = 0.513, p = 0.486, np^2 = 0.035).

Physiological Responses during Heat Acclimation (days 1-5 and 5-10)

Thermoregulatory Responses

There was a main effect of HA day on resting Tr (Tr_{rest}) ($F_{(2, 28)}$ = 37.281, $p \leq 0.001$, np^2 = 0.727). There was a reduction in Tr_{rest} from Day 1 to Day 5 (-0.26 ± 0.19 °C, p = 0.001), from Day 5 to Day 10 (-0.21 ± 0.28, p = 0.002), and from Day 1 to Day 10 (-0.47 ± 0.20, p ≤ 0.001). There was no interaction effect of HA day and sex on Tr_{rest} ($F_{(2, 28)}$ = 1.732, p = 0.195, np^2 = 0.110).

There was no main effect of HA phase on mean rectal temperature (Tr_{mean}) ($F_{(1, 14)}$ = 0.000, p = 0.988, np^2 = 0.000). Furthermore, there was no interaction effect of HA phase and sex on Tr_{mean} $F_{(1, 14)}$ = 0.872, p = 0.366, np^2 = 0.059).

Cardiovascular Responses

There was a main effect of HA day on resting heart rate (HR_{rest}) ($F_{(2, 28)}$ = 24.137, $p \leq 0.001$, np^2 = 0.633). There were no changes in HR_{rest} from Day 1 to Day 5 (-4 ± 6 beats.min^{-1}, p = 0.070). There was a reduction in HR_{rest} from Day 5 to Day 10 (-6 ± 4 beats.min^{-1} $p \leq 0.001$) and from Day 1 to Day 10 (-10 ± 7 beats.min^{-1}, $p \leq 0.001$). There was no interaction effect of HA day and sex on HR_{rest} ($F_{(2, 28)}$ = 2.117, p = 0.139, np^2 = 0.131).

There was no main effect of HA phase on mean heart rate (HR_{mean}) ($F_{(1, 14)}$ = 3.059, p = 0.102, np^2 = 0.179). Furthermore, there was no interaction effect of HA phase and sex on HR_{mean} ($F_{(1, 14)}$ = 0.716, p = 0.412, np^2 = 0.049).

Sudomotor Responses

There was a main effect of HA day on SR relative to body surface area (SR_{BSA}) ($F_{(2, 28)}$ = 16.266, $p \leq 0.001$, np^2 = 0.537). There was an increase from Day 1 to Day 5 (89 ± 144 g.hr^{-1}.m^2, p = 0.043), from Day 5 to Day 10 (87 ±

114 g.hr^{-1}.m^2, p = 0.014) and from Day 1 to Day 10 (177 ± 134 g.hr^{-1}.m^2, $p \leq 0.001$). There was no interaction effect for HA day and sex on SR_{BSA} ($F_{(2, 28)}$ = 2.806, p = 0.077, np^2 = 0.167).

There was a main effect of HA phase on SR_{BSA} ($F_{(1, 14)}$ = 21.737, $p \leq 0.001$, np^2 = 0.608). SR_{BSA} was lower in STHA (277 ± 58 g.hr^{-1}.m^2) compared with LTHA (329 ± 79 g.hr^{-1}.m^2). There was no interaction effect of HA phase and sex on SR_{BSA} ($F_{(1, 14)}$ = 0.987, p = 0.337, np^2 = 0.066).

Thermoregulatory Response to Short Term and Long Term Heat Acclimation

Table 3 presents the mean ± SD data for males and females physiological responses during RHTT1, RHTT2, RHTT3.

Resting Rectal Temperature

There was a main effect of RHTT for Tr_{rest} ($F_{(2, 28)}$ = 26.084, $p \leq 0.001$, np^2 = 0.651). Tr_{rest} reduced following STHA $_{(RHTT1\ to\ RHTT2)}$ (-0.13 ± 0.16°C, p = 0.002), LTHA $_{(RHTT2\ to\ RHTT3)}$ (-0.13 ± 0.16°C, p = 0.006) and LTHA $_{(RHTT1\ to\ RHTT3)}$ (-0.26 ± 0.16°C, $p \leq 0.001$). There was an interaction effect of RHTT and sex for Tr_{rest} ($F_{(2, 28)}$ = 5.282, p = 0.011, np^2 = 0.274). Tr_{rest} reduced following STHA $_{(RHTT1\ to\ RHTT2)}$ in males (-0.24 ± 0.16°C, $p \leq 0.001$), but no differences were observed in females (-0.02 ± 0.08°C, p = 0.597). Tr_{rest} reduced following LTHA $_{(RHTT2\ to\ RHTT3)}$ for females (-0.22 ± 0.12°C, $p \leq 0.001$), but no differences were observed in males (-0.04 ± 0.15°C, p = 0.459). Tr_{rest} reduced following LTHA $_{(RHTT1\ to\ RHTT3)}$ for both males (-0.28 ± 0.17°C, $p \leq 0.001$) and females (-0.24 ± 0.17°C, p = 0.001).

Peak Rectal Temperature

There was a main effect of RHTT for peak rectal temperature (Tr_{peak}) ($F_{(2, 28)}$ = 17.972, $p \leq 0.001$, np^2 = 0.532). Tr_{peak} reduced following STHA $_{(RHTT1\ to\ RHTT2)}$ (-0.23 ± 0.32°C, p = 0.018), LTHA $_{(RHTT2\ to\ RHTT3)}$ (-0.26 ± 0.30°C, p = 0.008) and LTHA $_{(RHTT1\ to\ RHTT3)}$ (-0.46 ± 0.36°C, p = 0.001). There was an interaction effect of RHTT and sex for Tr_{peak} ($F_{(2, 28)}$ = 3.339, p = 0.050, np^2 = 0.193). Tr_{peak} reduced following STHA $_{(RHTT1\ to\ RHTT2)}$ for males (-0.39 ± 0.36°C, $p \leq 0.001$), but no differences were observed in females (-0.07 ± 0.18, p = 0.504). Tr_{peak} reduced following LTHA $_{(RHTT2\ to\ RHTT3)}$ for females (-0.41 ± 0.24°C, $p \leq 0.001$), but no differences were observed in males (-0.05 ± 0.26°C, p = 0.590). Tr_{peak} reduced following LTHA $_{(RHTT1\ to\ RHTT3)}$ for both males (-0.44 ± 0.45°C, p = 0.005) and females (-0.48 ± 0.27°C, p = 0.003).

Rectal Temperature at 5 Minute Intervals

Figure 1 presents the Tr 5 minute interval data for both males and females. There was a main effect of RHTT and time on Tr ($F_{(12, 168)}$ = 2.343, p = 0.008, np^2 0.143). Following STHA $_{(RHTT1\ to\ RHTT2)}$ there was a reduction in Tr at 5 ($p \leq 0.001$), 10 (p = 0.008), 15 (p = 0.027), 20 (p = 0.007) and 25 (p = 0.018) minutes. Following LTHA $_{(RHTT2\ to\ RHTT3)}$ there were no differences in Tr at 5 (p = 0.265), 10 (p = 0.347), 15 (p = 0.138), 20 (p = 0.346) and 25 (p = 113) minutes. Following LTHA $_{(RHTT1\ to\ RHTT3)}$ there was a reduction in Tr at 5 (p = 0.002), 10 (p = 0.005), 15 (p = 0.006), 20 (p = 0.002) and 25 (p = 0.001) minutes. There was no interaction effect of RHTT and Time and sex for Tr ($F_{(12,168)}$ = 1.055 p = 0.402, np^2 = 0.070).

Change in Rectal Temperature

There was no main effect of RHTT for change in rectal temperature (Tr_{change}) ($F_{(2,28)}$ = 2.502, p = 0.100, np^2 = 0.152). There was no interaction effect of RHTT and sex observed for Tr_{change} ($F_{(2,28)}$ = 0.513, p = 0.604, np^2 = 0.035).

Peak Skin Temperature

There was a main effect of RHTT for peak skin temperature ($Tskin_{peak}$) ($F_{(2,28)}$ = 19.085, $p \leq 0.001$, np^2 = 0.577). $Tskin_{peak}$ reduced following STHA $_{(RHTT1\ to\ RHTT2)}$ (-0.45 ± 0.62°C; p = 0.038), LTHA $_{(RHTT2\ to\ RHTT3)}$ (-0.60 ± 0.62°C, p = 0.007) and LTHA $_{(RHTT1\ to\ RHTT3)}$ (-1.05 ± 0.74°C, $p \leq 0.001$). There was no interaction effect of RHTT and sex observed for $Tskin_{peak}$ ($F_{(2,28)}$ = 0.088, p = 0.916, np^2 = 0.006).

Cardiovascular Response to Short Term and Long Term Heat Acclimation

Resting Heart Rate

There was a main effect of RHTT for HR_{rest} ($F_{(2,28)}$ = 11.177, $p \leq 0.001$, np^2 = 0.444). Following STHA $_{(RHTT1\ to\ RHTT2)}$ there were no observed differences in HR_{rest} (-6 ± 11 beats.min^{-1}, p = 0.117). HR_{rest} reduced following LTHA $_{(RHTT2\ to\ RHTT3)}$ (-6 ± 8 beats.min^{-1}, p = 0.027) and LTHA $_{(RHTT1\ to\ RHTT3)}$ (-12 ± 12 beats.min^{-1}, p = 0.003). There was no interaction effect of RHTT and sex observed for HR_{rest} ($F_{(2,28)}$ = 0.942, p = 0.402, np^2 = 0.063).

Peak Heart Rate

There was a main effect of RHTT for heart rate peak (HR_{peak}) ($F_{(2, 28)}$ = 19.916, $p \leq 0.001$, np^2 = 0.587). HR_{peak} reduced following STHA $_{(RHTT1\ to\ RHTT2)}$ (-9 ± 10 beats.min^{-1}, p = 0.002), showed no differences following LTHA $_{(RHTT2\ to\ RHTT3)}$ (-2 ± 8 beats.min^{-1}, p = 1.000), but reduced following LTHA $_{(RHTT1\ to\ RHTT3)}$ (-11 ± 6 beats.min^{-1}, $p \leq 0.001$). There was an interaction effect of RHTT and sex for HR_{peak} ($F_{(2, 28)}$ = 3.598, p = 0.041, np^2 = 0.204). HR_{peak} reduced following STHA $_{(RHTT1\ to\ RHTT2)}$ in males (-14 ± 12 beats.min^{-1}, $p \leq 0.001$), no differences were observed in females (-5 ± 3 beats.min^{-1}, p = 0.164). There were no differences observed for HR_{peak} following LTHA $_{(RHTT2\ to\ RHTT3)}$ in both males (2 ± 10 beats.min^{-1}, p = 0.505) and females (-5 ± 5 beats.min^{-1}, p = 0.076). HR_{peak} reduced following LTHA $_{(RHTT1\ to\ RHTT3)}$ for both males (-13 ± 7 beats.min^{-1}, $p \leq 0.001$) and females (-10 ± 6 beats.min^{-1}, p = 0.001).

Heart Rate at 5 Minute Intervals

Figure 2 presents the HR 5 minute interval data for both males and females. There was no main effect of RHTT and time on HR ($F_{(12, 168)}$ = 0.845, p = 0.604, np^2 = 0.057). There was no interaction effect of RHTT and Time and sex for Tr ($F_{(12,168)}$ = 1.055 p = 0.401, np^2 = 0.070).

Sudomotor Response to Short Term and Long Term Heat Acclimation

Sweat Rate

There was a main effect of RHTT for SR ($F_{(2, 28)}$ = 12.207, $p \leq 0.001$, np^2 = 0.466). SR increased following STHA $_{(RHTT1\ to\ RHTT2)}$ (334 ± 590 g.hr^{-1}, p = 0.042). There were no differences observed for LTHA $_{(RHTT2\ to\ RHTT3)}$ for SR (334 ±636 g.hr^{-1}, p = 0.131) however, an increase was observed following LTHA $_{(RHTT1\ to\ RHTT3)}$ (668 ± 529 g.hr^{-1}, p = 0.001). There was an interaction effect of RHTT and sex for SR ($F_{(2, 28)}$ =3.661, p = 0.039, np^2 = 0.270). SR increased following STHA $_{(RHTT1\ to\ RHTT2)}$ in females (691 ± 412 g.hr^{-1}, p = 0.001), but no differences were observed in males (-22 ± 533 g.hr, p = 0.896). SR increased following LTHA $_{(RHTT2\ to\ RHTT3)}$ for males (583 ± 638 g.hr^{-1}, p = 0.016), but no differences were observed in females (85 ± 564 g.hr^{-1}, p = 0.696). SR increased following LTHA $_{(RHTT1\ to\ RHTT3)}$ for both males (560 ± 594 g.hr^{-1}, p = 0.010) and females (776 ± 470 g.hr^{-1}, p = 0.001).

Sweat Rate Relative to Body Surface Area

There was a main effect of RHTT for SR_{BSA} ($F_{(2, 28)}$ = 11.947, $p \leq 0.001$, np^2 = 0.460), increasing following STHA $_{(RHTT1\ to\ RHTT2)}$ (334 ± 590 $g.hr^{-1}.m^2$; p = 0.029). There were no differences observed for LTHA $_{(RHTT2\ to\ RHTT3)}$ (334 ± 636 $g.hr^{-1}.m^2$, p = 0.210) and LTHA $_{(RHTT1\ to\ RHTT3)}$ (668 ± 529 $g.hr^{-1}.m^2$, $p \leq 0.001$). There was an interaction effect of RHTT and sex on SR_{BSA} ($F_{(2, 28)}$ = 3.939, p = 0.031, np^2 = 0.220). SR_{BSA} increased following STHA $_{(RHTT1\ to\ RHTT2)}$ for females (428 ± 269 $g.hr^{-1}.m^2$, p = 0.001), but no differences were observed in males (-11 ± 286 $g.hr^{-1}.m^2$, p = 0.909). SR_{BSA} increased following LTHA $_{(RHTT2\ to\ RHTT3)}$ for males (308 ± 346 $g.hr^{-1}.m^2$, p = 0.029), but no differences were observed in females (44 ± 373 $g.hr^{-1}.m^2$, p = 0.733). SR_{BSA} increased following LTHA $_{(RHTT1\ to\ RHTT3)}$ for both males (297 ± 314 $g.hr^{-1}.m^2$, p = 0.015) and females (472 ± 291 $g.hr^{-1}.m^2$, p = 0.001).

Discussion

Heat acclimation literature in humans is primarily based on male participants, which limits the interpretation to females owing to the sex differences in thermoregulation. We examined the sex differences in the temporal patterning to short and long term heat acclimation. Our data demonstrates that both males and females achieve partial adaptation following STHA; with males demonstrating a reduction in thermoregulatory and cardiovascular strain and females demonstrating an increased sudomotor function. Following LTHA, both males and females achieved additional adaptation; with females demonstrating a reduction in thermoregulatory strain and males an increased sudomotor function. These results suggest that both males and females respond to STHA however, females require LTHA to establish thermoregulatory and cardiovascular stability.

Short term heat acclimation

Following STHA, approximately 70% of adaptations have been reported to be achieved (Poirier et al. 2014). In the current study, males demonstrated more adaptation following STHA compared with females with a reduction in Tr_{rest} (-0.24 ± 0.16°C) and Tr_{peak} (-0.32 ± 0.36°C); these changes did not result in any changes in Tr_{change}. The magnitude of reduction in Tr is very similar to the 0.3°C observed by Garrett and colleagues (2012) following 5 days controlled hyperthermia. Endurance performance is markedly impaired in hot compared to temperate environment due to an increase in core temperature causing a decrease in central activation (Nybo & Nielsen 2001). Attenuation of the Tr_{peak} may lessen or delay the likelihood of individuals obtaining or

expressing signs of heat-related illnesses when training, working or competing in the heat, demonstrating the effectiveness of STHA in males.

In the current study, exercise intensity, exercise duration and total work performed was higher during LTHA compared with STHA. This increased exercise intensity was administered to elicit and maintain the target core temperature of 38.5°C. The higher exercise intensity during LTHA would result in a higher metabolic heat production compared with STHA. Since total heat loss during exercise is predominantly a function of evaporative heat loss, a greater rate of metabolic heat production in LTHA, with comparable Tr values achieved, suggests an increase in evaporative heat loss and thus reduced heat storage. These findings support that partial heat adaptation was achieved during STHA.

Cardiac stability was achieved following STHA in male participants evidenced by a reduction in $HR_{peak.}$ The 14 ± 12 beats.min^{-1} reduction in HR_{peak} is in accordance with previous observations following STHA using controlled hyperthermia (Garrett et al. 2012; Patterson et al. 2004). The reduction in cardiovascular strain is potentially due to an increase in blood volume, preserving stroke volume and reducing heat transfer from the body's core to the skin and ultimately to the external environment.

Sunderland and colleagues (2008) assessed the effect of four days HA on female games players intermittent sprint performance in the heat. Intermittent sprint performance increased by 33%, following 4 days HA, however there were no differences in Tr_{peak}, HR_{peak} and SR. The self-regulated nature of the intermittent sprint protocol used provides no standardised endogenous thermal load, potentially constraining adaptation in some individuals (Taylor & Cotter 2006). Furthermore, any reduction in thermoregulatory and cardiovascular strain may have been negated due to participants performing more work following HA. The findings in the current study are in agreement with these previous reports, with no reductions in cardiovascular and thermoregulatory strain during STHA in female participants.

Increased SR have been reported to occur following STHA (Patterson et al. 2004; Buono et al. 2009; Poirier et al. 2014). Patterson and colleagues (2004a) reported that only six HA sessions were required to elicit partial sudomotor adaptation evidenced by an elevated local SR. The findings in the current study support previous findings with a 428 ± 269 g.hr^{-1}.m^2 increase in SR in female participants following STHA. Although females in the

current study had a lower stimulus for sweat production, due to potentially exercising at a lower metabolic heat production, they nonetheless improved sweat production to a greater extent compared to males, particularly following STHA. This is surprising, based on previous findings which suggest that the production of sweat is the main drive for improvements in sweating during heat acclimation (Buono et al. 2009). As such, it could be argued that females adapt during STHA to a greater extent than males. However, the observed increase in SR did not result in a reduced thermal strain in female participants. No plateau was observed in Tr during the RHTT in female participants, thus, the enhance SR did not offset the uncompensable rate of metabolic heat production observed.

An increase in whole body SR observed in females following STHA in the current study suggests either an altered afferent neural activity from the peripheral or central thermo receptors causing different integration of thermal information, an altered efferent neural activity for a given level of afferent input or an altered effector response. Sex modulates peripheral control of the sudomotor function, this is evidenced by a reduced thermosensitivity; resulting in females having a reduced SR compared with males (Gagnon & Kenny 2011). Consequently, it may be hypothesised that the enhanced sudomotor function in the female participants following STHA in the current study is as a result of peripheral changes to the thermosensitivity of the eccrine sweat glands. The potential mechanisms for this include an increased cholinergic sensitivity of the eccrine sweat gland and increase glandular hypertrophy (Buono et al. 2009; Lorenzo & Minson 2010).

In the current study there were no observed changes in SR during $STHA_{(RHTT1\ to\ RHTT2)}$ in male participants. These findings may be due to a lower Tr_{peak} in RHTT2 compared with RHTT1. Specifically, in male participants the Tr_{peak} in RHTT1 was 38.67 ± 0.25°C which produced a SR_{BSA} of 838 ± 215 $g.hr^{-1}.m^2$. The Tr_{peak} in RHTT2 was 38.28 ± 0.24 °C which produced a SR_{BSA} of 827 ± 168 $g.hr^{-1}.m^2$. It is well reported that an increase in core body temperature stimulate sudomotor function during exercise in the heat (Sawka et al. 1989). Recent findings suggest that whole body SR may underestimate the true adaptation that occurred to the sweat gland function following HA (Buono et al. 2009; Inoue et al. 1999). Buono et al. (2009) reported 20% increase in whole body SR, while pilocarpine induced SR increased by 63% following 8 day HA. Furthermore, Inoue et al. (1999) reported no changes in whole body SR, while a significant improvement was observed for methycholine induced SR following 8 day HA.

Long term heat acclimation

The adaptive effects of LTHA are well established, such that the extent to which an individual physiologically adapts to HA is dependent upon the length of exposure to heat stress conditions. In the current study, there was a reduction in the combined thermoregulatory and cardiovascular strain, an enhance sudomotor function following LTHA in all participants. These findings are in accordance to the results reported by Avellini and colleagues (1980) when assessing sex differences in adaptation to 10 days fixed intensity HA. Males and females were reported to express similar adaptive response evidence by reduction in Tr, an increased SR and an improved exercise capacity. Participants worked at the same absolute exercise intensity during the HA session, therefore, there may have been variety in the physiological strain placed on participants. When work rate remains constant, thermal strain during sequential exposures progressively declines, constraining adaptation (Taylor & Cotter 2006)

Since controlled hyperthermia ensures equal thermal strain during every session, it can be assumed that adaptation was not constrained during sequential sessions in the current study, establishing more complete adaptation (Taylor & Cotter 2006). Additional adaptations were observed during LTHA for the female participant's evidence by reductions in measure of thermoregulatory and cardiovascular strain from RHTT2 to RHTT3. These observed differences were not present in the male participants thus females require LTHA to establish thermoregulatory and cardiovascular stability.

LTHA established an improved sudomotor response in the current study. Increased SR is not unique to the current study with HA known to improve peripheral and central mechanisms involved in sudomotor function, via enhanced sweat gland sensitivity (Buono et al. 2009) and reductions in the onset threshold for sweating, enhancing evaporative heat loss (Yamazaki & Hamasaki 2003). Complete sudomotor adaptation has been suggested to take between 10 and 14 days to establish (Armstrong & Maresh 1991) but this biphasic adaptation may be more protocol dependent. In the current study females obtained no additional benefit to the sudomotor function as a result of LTHA, however, an increase in SR was observed from RHTT2 to RHTT3 in male participants.

Limitations

The exercise elicited during the heat acclimation sessions was performed at 65% of $\dot{V}O_2$max. Females in the current study had a lower absolute $\dot{V}O_2$max compared with the males participants, consequently they worked

at a lower metabolic heat production providing a lower stimulus for sweat production (Gagnon et al. 2008; Gagnon et al. 2013; Cramer & Jay 2014). Furthermore, females in the current study had a lower body mass compared with the male participants which entails less heat storage, and therefore a lower exercise intensity is required to increase their body temperature to 38.5°C (Gagnon et al. 2009). Consequently, the stress imposed during the HA sessions was likely lower in female participants, potentially constraining adaptation and influencing the time course of adaptation, due to inadequate endogenous heat strain. Future research is warranted to quantify these potential differences between males and females. Future work should involve the implementation of a controlled hyperthermia protocol where workload in administered using relative heat production. This may further optimise adaptation to HA by reducing individual variability associated with metabolic heat production (Cramer & Jay 2014)

Future work should implement greater control over hormonal alterations which alter thermoregulatory responses associated with the menstrual cycle between repeated trials. An elevation in progesterone concentration, associated with the luteal phase of the menstrual cycle, alters resting body temperature, the threshold for sweating, and cutaneous vasodilation and consequently, tolerance to exercise heat stress (Inoue et al. 2005). In the current study, the minimum number of days the protocol required for completion was 16 thus, crossing over the two menstrual cycle phases. For those participants not using oral contraception, RHTT1 and RHTT2 were performed during the follicular phase of their self-reported menses, when resting core temperature and the threshold for the onset of sweating and cutaneous vasodilation is lower compared with the luteal phase. However, RHTT3 was performed during the luteal phase of their self-reported menses. Consequently, the extent of the adaptation reported in females may have been smaller due to alterations in hormone concentrations of progesterone associated with the menstrual cycle (Inoue et al. 2005). Controlling for the hormonal alteration associated with the menstrual cycle throughout heat acclimation would provide a greater understanding into the true adaptation present.

Furthermore, in the current study, changes in plasma volume and fluid regulation were not measured, both of which assist in the maintenance of an elevated SR and a reduced cardiovascular strain associated with heat acclimation (Taylor 2014). Consequently, the effect of these adaptations on the improvement in SR and reduction in cardiovascular strain in the current is not known.

Perspectives

In the current study, HA was effective in attenuating physiological strain and improving exercise-heat tolerance in both males and females and thus, may reduce the likelihood of obtaining a heat-related illness during training or competition in the heat. STHA is a preferred regime for athletes since it is easier to adopt when sustaining quality training and tapering performance in the weeks before competition. These findings suggest that whilst STHA may be effective in achieving partial adaptation in males and females, females require LTHA to establish reductions in cardiovascular and thermoregulatory strain. Thus, HA protocols should be tailored to target sex differences, since sex has been shown to modulate the temporal patterning of HA.

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Conflict of Interest

The authors declare that they have no competing interests such as funding or personal financial interest.

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Table 1. Mean ± standard deviation participant characteristics

	Males	Females
Number	8	8
Age (years)	22 ± 6	20 ± 1
Height (cm)	178 ± 6	164 ± 7*
Body Mass (kg)	74.16 ± 6.92	58.89 ± 7.70*
Sum of 4 skin folds	34 ± 5	45 ± 13*
Absolute $\dot{V}O_2$ peak ($ml.min^{-1}$)	3.63 ± 0.69	2.69 ± 0.30*
End Power output (W)	299 ± 33	200 ± 25*

$\dot{V}O_2$ peak = peak oxygen consumption

*denotes significant difference between sexes ($p \leq 0.05$).

Table 2 Mean ± SD Performance and Physiological responses during short term heat acclimation and long term heat acclimation

	STHA		LTHA	
	Males	Females	Males	Females
Exercise Duration (min)	69 ± 7	70 ± 9	76 ± 7	74 ± 7
Mean Exercise Intensity (%$\dot{V}O_2$ max)	55 ± 7	59 ± 5	58 ± 6	60 ± 4
Total Work Done (kJ)	562 ± 70	413 ± 79	653 ± 116	487 ± 63
Time > 38.5°C (min)	48 ± 9	51 ± 7	45 ± 8	47 ± 9
Mean Tr (°C)	38.20 ± 0.10	38.19 ± 0.21	38.17 ± 0.14	38.23 ± 0.10
Mean HR (beats.min^{-1})	150 ± 9	151 ± 9	151 ± 9	154 ± 8
SR_{BSA} (g.hr^{-1}/m^2)	276 ± 65	278 ± 53	317 ± 65	341 ± 194

Notes STHA = short term heat acclimation, LTHA = long term heat acclimation, Tr = rectal temperature, HR = heart rate and SR_{BSA} = Sweat rate relative to body surface area

Table 3 Mean ± SD physiological variables during baseline testing (RHTT1), following 5 days heat acclimation (RHTT2) and following 5 days heat acclimation (RHTT3).

	RHTT1		RHTT2		RHTT3	
	Males	Females	Males	Females	Males	Females
Tr_{rest} (°C)	37.16 ± 0.11	37.23 ± 0.29	36.92 ± 0.19*	37.21 ± 0.34	36.88 ± 0.18*	36.94 ± 0.29*†
Tr_{peak} (°C)	38.67 ± 0.25	39.18 ± 0.39	38.28 ± 0.24*	39.12 ± 0.44	38.24 ± 0.40*	38.71 ± 0.42*†
Tr_{change} (°C)	1.51 ± 0.26	1.95 ± 0.31	1.37 ± 0.25	1.91 ± 0.27	1.35 ± 0.41	1.72 ± 0.38
$Tskin_{peak}$	37.30 ± 0.53	37.66 ± 0.95	36.80 ± 0.36	37.25 ± 0.55	36.27 ± 0.77	36.58 ± 0.72*†
HR_{rest} (beats.min^{-1})	71 ± 9	78 ± 10	66 ± 8	71 ± 7	63 ± 4	62 ± 7
HR_{peak} (beats.min^{-1})	185 ± 11	190 ± 11	170 ± 12*	185 ± 11	172 ± 10*	180 ± 11*
SR_{BSA} (g.hr^{-1}.m^{2})	838 ± 215	326 ± 156	827 ± 168	754 ± 260*	1135 ± 324*†	798 ± 229*

Notes Tr = rectal temperature, HR = heart rate, Tskin = skin temperature, and SR_{BSA} = Sweat rate relative to body surface area

*denotes significant difference to RHTT1 ($P \leq 0.05$).

†denotes significant difference to RHTT2 ($p \leq 0.05$).

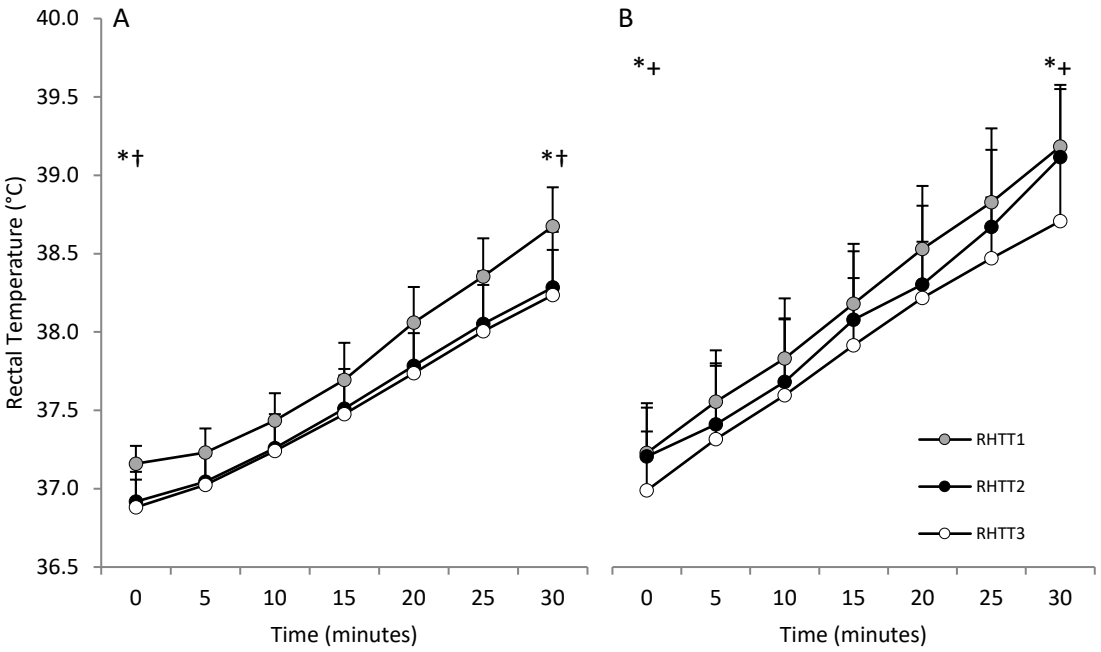

Figure 1. Mean ± SD rectal temperature at 5 minute intervals during the running heat tolerance test for males (1A) and females (1B). Grey markers represent the RHTT1, black markers RHTT2 and white marker RHTT3.

*denotes significant difference in STHA $_{(RHTT1\ to\ RHTT2)}$ ($P \leq 0.05$).

+ denotes significant difference in LTHA $_{(RHTT2\ to\ RHTT3)}$ ($P \leq 0.05$).

† denotes significant difference in LTHA $_{(RHTT1\ to\ RHTT3)}$ ($p \leq 0.05$).

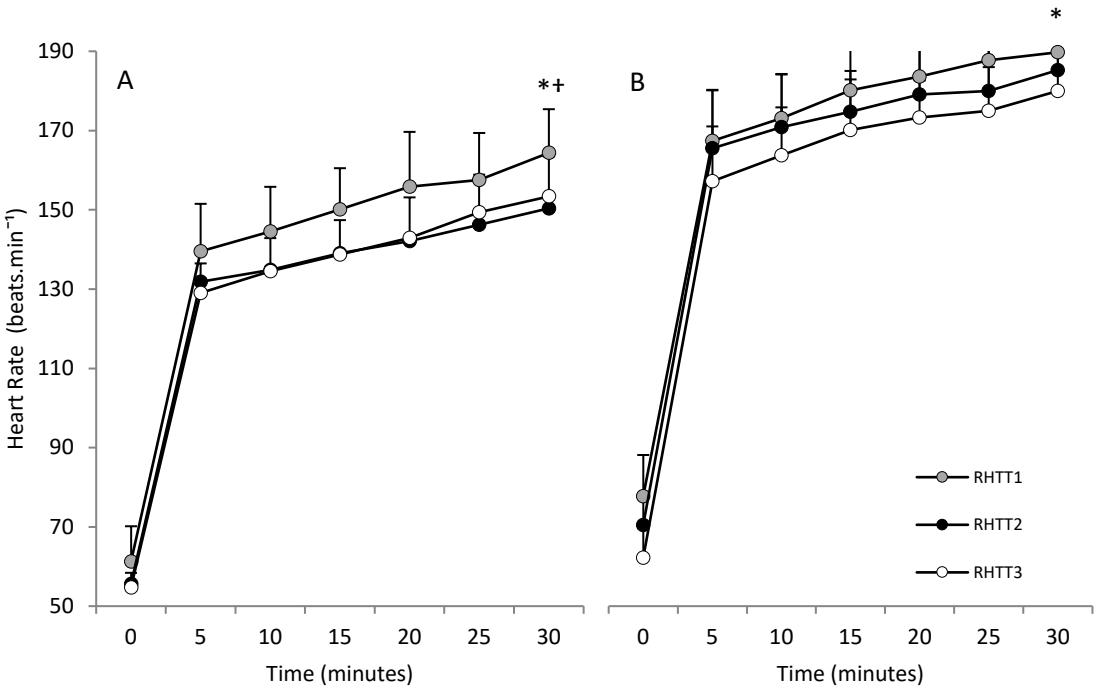

Figure 2. Mean ± SD heart rate at 5 minute intervals during the running heat tolerance test for males (2A) and females (2B). Grey markers represent the RHTT1, black markers RHTT2 and white marker RHTT3.

*denotes significant difference in STHA $_{(RHTT1\ to\ RHTT2)}$ ($P \leq 0.05$).

† denotes significant difference in LTHA $_{(RHTT1\ to\ RHTT3)}$ ($p \leq 0.05$).