Sauna exposure immediately prior to short-term heat acclimation accelerates phenotypic adaptation in females

Jessica A. Mee[a,b], Sophie Peters[a], Jonathan H. Doust[a], and Neil S. Maxwell[a]

[a] Centre for Sport and Exercise Science and Medicine (SESAME), University of Brighton, Eastbourne, UK

[b] School of Sport Health and Exercise Science (SSHES), Bangor University, Bangor, Gwynedd, UK

Corresponding author: Dr Jessica A. Mee; School of Sport Health and Exercise Science (SSHES), Bangor University, Bangor, Gwynedd, UK; j.a.mee@bangor.ac.uk

Abstract word count: 246 words

Text only word count: 3,261 words

Number of figures and tables: 2 tables, 2 Figures

Key words

Controlled hyperthermia; sweat sodium chloride; 17-β estradiol; progesterone; thermoregulation

Sauna exposure immediately prior to short-term heat acclimation accelerates phenotypic adaptation in females

Abstract

Objectives: Investigate whether a sauna exposure prior to short-term heat acclimation (HA) accelerates phenotypic adaptation in females.

Design: Randomised, repeated measures, cross-over trial.

Methods: Nine females performed two 5-d HA interventions (controlled hyperthermia $T_{re} \geq 38.5$°C), separated by 7-wk, during the follicular phase of the menstrual cycle confirmed by plasma concentrations of 17-β estradiol and progesterone. Prior to each 90-min HA session participants sat for 20-min in either a temperate environment (20°C, 40% RH; HA_{temp}) wearing shorts and sports bra or a hot environment (50°C, 30% RH) wearing a sauna suit to replicate sauna conditions (HA_{sauna}). Participants performed a running heat tolerance test (RHTT) 24-hr pre and 24-hr post HA.

Results: Mean heart rate (HR) (85±4 vs. 68±5 bpm, $p \leq 0.001$), sweat rate (0.4±0.2 vs. 0.0±0.0 $L.hr^{-1}$, $p \leq 0.001$), and thermal sensation (6±0 vs. 5±1, $p = 0.050$) were higher during the sauna compared to temperate exposure. Resting rectal temperature (T_{re}) (-0.28±0.16°C), peak T_{re} (-0.42±0.22°C), resting HR (-10±4 bpm), peak HR (-12±7 bpm), T_{re} at sweating onset (-0.29±0.17°C) ($p \leq 0.001$), thermal sensation (-0.5±0.5; $p = 0.002$), and perceived exertion (-3±2; $p \leq 0.001$) reduced during the RHTT, following HA_{sauna}; but not HA_{temp}. Plasma volume expansion was greater following HA_{sauna} (HA_{sauna}, 9±7%; HA_{temp}, 1±5%; $p = 0.013$). Sweat rate ($p \leq 0.001$) increased and sweat NaCl ($p = 0.006$) reduced during the RHTT following HA_{sauna} and HA_{temp}.

Conclusions: This novel strategy initiated HA with an attenuation of thermoregulatory, cardiovascular, and perceptual strain in females due to a measurably greater strain in the sauna compared to temperate exposure when adopted prior to STHA.

Key words

Controlled hyperthermia; sweat sodium chloride; 17-β estradiol; progesterone; thermoregulation

Introduction

Heat acclimation (HA) improves thermal comfort[1], submaximal exercise performance and maximal aerobic capacity in the heat[2]. The benefits arise from enhanced sudomotor and skin blood flow responses, cardiovascular stability, and an improved fluid balance[3]. As a result, HA is the consensus recommendation strategy to attenuate the physiological strain associated with training and competing in the heat[4]. However, there remains a paucity of data concerning best practice for HA in females, who appear to take longer to adapt compared to males[5]. Consequently, female athletes are implementing HA strategies based upon research in male participants. Accordingly, females may be adopting sub-optimal strategies for alleviating physiological strain.

Sunderland and colleagues reported improved thermal comfort and an increase in distance covered during a high intensity intermittent running test in females, following four intermittent HA sessions over 10-d, despite no differences in typical adaptive response, including resting rectal temperature (T_{re}), heart rate (HR), and whole body sweat rate (SWR)[6]. Furthermore, following short-term controlled hyperthermia HA (T_{re} = 38.5°C)[5], an optimised HA method due to the maintenance of the endogenous stimulus[7,8,] females only demonstrated partial adaptation[5], evidenced by an increase in SWR[5] and significant increases in Hsp72 mRNA[9] despite no changes in cardiovascular and thermoregulatory responses, when compared to males. An attenuation of thermoregulatory and cardiovascular strain was only observed in females following long-term HA[5]. Despite controlling for the endogenous stimulus for adaptation (T_{re} = 38.5°C), the controlled hyperthermia HA method may constrain adaptation in females compared with males due to a lower metabolic heat production and thus evaporative requirement during the HA sessions since exercise intensity was set to 65% of $\dot{V}O_2$ max. The absence of evidence concerning short-term HA (≤5-d) protocols that are effective for female athletes' is problematic since it is a preferred regime that incurs less disruption to quality training prior to competition compared with more traditional HA protocols[8]. A HA method for accelerating the adaptive response in females, without further disrupting training is desirable.

A novel, yet unexplored approach to accelerating heat adaptation over a short-term timescale is combining passive heat exposures with controlled hyperthermia HA. Related support for this practice comes from studies demonstrating thermoregulatory adaptation and performance benefits following passive HA using sauna exposures[10,11] and hot water immersion[12]. However, these methods demonstrate similar adaptation responses to that observed following controlled hyperthermia HA alone. Combining a passive exposure to sauna conditions with controlled hyperthermia HA may develop a greater stimulus for adaptation in females. The use of sauna suits during training results in a reduction in resting heart rate (-4 bpm), systolic (-2 mmHg) and diastolic (-3 mmHg) blood pressure, combined with an improved ventilatory threshold (+5.6% of $\dot{V}O_2$ max) and $\dot{V}O_2$ max (4.3 $mL.kg^{-1}.min^{-1}$)[13]. Thus, sauna suits may offer a practical and portable surrogate for classical HA via chamber acclimation or in-situ acclimatization compare to other thermal treatments.

It is essential to establish effective short-term HA methods for females, who appear to take longer to adapt compared to males[5] whilst limiting the exercise stimulus, to reduce interference with daily training. In the present study, we investigated thermoregulatory, cardiovascular, and sudomotor responses following a period of adaptation to sauna conditions immediately prior to controlled hyperthermia HA over a short-term timescale. It was hypothesised that a sauna exposure immediately prior to HA would result in a greater adaptation compared with HA alone.

Methods

Nine female athletes (Mean ± SD: age 22 ± 4-yr; body mass 59.33 ± 11.55 kg; height 1.64 ± 0.01 m; $\dot{V}O_2$-max 50 ± 4 $mL.kg^{-1}.min^{-1}$) provided written informed consent prior to their participation in the current study. Participants regularly undertook 6 ± 2 $h.wk^{-1}$ of endurance exercise; during the testing period participants were required to reduce this by 2.5 $h.wk^{-1}$ which was the typical time spent cycling to attain the target core temperature of 38.5°C. Confounding variables of smoking, caffeine, alcohol, generic supplementation and prior thermal exposure were controlled in line with previous work in the field[14]. To reduce the influence of hormonal fluctuations across the menstrual cycle on thermoregulatory responses, participants performed all trials between the third and tenth day after the onset of their self-

reported menstrual cycle. Female participants (n = 4) using oral contraceptives performed the experimental session during the no pill/ placebo phase of oral contraceptive use. To confirm hormonal status a venous blood sample was taken on the day of each experimental trial. No experimental sessions had to be withdrawn following analysis of the blood samples. This study was approved by the Institution's ethics committee for human investigation and conducted in accordance with the Declaration of Helsinki (2013).

In a randomised, cross-over design, participants completed two 5-d HA interventions separated by 7-wk to ensure the decay of adaptation[15] (Figure 1). Participants completed a running heat tolerance test (RHTT) 24-hr prior ($RHTT_1$) and 24-hr following ($RHTT_2$) HA. All trials were completed between December and March (mean temperature 5°C) and scheduled for the same time of day (0700 – 0900-hr).

Prior to assessment of maximal aerobic capacity ($\dot{V}O_2$-max), nude body mass was measured to the nearest 0.01 kg (ADAM GFK 150, USA). An incremental cycling test was performed in temperate laboratory conditions (20°C, 40% relative humidity [RH]) using a cycle ergometer (Monark e724, Vansbro, Sweden) to determine $\dot{V}O_2$-max. Power output was set to 80 W, and increased by 20 $W.min^{-1}$ until volitional exhaustion. $\dot{V}O_2$-max was determined as the highest $\dot{V}O_2$ (Metalyser 3B, Cortex, Leipzig, Germany) averaged over a 30-s period. $\dot{V}O_2$-max was confirmed via the attainment of a HR (Polar Electro Oyo, Kempele, Finland) within 10 bpm of age-predicted maximum, a respiratory exchange ratio ≥ 1.15, and a capillary lactate value ≥ 8 $mMol.L^{-1}$. A regression equation for oxygen consumption and power was solved to provide the required intensity (65% $\dot{V}O_2$-max) for the experimental exercise bouts.

Prior to all experimental trials and heat acclimation sessions a standardised pre-trial preparation occurred. Two hours prior to arrival to the laboratory, participants consumed 3-5 $mL.kg^{-1}$ of water. Euhydration was confirmed when body mass was within 1% of the participants daily average and osmolality was ≤ 700 $mOsm.kg^{-1}$ following analysis of a mid-flow urine sample. This experimental control was not violated for any trials. Towel-dried, nude body mass was recorded to the nearest gram

before and after all trials as a measure of SWR; with no fluid permitted between measurements. A rectal thermometer (Henley, Reading, UK) inserted 10 cm past the anal sphincter was used to monitor T_{re} and a HR monitor was affixed to the chest (Polar Electro Oyo, Kempele, Finland) to monitor HR. Exercise was terminated if $T_{re} \geq 39.7$°C.

In addition to the standardised pre-trial preparation and prior to the RHTT, skin thermistors (Eltek Ltd, Cambridge, UK) were attached to the mid-belly of the pectoralis major, triceps brachii, rectus femoris, and gastrocnemius and connected to a data logger (Squirrel 1000 series, Eltek Ltd., UK). Mean skin temperature was subsequently calculated[16]. Following 20-min seated rest in temperate conditions (20°C, 40% RH), capillary blood samples were collected in duplicate to estimate plasma volume expansion[17]. In addition, a 6 mL whole blood sample was taken from the antecubital vein into an EDTA tube. Plasma was immediately separated and stored in -83°C for subsequent analysis. Plasma concentrations of 17β-estradiol and progesterone were quantified in duplicate using commercially available ELIZA assay kits (Abcam plc, UK) and analysed in accordance with manufacturer's guidelines to confirm that trials occurred in the follicular phase of the menstrual cycle.

Participants entered an environmental chamber (WatFlow control system, TISS, Hampshire, UK) for the RHTT which involved 30-min running at 9 $km.hr^{-1}$, 2% gradient in 40°C and 40% RH[18]. Breath-by-breath expired air was measured using an online gas analyser (Metalyser 3B, Cortex, Leipzig, Germany) and metabolic heat production was subsequently calculated in accordance with the guidelines of Cramer and Jay[19]. At 5-min intervals T_{re}, HR, and skin temperature were recorded. At 10-min intervals thermal sensation (TS) and ratings of perceived exertion (RPE) were recorded.

Forearm sweat samples were collected during the first 15-min of the RHTT using a Macroduct sweat collector (Wescor, Logan, UT). The skin was cleaned with deionized water and dried before securing the sweat collector at the midpoint of the anterior forearm using a Velcro strap, which prevented leakage and sample contamination. When sweat entered the spiral tubing of the sweat collector it mixed with blue dye for simple visual identification of the onset of sweating. The forearm sweat samples were

analysed in duplicate for sodium chloride (NaCl) using a sweat conductivity analyser (Sweat Chek 3120, Wescor, UK).

Heat acclimation sessions were preceded by 20-min seated rest in either in a temperate environment (20°C, 40% RH) wearing shorts and a sports bra (HA_{temp}) or a hot environment (50°C, 30% RH), wearing a 100% Vinyl sauna suit (HA_{sauna}). Participants' nude body mass was recorded prior to participants completing the 90-min controlled hyperthermia HA session in 40°C, 40% RH wearing shorts and a sports bra. Exercise intensity was set at 65% $\dot{V}O_2$-max from the outset and adjusted to establish and maintain a $T_{re} \geq 38.5$°C. Measures of T_{re} and HR were recorded at 5-min intervals.

All data were checked for normality and sphericity. Two-way repeated measures analysis of variance (ANOVA; intervention x trial) were used to assess differences in physiological measures pre and post HA. Paired samples t-tests were performed to assess differences between training parameters and plasma volume expansion. When a main effect or interaction effect was observed, Bonferroni corrected pair-wise comparisons revealed where the differences occurred. All other data were analysed using a standard statistical package (SPSS version 20.0, IBM, USA) and reported as mean ± SD unless otherwise stated. Statistical significance was accepted at the level of $p \leq 0.05$. Effect sizes for main effects and interactions are presented as partial eta squared (ηp^2) while Cohen's d was used to evaluate differences between two related samples.

In addition to null hypothesis testing, magnitude based inferences were also used for analysis. For the RHTT data only, 90% confidence interval (CI) and the probabilities of whether the true (unknown) differences were lower, similar or higher than the smallest worthwhile change was calculated using Hopkins[20] spreadsheet. The qualitative chances of either higher or lower differences were evaluated as follows; 1%, almost certainly not; 1–5%, very unlikely; 5–25%, unlikely; 25–75%, possible; 75–95%, likely; 95–99%, very likely; >99%, almost certain. The smallest worthwhile change for each variable was determine from a recent meta-analysis on heat acclimation responses[21] for resting T_{re} (-0.18ºC), peak T_{re} (-0.31ºC), peak skin temperature (-0.57ºC), HR rest (–6 bpm), HR peak (-12 bpm), PV (4.3%), SWR (38%), sweat NaCl (-22), T_{re} at onset of sweating (-0.28ºC), thermal sensation (-0.9), and RPE (-

1).

Results

Table 1 presents the physiological, perceptual and performance responses between HA_{temp} and HA_{sauna}. Mean HR ($t_{(8)}$ = 6.143, $p \leq 0.001$, d = 2.047), SWR ($t_{(8)}$ = 6.340, $p \leq 0.001$, d = 2.113), and TS ($t_{(8)}$ = 2.309, p = 0.050, d = 0.770) were higher during the 20-min sauna compared to the temperate exposure. There were no differences in mean T_{re} between the two interventions during the 20-min sauna and temperate exposure ($t_{(8)}$ = 2.121, p = 0.067, d = 0.707).

During the heat acclimation sessions no differences were observed between interventions for duration spent with a $T_{re} \geq 38.5°C$ ($t_{(8)}$ = 0.160, p = 0.876, d = 0.053), exercise duration ($t_{(8)}$ = 0.448, p = 0.666, d = 0.149), relative exercise intensity ($t_{(8)}$ = 2.147, p = 0.064, d = 0.716), total work ($t_{(8)}$ = 0.954, p = 0.368, d = 0.318), mean T_{re} ($t_{(8)}$ = 0.654, p = 0.532, d = 0.218), and mean HR ($t_{(8)}$ = 0.410, p = 0.693, d = 0.137) during the controlled hyperthermia HA sessions. However, sweat NaCl concentrations were higher during the HA_{temp} sessions compared to HA_{sauna} ($t_{(8)}$ = 3.345, p = 0.010, d = 1.115).

There were no differences between the RHTT trials for measures of urine osmolality ($F_{(3, 24)}$ = 0.507, p = 0.981, ηp^2 = 0.060), progesterone ($F_{(3, 24)}$ = 3.452, p = 0.057, ηp^2 = 0.056) and 17β-estradiol concentrations ($F_{(3, 24)}$ = 2.348, p = 0.098, ηp^2 = 5.180).

Table 2 presents the physiological and perceptual changes from $RHTT_1$ to $RHTT_2$ in the HA_{sauna} and HA_{temp} and the quantitative chance that the true difference was higher/trivial/lower. There was an interaction effect between RHTT and intervention for resting T_{re} ($F_{(1, 8)}$ = 24.636, $p \leq 0.001$, ηp^2 = 0.755), peak T_{re} ($F_{(1, 8)}$ = 18.951, p = 0.002, ηp^2 = 0.703) and peak skin temperature ($F_{(1, 8)}$ = 7.409, p = 0.026, ηp^2 = 0.481). No differences were observed in resting T_{re} (p = 0.528, d = 0.201), peak T_{re} (p = 0.888, d = 0.041), or peak skin temperature (p = 0.091, d = 0.512) during $RHTT_1$. Resting T_{re} ($p \leq 0.001$, d = 1.308; Figure 2A), peak T_{re} ($p \leq 0.001$, d = 1.857; Figure 2B) and peak skin temperature (p = 0.015, d = 1.123) reduced from $RHTT_1$ to $RHTT_2$ in HA_{sauna}; but not HA_{temp} ($p > 0.05$).

There was an interaction effect between RHTT and intervention for HR rest ($F_{(1, 8)}$ = 6.545, p = 0.035, ηp^2 = 0.447) and HR peak ($F_{(1, 8)}$ = 8.983, p = 0.017, ηp^2 = 0.406). No differences were observed in HR rest (p = 0.172, d = 0.492) and HR peak (p = 0.657, d = 0.153) during $RHTT_1$. HR rest (p ≤ 0.001, d = 2.311; Figure 2C) and HR peak (p ≤ 0.001, d = 1.638; Figure 2D) reduced from $RHTT_1$ to $RHTT_2$ in HA_{sauna}; but not HA_{temp} (p > 0.05; Figure 2). Plasma volume expansion was greater in HA_{sauna} compared to HA_{temp} ($t_{(8)}$ = -3.163, p = 0.013, d = 1.071). Metabolic heat production reduced from $RHTT_1$ to $RHTT_2$ following both interventions (HAsauna -0.8 ± 1.0 $W.kg^{-1}$; HAtemp -0.3 ± 0.7 $W.kg^{-1}$; $F_{(1, 8)}$ = 10.896, p = 0.011, ηp^2 = 0.577).

SWR increased ($F_{(1, 8)}$ = 49.982, p ≤ 0.001, ηp^2 = 0.862) and sweat NaCl reduced ($F_{(1, 8)}$ = 32.900, p ≤ 0.001, ηp^2 = 0.804; Figure 2E) from $RHTT_1$ to $RHTT_2$ following both interventions. There was an interaction effect between RHTT and intervention for T_{re} at onset of sweating ($F_{(1, 8)}$ = 12.386, p = 0.008, ηp^2 = 0.608). No differences were observed in the T_{re} at onset of sweating (p = 0.989, d = 0.011) during $RHTT_1$, however T_{re} at onset of sweating reduced from $RHTT_1$ to $RHTT_2$ in HA_{sauna} (p ≤ 0.001, d = 1.744; Figure 2F); but not HA_{temp} (p = 0.137, d = 1.550).

There was an interaction effect between RHTT and intervention for TS ($F_{(1, 8)}$ = 67.600, p ≤ 0.001, ηp^2 = 0.894) and RPE ($F_{(1, 8)}$ = 24.143, p = 0.001, ηp^2 = 0.751). No differences were observed in TS (p = 1.000, d = 0.000) and RPE (p = 0.347, d = 0.333) during $RHTT_1$. TS (p = 0.002, d = 1.168) and RPE (p ≤ 0.001, d = 2.002) reduced from $RHTT_1$ to $RHTT_2$ in HA_{sauna}; but not HA_{temp} (p > 0.05; TS).

Discussion

The aim of this study was to investigate the effect of a sauna exposure immediately prior to short-term HA on thermal adaptation in females. Our novel findings indicate such sauna exposures to be an effective strategy to accelerate adaptation compared to controlled hyperthermia HA alone, evidenced by a reduction in resting T_{re} (-0.28°C) and HR (-10 bpm), exercising T_{re} (-0.42°C), HR (-12 bpm), TS (-1.0), and RPE (-2), combined with plasma volume expansion (+9%), a reduction in the onset of

sweating, an increase in SWR (-0.29°C), and a decrease in sweat NaCl concentrations (-16 mMol.L^{-1}). These adaptations are likely mediated in part by the accumulative 100 min additional heat exposure, in addition to the combined stimulus of an elevated HR, SWR, and TS during the sauna exposure compared to the temperate exposure. These findings are largely in keeping with other research employing controlled hyperthermia HA regimes in males[8,22,23] and suggest an altered autonomic control for heat balance. The importance of these findings is that HA_{sauna} accelerates adaptation in females who appear to take longer to adapt compared to males[5], whilst avoiding further interference with daily training that may occur when using long-term HA.

In the current study, there was a 9% plasma volume expansion when participants had a sauna exposure immediately prior to HA. This plasma volume expansion is similar to that observed by others following long-term exercise (+15%)[24] and sauna (+18%)[10] HA in males. However, short term heat acclimation alone was not able to elicit an expansion in plasma volume. These findings are in accordance with other short-term controlled hyperthermia HA investigations using male participants[22,23] who report a 1.8 - 4.2% non-significant increase in plasma volume. Sauna exposures are suggested to act as an independent stimulus for HA by augmenting plasma volume expansion[10,11]; causing an increase in vascular filling to support cardiovascular stability, an increase in the specific heat capacity of blood, and an attenuation of skin blood flow responses[3]. The reduction in cardiovascular strain observed in the current study was likely mediated by plasma volume expansion, which may be caused by an increase in aldosterone and arginine vasopressin secretion in combination with NaCl conservation[25], thus alleviating sub maximal exercise performance decerements[26] typically observed exercise in the heat.

This is the first study to provide data on an altered thermo-effector response of sudomotor function following HA in females, evidenced by a reduction in the T_{re} at the onset of sweating following HA_{sauna}. This observation may be explained by the increase in SWR; which is likely mediated by an increase in the cholinergic sensitivity of the eccrine sweat gland[27], altering the afferent neural activity of the central thermo-receptors[27], resulting in an altered integration of thermal information[27], in addition to glandular hypertrophy[28]. This is also the first study to provide data on sweat mineral concentrations following

controlled hyperthermia short-term HA in females. Both interventions resulted in a reduction in sweat concentrations of NaCl. The reduction observed following the HA_{sauna} interventions exceeded that reported previously in males following short-term controlled hyperthermia HA[23]. The mechanism of sweat mineral conservation are unclear, although previous data suggests sweat sodium conservation following HA involves increased sodium ion reuptake within the re-absorptive duct of the sweat gland[29] due to an increase in aldosterone secretion. This is a novel finding with practical relevance to female athletes who may experience chronic perfuse sweating during training which may raise the potential for mineral deficiencies[30].

Sauna exposures immediately prior to HA improved TS and RPE whilst running in the heat. These findings may have implications for free-paced exercise in the heat, since thermal comfort is a main driver of behavioural thermoregulation[31]. Evidence that TS modulates self-selected exercise intensity comes from data determining that menthol mouth rinse and facial spray are able to match the ergogenic effect of cold water immersion with the induction of similar biochemical shifts including prolactin[32]; supporting the practical relevance of these findings for athletes preparing to compete under environmental conditions posing a potentially limiting thermal burden. Further investigations are required to determine the effect of short-term HA, with or without additional sauna stress on pacing and time trial performance in females.

Conclusions

These data demonstrate an effective HA method to alleviate heat strain in females over a short-term timescale. A sauna exposure immediately prior to controlled hyperthermia HA reduced thermoregulatory, cardiovascular, and perceptual strain during running in the heat. Exposure to sauna conditions immediately prior to controlled hyperthermia HA overcomes the practical limitations with current HA interventions by offering a time-efficient method using easily accessible equipment to accelerate thermal, cardiovascular, and perceptual adaptions whilst limiting the disruption from training.

Practical implications

- Exposure to sauna-like conditions immediately prior to controlled hyperthermia HA attenuates thermoregulatory and cardiovascular strain in females during the follicular phase of the menstrual cycle.
- Combining sauna exposures with controlled hyperthermia HA alleviates heat strain and potentially susceptibility to heat illness in females.
- Combining sauna exposures with controlled hyperthermia HA should be considered when long-term HA is not available in females to alleviate heat strain.

Acknowledgements

The authors would like to thank the volunteers for their participation in this investigation and the University of Brighton technical support. There has been no financial assistance with the project.

References

1 Gonzalez RR, Gagge AP. Warm discomfort and associated thermoregulatory changes during dry and humid heat acclimatization. *Isr J Med Sci* 1976; 12(8):804–807.

2 Lorenzo S, Halliwill JR, Sawka MN, et al. Heat acclimation improves exercise performance. *J Appl Physiol* 2010; 109(4):1140–1147. Doi: 10.1152/japplphysiol.00495.2010.

3 Sawka MN, Leon LR, Montain SJ, et al. Integrated physiological mechanisms of exercise performance , adaptation , and maladaptation to heat stress. *Compr Physiol* 2011; 1:1883–1928.

4 Racinais S, Alonso JM, Coutts AJ, et al. Consensus recommendations on training and competing in the heat. *Br J Sports Med* 2015; 49(18):1164–1173.

5 Mee JA, Gibson OR, Doust J, et al. A comparison of males and females' temporal patterning to short- and long-term heat acclimation. *Scand J Med Sci Sports* 2015; 25:250–258. Doi: 10.1111/sms.12417.

6 Sunderland C, Morris JG, Nevill ME. A heat acclimation protocol for team sports. *Br J Sports Med* 2008; 42:327–333.

7 Taylor NS. Human heat adaptation. *Compr Physiol* 2014; 4(1):325–365. Doi: 10.1002/cphy.c130022.

8 Gibson OR, Mee JA, Tuttle JA, et al. Isothermic and fixed intensity heat acclimation methods induce similar heat adaptation following short and long-term timescales. *J Therm Biol* 2015; 49–50:55–65. Doi: 10.1016/j.jtherbio.2015.02.005.

9 Mee JA, Gibson OR, Tuttle JA, et al. Leukocyte Hsp72 mRNA transcription does not differ between males and females during heat acclimation. *Temperature* 2016. Doi: 10.1080/23328940.2016.1214336.

10 Stanley J, Halliday A, D'Auria S, et al. Effect of sauna-based heat acclimation on plasma volume and heart rate variability. *Eur J Appl Physiol* 2015; 115(4):785–794.

11 Scoon GSM, Hopkins WG, Mayhew S, et al. Effect of post-exercise sauna bathing on the endurance performance of competitive male runners. *J Sci Med Sport* 2007; 10(4):259–262.

12 Zurawlew MJ, Walsh NP, Fortes MB, et al. Post-exercise hot water immersion induces heat acclimation and improves endurance exercise performance in the heat. *Scand J Med Sci Sport* 2016; 26(7):745–754. Doi: 10.1111/sms.12638.

13 Van de Velde SS, Pierre IA, Fargo JS, et al. Effects of Exercise Training with a Sauna Suit on Cardiovascular Health : a Proof-of-Concept Study. *Int J Res Exerc Physiol* 2016; 11(1):1–10.

14 Taylor L, Midgley AW, Chrismas B, et al. Daily hypoxia increases basal monocyte HSP72 expression in healthy human subjects. *Amino Acids* 2011; 40(2):393–401.

15 Pandolf KB. Time course of heat acclimation and its decay. *Int J Sports Med* 1998; 19(2):S157–S160.

16 Ramanathan NL. A new weighting system for mean surface temperature of the human body. *J Appl Physiol* 1964; 19:531–533.

17 Dill DB, Costill DL. Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *J Appl Physiol* 1974; 37(2):247–248.

18 Mee J, Doust J, Maxwell N. Repeatability of a running heat tolerance test. *J Therm Biol* 2015; 49–50:91–97.

19 Cramer MN, Jay O. Selecting the correct exercise intensity for unbiased comparisons of thermoregulatory responses between groups of different mass and surface area. *J Appl Physiol* 2014; 116(9):1123–1132.

20 Hopkins WG. Spreadsheets for analysis of controlled trials with adjustment for a subject characteristic. *Sport Sci* 2006; 10:46–50.

21 Tyler CJ, Reeve T, Hodges GJ, et al. The effects of heat adaptation on physiology, perception and exercise performance in the heat: A meta-analysis. *Sport Med* 2016; 46(11):1699–1724. Doi: 10.1007/s40279-016-0538-5.

22 Garrett AT, Goosens NG, Rehrer NJ, et al. Induction and decay of short-term heat acclimation. *Eur J Appl Physiol* 2009; 107(6):659–670. Doi: 10.1007/s00421-009-1182-7.

23 Neal RA, Corbett J, Massey HC, et al. Effect of short-term heat acclimation with permissive dehydration on thermoregulation and temperate exercise performance. *Scand J Med Sci Sports* 2015; 26(8):875–884. Doi: 10.1111/sms.12526.

24 Gibson OR, Turner G, Tuttle JA, et al. Heat Acclimation attenuates physiological strain and the Hsp72, but not Hsp90α mRNA response to acute normobaric hypoxia. *J Appl Physiol* 2015; 119:889–899.

25 Garrett AT, Rehrer NJ, Patterson MJ. Induction and decay of short-term heat acclimation in moderately and highly trained athletes. *Sport Med* 2011; 41(9):757–771.

26 Lorenzo S, Minson CT. Heat acclimation improves cutaneous vascular function and sweating in trained cyclists. *J Appl Physiol* 2010; 109:1736–1743. Doi: 10.1152/japplphysiol.00725.2010.

27 Charkoudian N. Mechanisms and modifiers of reflex induced cutaneous vasodilation and vasoconstriction in humans. *J Appl Physiol* 2010; 109(4):1221–1228.

28 Buono M, Numan T, Claros R, et al. Is active sweating during heat acclimation required for improvements in peripheral sweat gland function? *Am J Physiol Regul Integr Comp Physiol* 2009; 297(4):R1082-5.

29 Sato K, Dobson RL. Regional and individual variations in the function of the human eccrine sweat gland. *J Investig Dematology* 1970; 54(6):443–449.

30 Chinevere TD, Kenefick RW, Cheuvront SN, et al. Effect of heat acclimation on sweat minerals. *Med Sci Sport Exerc* 2008; 40(5):886–891.

31 Flouris AD, Schlader ZJ. Human behavioral thermoregulation during exercise in the heat. *Scand J Med Sci Sport* 2015; 25(S1):52–64.

32 Stevens CJ, Thoseby B, Sculley D V., et al. Running performance and thermal sensation in the heat are improved with menthol mouth rinse but not ice slurry ingestion. *Scand J Med Sci Sports* 2015. Doi: 10.1111/sms.12555.

Table 1. Physiological and performance responses during the HA_{sauna} and HA_{temp} intervention. Data presented as Mean ± SD

Measures	HA_{sauna}	HA_{temp}
20 min Sauna and Temperate exposure		
Mean T_{re} (°C)	37.12 ± 0.14	37.26 ± 0.17
Mean HR (bpm)	85 ± 4 *	68 ± 5
SWR ($L.hr^{-1}$)	0.4 ± 0.2 *	0.0 ± 0.0
TS	6 ± 0 *	5 ± 1
90 min Controlled Hyperthermia HA		
Duration $T_{re} \geq 38.5$°C (min)	52 ± 10	51 ± 7
Exercise duration (min)	65 ± 4	67 ± 2
Relative exercise intensity (%$\dot{V}O_2$ max)	63 ± 4	58 ± 4
Total work (kJ)	379 ± 39	348 ± 36
Mean T_{re} (°C)	38.23 ± 0.14	38.26 ± 0.06
Mean HR (bpm)	145 ± 8	146 ± 8
Sweat NaCl ($mMol.L^{-1}$)	40 ± 14 *	47 ± 13

Notes. HR heart rate; NaCl sodium chloride; SWR sweat rate; T_{re} rectal temperature; TS thermal sensation. * denotes significant difference between HA_{temp} and HA_{sauna} ($p \leq 0.05$).

Table 2. Delta change for physiological and perceptual responses from $RHTT_1$ to $RHTT_2$ in the HA_{sauna} and HA_{temp}. Data presented as Mean ± SD (90% confidence intervals) and the quantitative chance that the true difference was higher, trivial, lower than the smallest worthwhile change.

Measures	HA_{sauna}		HA_{temp}	
	Mean ± SD (90% CI)	**Higher/ trivial/ lower (%)**	**Mean ± SD (90% CI)**	**Higher/ trivial/ lower (%)**
Resting T_{re} (°C)	-0.28 ± 0.16 (-0.38; -0.18) $^{+}$*	0, 5, 95	-0.07 ± 0.17 (-0.18; 0.04)	0, 95, 5
Peak T_{re} (°C)	-0.42 ± 0.23 (-0.57; -0.27) $^{+}$*	0, 11, 89	-0.05 ± 0.17 (-0.15; 0.05)	0, 100, 0
Peak skin temperature (°C)	-0.89 ± 0.86 (-1.4; -0.35) $^{+}$*	0, 15, 85	+0.03 ± 0.6 (0.01; 0.05)	0, 100, 0
Resting HR (bpm)	-10 ± 4 (-14; -6) $^{+}$*	0, 4, 96	-4 ± 5 (-7; -1)	0, 85, 15
Peak HR (bpm)	-12 ± 7 (-16; -8) $^{+}$*	0, 50, 50	-3 ± 4 (-5; -1)	0, 100, 0
Plasma volume expansion (%)	+9 ± 7 (4; 14) *	93, 7, 0	+1 ± 3 (0.4; 1.6)	0, 100, 0
SWR (%)	+81 ± 54 (51; 110) *	99, 1, 0	+58 ± 41 (42; 92) *	94, 6, 0
Sweat NaCl ($mMol.L^{-1}$)	-16 ± 10 (-22; -10) *	0, 95, 5	-5 ± 2 (-7; -3) *	0, 100, 0
T_{re} at onset of sweating (°C)	-0.29 ± 0.15 (-0.4; -0.18) $^{+}$*	0, 43, 57	-0.08 ± 0.15 (-0.17, 0.01)	0, 100, 0
TS	-1.0 ± 0.5 (-1.4; -0.6) $^{+}$*	0, 31, 69	0.0 ± 0.5 (0; 0)	1, 99, 0
RPE	-2 ± 1 (-3; -1) $^{+}$*	0, 2, 98	-1 ± 1 (-2; 0)	0, 50, 50

Notes. HR heart rate; NaCl sodium chloride; RPE rating of perceived exertion; SWR sweat rate; T_{re} rectal temperature; TS thermal sensation. $^{+}$ denotes an interaction effect between HA_{temp} and HA_{sauna}; * denotes significant difference between $RHTT_1$ and $RHTT_2$ ($p \leq 0.05$). The qualitative chances of either higher or lower differences were evaluated as follows; 1%, almost certainly not; 1–5%, very unlikely; 5–25%, unlikely; 25–75%, possible; 75–95%, likely; 95–99%, very likely; >99%, almost certain.

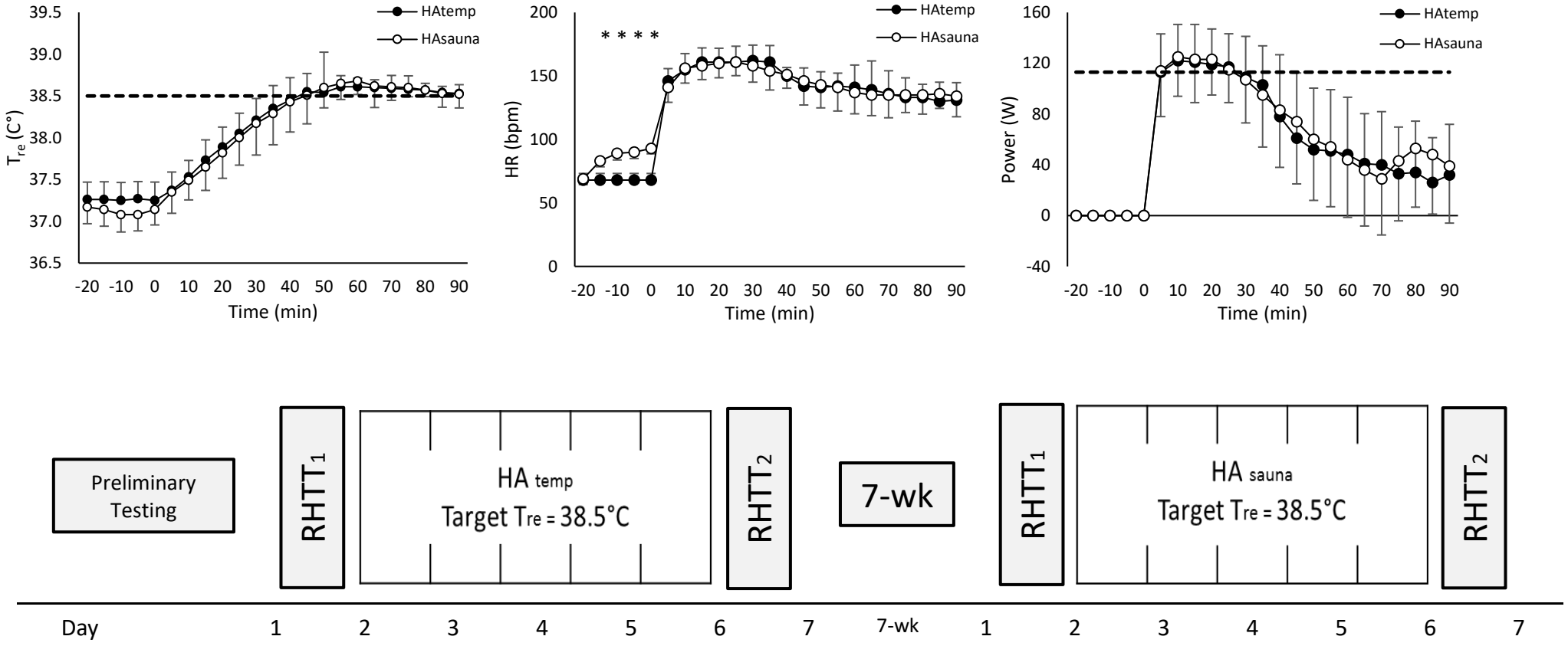

Figure 1: Experimental Schematic. HA_{temp} and HA_{sauna} completed in a randomised cross over design, separated by 7 wk. Figures represent mean ± SD data for rectal temperature (T_{re}), heart rate (HR), and power during the HA_{temp} (closed markers) and HA_{sauna} (open markers). See text for full details.

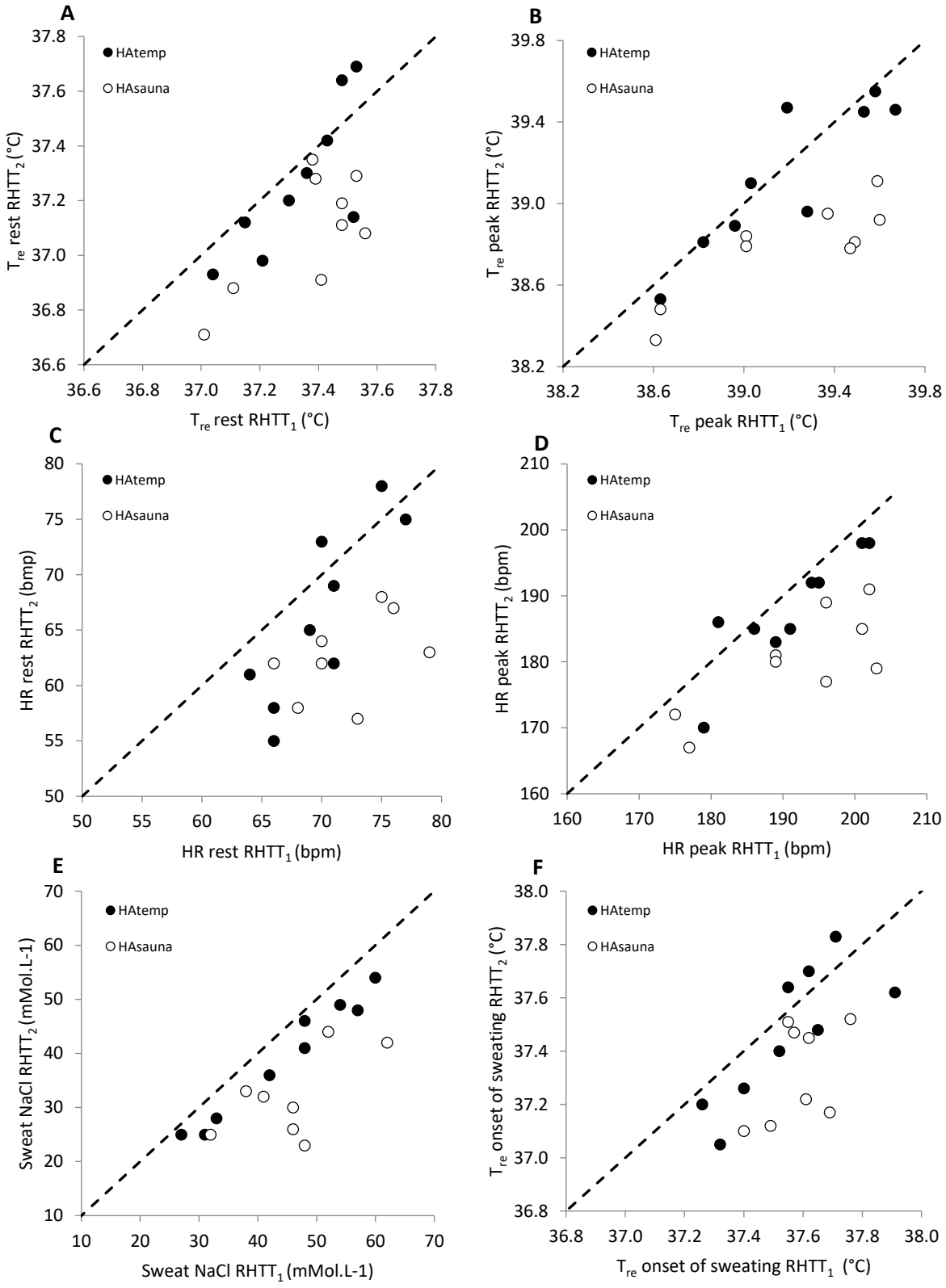

Figure 2. Resting rectal temperature (T_{re} rest; A), Peak rectal temperature (T_{re} peak; B), Resting heart rate (HR rest; C), Peak heart rate (HR peak; D), Sweat sodium chloride (NaCl; E), and Rectal temperature (T_{re}) at onset of sweating (F) in HA_{temp} (closed markers) and HA_{sauna} (open markers). Dotted line represents line of equality.