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A.G.B. Willmott, O.R. Gibson, M. Hayes, N.S. Maxwell

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Running Title:

Single versus twice daily short term heat acclimation

Authors:

A.G.B. Willmott[1], O.R. Gibson[12], M. Hayes[1], N.S. Maxwell[1]

Address for all authors:

[1]Centre of Sport and Exercise Science and Medicine (SESAME), Environmental Extremes Laboratory, School of Sport and Service Management, University of Brighton, Eastbourne, UK

[2]Centre for Sports Medicine and Human Performance (CSMHP), Brunel University, London, UK

Email Correspondence:

Corresponding author - Ashley Willmott A.G.Willmott@brighton.ac.uk

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Abstract

Endurance performances are impaired under conditions of elevated heat stress. Short term heat acclimation (STHA) over 4-6 days can evoke rapid adaptation, which mitigate decrements in performance and alleviate heat strain. This study investigated the efficacy of twice daily heat acclimation (TDHA) compared to single session per day heat acclimation (SDHA) and normothermic training, at inducing heat acclimation phenotype and its impact upon running performance in hot, humid conditions.

Twenty one, moderately trained males were matched and assigned to three groups; SDHA (mean ± SD) (peak oxygen consumption [$\dot{V}O_{2peak}$] 45.8±6.1 $mL.kg^{-1}.min^{-1}$, body mass 81.3±16.0 kg, stature 182±3 cm), TDHA (46.1±7.0 $mL.kg^{-1}.min^{-1}$, 80.1±11.9 kg, 178±4 cm) or control (CON) (47.1±3.5 $mL.kg^{-1}.min^{-1}$, 78.6±16.7 kg, 178±4 cm). Interventions consisted of 45 min cycling at 50% $\dot{V}O_{2peak}$, once daily for 4d (SDHA) and twice daily for 2d (TDHA), in 35°C, 60% relative humidity (RH), and once daily for 4d (CON) in 21°C, 40% RH. Participants completed a pre- and post-intervention 5 km treadmill run trial in 30°C, 60% RH, where the first 2 km were fixed at 40% $\dot{V}O_{2peak}$ and the final 3 km was self-paced.

No statistically significant interaction effects occurred within- or between-groups over the 2-4d intervention. While within-group differences were found in physiological and perceptual measures during the fixed intensity trial post-intervention, they did not statistically differ between-groups. Similarly, TDHA (-36±34s [+3.5%]) and SDHA (-26±28s [+2.8%]) groups improved 3 km performances ($p=0.35$), but did not differ from CON (-6±44s [+0.6%]).

This is the first study to investigate the effects of HA twice daily and compare it with traditional single session per day STHA. These STHA protocols may have the ability to induce partial adaptive responses to heat stress and possibly enhance performance in environmentally challenging conditions, however, future development is warranted to optimise the administration to provide a potent stimuli for heat adaptation in athletic and military personnel within a rapid regime.

Key words:

Heat Acclimation; Short Term Heat Acclimation; Heat stress; Twice daily; Thermoregulation; Athletics; Military

1. Introduction

Endurance performances across a spectrum of distances are impaired by heat stress (McCann and Williams, 1997; Guy et al., 2014). While exercising or physically active within the heat, systemic metabolic heat production ($\dot{H}_{prod}$) is typically balanced by heat dissipation though enlarged skin blood flow. This process consequently increases skin temperature and augments sweating, which ultimately facilitates evaporation (Poirier et al., 2015). Reductions in self-paced exercise or fixed intensity military tasks, are a result of physiological strain and, or behavioural thermoregulation during heat stress (Sawka et al., 2011; Racinais et al., 2015). To compete or serve optimally under heat stress, heat acclimation or acclimatization is typically recommended (Racinais et al., 2015), as repeated heat strain confer physiological and metabolic adaptations, in addition to reducing perceptual feelings, which alleviate physiological strain and enhance physical performance (Sawka et al., 2011; Taylor, 2014; Racinais et al., 2015). To induce such adaptations, repeated daily exercise is performed in hot environmental conditions, which may be simulated (i.e. heat acclimation [HA]) or naturally occurring (i.e. heat acclimatization). Long-term HA (LTHA) consists of 60 to 120 minutes of exercise at moderate intensities (~50% relative to maximal oxygen uptake [$\dot{V}O_{2max}$]) over 10 to 14 days, within target environmental conditions (Armstrong and Maresh, 1991). However, despite total phenotypic adaptations occurring within 14 to 21 days during LTHA, ≤ 5 daily exposures during short term HA (STHA) facilitates practically significant thermoregulatory and cardiovascular (~75%) adaptations (Pandolf, 1998), over a more applicable duration for athletes and military personnel. Previous STHA studies (Cotter et al., 1997; Patterson et al., 2004; Sunderland et al., 2008; Garrett et al., 2009, 2011; Costa et al., 2014; Mee et al., 2015; Gibson et al., 2015) have reported physiological and athletic performance improvements within hot conditions.

During training, prior to competition or in the lead up to military deployment, STHA may appear more feasible for inclusion in established schedules due to lesser disruption and costs, particularly when tapering (Gibson et al., 2015) and will avoid unnecessary reductions in the quality of training or heat-related illnesses. Rapid examples of STHA prior to hockey (Sunderland et al., 2008), cricket (Petersen et al., 2010) and cycling (Brade et al., 2012) performance, have reported partial adaptations over 4-5 consecutive (Petersen et al., 2010; Brade et al., 2012) and 10 non-consecutive days (Sunderland et al., 2008), within hot environmental conditions. These convenient methods of HA consisted of short (30-45 minutes), high intensity or sport specific training within 30-35^{o}C, and are similar to Houmard et al. (1990) recommendations of short-duration (30-35 min), moderate intensity (75% $\dot{V}O_{2max}$) exercise in the heat to induce adaptation. Although only partial HA adaptions were reported in physiological and perceptual measures, improvements in exercise performance were observed (+5% [Brade et al., 2012] and +33% [Sunderland et al., 2008]). Therefore, these prompt STHA methods may be more appealing and practical when applied to the competing athlete or military personnel.

Contrary to pre-planned, self-paced exercise performances, which have definitive start and end times, with readily available fluids and reside in safe, sporting surroundings, such as the Olympics, military personnel face an array of extreme situations. The increased risk of heat-related illnesses may arise from requirements to carry and wear heavy protective clothing, sleep deprivation, dehydration and the uncertainty surrounding time before deployment and prolonged durations to perform optimally in hazardous, combat and adverse environmental circumstances (Sawka et al., 2011). Therefore, due to practical constraints such as deployment times, prompt STHA should be applied to military soldiers, if they are required to heat acclimate to target conditions within a matter of days. Likewise, athletes may prefer rapid STHA to reduce the negative impact upon their quality of training during a tapering phase. It has been demonstrated that during once versus twice daily acclimatisation, no between-group difference exists in core temperature (T_{re}) or body mass change, while investigating the risk of heat injury in American football players over a two week training period (Herzog et al., 2009).

Consequently, the plausibility of further reducing the duration of STHA interventions by performing twice daily heat acclimation, warrants investigation. **The aim of this study was to investigate the physiological and perceptual adaptions, and 3km running performance in hot, humid conditions, after four days of single session HA, two days of twice daily HA and four days of single session normothermic training. It was hypothesised that both HA protocols would induce similar adaptations, as opposed to no changes after temperate training. It was further hypothesised there would be no statistically significant difference in adaptation and running performance between HA protocols.**

2. Methods

2.1 Participants

Twenty one moderately trained (performance level 2) (De Pauw et al., 2013) males volunteered and provided written consent after being informed of the purpose and associated risks of the experiment. The study was approved by the Institutional Research Ethics and Governance Committee and conducted in accordance to the Declaration of Helsinki of 1975, as revised in 2008. Participants were matched for physiological parameters (Table 1) and assigned to three groups; single session per day heat acclimation (SDHA), twice daily heat acclimation (TDHA) or control (CON).

2.2 Experimental Design

On the completion of a running and cycling peak oxygen uptake ($\dot{V}O_{2peak}$) test separated by 48 hours, and a running 5 km pre-loaded time trial (5 kmTT), participants were either assigned to; four consecutive days of HA (SDHA), or four consecutive days of temperate training (CON) or two consecutive days of twice daily HA (TDHA). Participants then completed a post training 5 kmTT 48 hours following completion of the intervention. Participants wore minimal clothing, including shorts, t-shirt and sports shoes, which remained constant throughout each trial.

2.3 Pre-experimental protocol and equipment

Participants avoided alcohol, caffeine and large food intake prior to each visit, and arrived in a euhydrated state (indicated by urine osmolality [U_{osm}] <700 mOsm.kg^{-1} and specific gravity [U_{sg}] <1.020) (Sawka et al., 2007). Stature was measured and nude body mass (NBM) assessed using physician scales (Detecto Scale Company, USA) to determine body surface area (BSA) (DuBois and DuBois, 1916). Fresh mid-flow urine samples were requested to determine fluid balance indices using a hand-held light refractometer (Atago Co., Tokyo, Japan) and Pocket Pal-Osmo (Vitech Scientific, Ltd). T_{re} was continually recorded on logging monitors (YSI, 4600 series, Hampshire, UK), using a single use probe (449H, Henleys Medical, Hertfordshire, UK), inserted 10 cm past the anal sphincter. This method of measuring core temperature is accurate (±0.13^{o}C) and prevents the likelihood of serious heat related-illnesses (Sawka et al., 2011). HR monitors were affixed to the chest (Accurex+, Polar Electro, Oy, Kempele, Finland) and monitored continuously during all exercise. Participants were familiarised to perceptual scales, including ratings of perceived exertion (RPE), from 6 (no exertion) to 20 (maximal exertion) (Borg, 1982) and thermal sensation (TSS), from 0 (unbearably cold) to 8 (unbearably hot) (Toner et al., 1986). Participants were aware they could stop exercising at any time and were removed from the heat if T_{re} reached ≥39.7°C (zero incidences). Non-urine fluid loss (NUFL) was estimated post exercise using weighing scales and the difference between pre and post exercise towel-dried NBM, corrected for urine output and fluid consumption (zero incidences), but not for respiratory or metabolic losses which were assumed similar and negligible between tests. Fingertip capillary blood samples were collected on arrival to HA and CON session 1 and 4 for haemoglobin concentration (in triplicate) using a microvette and analysed using the B-Hemoglobin analyser (Hemo Cue, Ltd), and haematocrit using a capillary tube (in triplicate), spun using a Haematospin 1300 (Hawksley) centrifuge and analysed using a Hawksley micro haematocrit reader. These measures were used to estimate changes in plasma volume (ΔPV) (Dill and Costill, 1974).

2.4 Peak oxygen uptake ($\dot{V}O_{2peak}$) tests

Running and cycling $\dot{V}O_{2peak}$ tests were performed within temperate (21.1±0.9°C and 38±4 % RH) conditions on a motorised treadmill (Woodway ELG2 treadmill GmbH) set at 1% incline (Jones and Doust, 1996) and on a cycle ergometer (Monark, 620, Ergodemic, Sweden), respectively. The running test started with 5 minutes warm up at 5 $km.hr^{-1}$, which increased to 8 $km.hr^{-1}$ and then by 0.8 $km.min^{-1}$ until exhaustion (Aoyagi et al., 1994). The cycling test started with 5 minutes warm up at an external mechanical intensity of 50 watts (W) and subsequently increased 24 $W.min^{-1}$ until exhaustion (Hayes et al., 2014). RPE and HR were recorded in the final 15 s of each stage. Expired air was collected into Douglas bags using open-circuit spirometry for 45 s during each stage. Pulmonary gases (oxygen [O_2] and carbon dioxide [CO_2]), temperature and volume were sampled using a gas analyser (Servomex International Ltd, Crowborough, UK; CV; O_2=1.5%; CO_2=1.9%). A two-point calibration using nitrogen and a mixture of gases of known O_2 and CO_2 quantities (BOC, UK) was undertaken prior to each test.

2.5 Pre-loaded 5 km treadmill run time trial

All participants performed a running 5 kmTT on a motorised treadmill (Woodway ELG2 treadmill GmbH) set at 1% incline (Jones and Doust, 1996) within 29.8±0.7°C and 58±3% RH pre- and post-intervention. This occurred inside a purpose-built environmental chamber, with temperature and humidity controlled using automated computer feedback (WatFlow control system; TISS, Hampshire, UK) and without the use of fans or direct heat stimuli. The initial 2 km was pre-loaded at a fixed speed to elicit 40% of $\dot{V}O_{2peak}$, which was lower than ventilatory threshold and similar to a military march pace (5-7 $km.hr^{-1}$) (van Dijk, 2009). The remaining 3 km was a self-paced performance time trial (3 kmTT). At 4 min intervals HR, T_{re}, TSS and RPE were recorded, in addition to NUFL estimation and 3 km performance time. Participants were only provided with kilometre distance remaining as feedback, with time blinded.

2.6 Heat acclimation and temperate training

Participants cycled at a fixed mechanical intensity to elicit 50% $\dot{V}O_{2peak}$ for 45 minutes (Nielsen et al., 1997; Saat, et al., 2005) once a day for 4 consecutive days within 35.2±0.5°C and 60±2% RH (SDHA), within 21.7±0.6°C and 39±5% (CON), or twice daily for two consecutive days within 35.4±0.8°C and 61±3% RH (TDHA). Cycling exercise was chosen as it is non-weight-bearing and practical, thus reducing injury risk and enabling 4 to 6 participants to train simultaneously. HR, T_{re}, RPE, TSS were recorded at 5 min intervals and post NUFL were estimated. **Fluid ingestion was restricted during HA and temperate training. Participants were instructed to replace 150% of NUFL with water *ad libitum,* after each HA session (Shirreffs and Maughan, 1998)**. Mean and peak physiological and perceptual data are reported **in** the results.

2.7 Metabolic heat production

Metabolic energy expenditure (*M*) was estimated using the equation of Nishi (1981):

$$M = \dot{V}O_2 \; \frac{\left(\frac{RER-0.7}{0.3}\mathrm{ec}\right)+\left(\frac{1-\mathrm{RER}}{0.3}\mathrm{ef}\right)}{60} \; \mathrm{x}\; 1000\ \mathrm{W}$$

where: the caloric equivalents per litre of O_2 consumed for the oxidation of carbohydrates and the oxidation of fat are ec (21.13 kJ) and ef (19.62 kJ), respectively. $\dot{H}_{prod}$ was determined by the difference between *M* and the external mechanical power output (W) and divided by body mass to obtain a relative fixed measure (W kg^{-1}).

2.8 Statistical analyses

All data are reported as mean ± standard deviation (SD) and were assessed for normality and sphericity prior to further statistical analyses. Pre-to-post rest and exercise physiological, perceptual and performance changes were analysed using a two way repeated measures design ANOVA (group*test) for intervention group (SDHA, TDHA and CON) and test (pre and post 2 km and 3 kmTT). The physiological and perceptual responses to 4 sessions of HA and temperate training were analysed using a two way repeated measures design ANOVA (group*session), for intervention group (SDHA, TDHA and CON) and session (1 to 4), with follow up Bonferroni-corrected post-hoc comparisons. A one way ANOVA was performed to determine differences in dependant variables between HA and control methods. Meaningful differences between and within-groups during interventions, 2 km and 3 kmTT were evaluated using Cohen's *d* with confidence intervals (CI) (Cumming, 2012). Effect sizes were estimated using partial Eta squared (η_p^2) in statistical ANOVA analysis, to analyse the magnitude and trends of the intervention (Nakagawa and Cuthill, 2007). Effect size was categorised as small (0.2), moderate (0.5) and large (0.8) (Cohen, 1988). Statistical significance was accepted as $P \leq 0.05$. Data were analysed using SPSS (version 22.0). The typical error of measurement (TEM) for the 3 kmTT was set at 2.1% (Rodriguez et al., 2007). Predefined analytical limits to highlight meaningful adaptations among typical STHA adaptations, assist in determining efficacy and robustness of the protocol (Atkinson & Nevill 1998). The *a priori* meaningful limits for adaptation in resting and exercising parameters for this study have been calculated from numerous STHA research studies (Cotter et al., 1997; Patterson et al., 2004; Sunderland et al., 2008; Garrett et al., 2009, 2011, 2014; Petersen et al., 2010; Brade et al., 2012; Mee et al., 2015; Gibson et al., 2015; Neal et al., 2015) and are; ΔT_{re} >0.20°C, ΔHR >5 b.min^{-1} and ΔPV >5%.

3. Results

3.1 Preliminary Participant Characteristics

There was no interaction effect between the groups across session 1 to 4 for resting HR ($F_{(6,54)}$=0.06, p=0.99, n_p^2=0.0), U_{osm} ($F_{(6,54)}$=1.00, p=0.43, n_p^2=0.1), U_{sg} ($F_{(6,54)}$=0.80, p=0.58, n_p^2=0.1) or NBM ($F_{(6,54)}$=1.60, p=0.16, n_p^2=0.2). Although there was a statistically significant interaction effect between group **for** resting T_{re} ($F_{(6,54)}$=4.39, p=0.00, n_p^2=0.3), this can be explained by circadian rhythm alterations within the TDHA group. This occurred between session 1 and 2 (p=0.02), 2 and 3 (p=0.00) and, 3 and 4 (p=0.03) (Figure 1). There was no interaction effect between-group for resting T_{re} ($F_{(2,18)}$=0.34, p=0.72, n_p^2=0.1), HR ($F_{(2,18)}$=0.13, p=0.88, n_p^2=0.1) or NBM ($F_{(2,18)}$=0.34, p=0.72, n_p^2=0.1) on arrival to pre and post 5 kmTT.

3.2 Single session per day and twice daily heat acclimation and temperate training

While analysing all mean data across training sessions, a between-group interaction effect was observed for mean HR ($F_{(2,18)}$=5.22, p =0.02, n_p^2=0.4), HR_{peak} ($F_{(2,18)}$=9.32, p =0.00, n_p^2=0.5) and TSS_{peak} ($F_{(2,18)}$=35.17, p =0.00, n_p^2=0.8). HR (p=0.03), HR_{peak} (p=0.01) and TSS_{peak} (p=0.00) were significantly **higher** across TDHA compared to CON training. Likewise, SDHA group's HR (p=0.05), HR_{peak} (p=0.01) and TSS_{peak} (p=0.00) were significantly **higher** than CON group, yet no differences were observed between HA methods (p=1.00 for all variables). No other differences we found for T_{re}, T_{repeak}, RPE_{peak} or NUFL as shown in Table 2.

While analysing individual training sessions, a main effect was reported **for** T_{re} ($F_{(6,54)}$=5.76, p=0.00, n_p^2=0.4) and T_{repeak} ($F_{(6,54)}$=4.25, p=0.00, n_p^2=0.3) during HA. This occurred between session 1 and 2 (p=0.01 and p=0.01) and, session 2 and 3 (p=0.00 and p=0.01) within the TDHA group, respectively. There was an interaction effect between TDHA and SDHA group's mean T_{re} (p=0.02) between session 1. **Moreover, a main effect for T_{repeak} (p=0.04) occurred between session 1 and 2 within the SDHA group.** Although improvements in physiological and perceptual measures transpired across sessions 1 to 4, with associated moderate effect size (*d*>0.5), no further statistically significant interaction effect between-groups **for** ΔT_{re} ($F_{(6,54)}$=1.70, p=0.14, n_p^2=0.2), HR ($F_{(6,54)}$=1.27, p=0.29, n_p^2=0.1) or HR_{peak} ($F_{(6,54)}$=0.68, p=0.67, n_p^2=0.1) occurred. Nor were there differences for RPE_{peak} ($F_{(6,54)}$=1.07, p=0.39, n_p^2=0.1), TSS_{peak} ($F_{(6,54)}$=0.80, p=0.58, n_p^2=0.1) or NUFL ($F_{(6,54)}$=0.66, p=0.68, n_p^2=0.1) (Table 3). ΔPV did not differ between-groups ($F_{(2,18)}$=1.03, p=0.38) across sessions 1 to 4 in the TDHA (4.8±3.5%), SDHA (5.2± 4.1%) or CON (2.9±1.7%) group. **An interaction effect was observed between group and time ($F_{(6,54)}$=2.97, p=0.01, n_p^2=0.3), for the time spent above 38.0°C, this occurred during session 1 of SDHA (32±13 mins) and TDHA (12±7 mins).** There was also a main effect for time in the TDHA group between session 1 and 2 (12±7 *vs.* 29±15 mins, p=0.01), and session 2 and 3 (29±15 *vs.*10±9 mins, p=0.01). Power output ($F_{(2,18)}$=0.12, p=0.87) and total work done ($F_{(2,18)}$=0.08, p=0.92) did not differ between-groups (Table 2).

3.3 Pre and post 2 km physiological and perceptual measures

Reductions in exercising T_{re} and HR, in addition to improved thermal comfort were present post HA interventions with moderate to large effect (*d*>0.5-0.8). However, no statistically significant interaction effect occurred between-groups, pre-to-post 2 km **for** T_{re} ($F_{(2,18)}$=0.39, p=0.68, n_p^2=0.1), T_{repeak} ($F_{(2,18)}$=0.68, p=0.52, n_p^2=0.1) or ΔT_{re} ($F_{(2,18)}$=1.34, p=0.29 n_p^2=0.1). Nor was there an interaction effect **between-groups for** HR ($F_{(2,18)}$=0.61 p=0.51, n_p^2=0.1), HR_{peak} ($F_{(2,18)}$=0.27, p=0.77, n_p^2=0.1), RPE_{peak} ($F_{(2,18)}$=1.60, p=0.21, n_p^2=0.2) or TSS_{peak} ($F_{(2,18)}$=3.00, p=0.08, n_p^2=0.3) (Table 4). The fixed speed to elicit 40% of $\dot{V}O_{2peak}$ ($F_{(2,18)}$=1.18, p=0.33), and therefore, absolute ($F_{(2,18)}$=1.27, p=0.31) and relative ($F_{(2,18)}$=0.23, p=0.80) $\dot{H}_{prod}$ between groups did not differ (Table 2).

3.4 Pre and post 3 kmTT physiological, perceptual and performance measures

HR_{peak} was significantly lower in the 3kmTT following 4 sessions in the CON condition ($F_{(2,18)}$=3.65, p=0.05, n_p^2=0.3). Although no statistical significance was found ($F_{(2,18)}$=1.14, p=0.35, n_p^2=0.1) in 3 kmTT performances, moderate to large effect size and enhancements above the predefined CV (2.1%) within TDHA (-36±34 s [+3.5%], *d*=0.6) and SDHA (-26±28 s [+2.8%], *d*=0.4) groups are reported (Figure 2). **No statistically significant interaction effects transpired between-groups for T_{repeak} ($F_{(2,18)}$=0.20, p=0.82, n_p^2=0.1), RPE_{peak} ($F_{(2,18)}$=0.94, p=0.41, n_p^2=0.1), TSS_{peak} ($F_{(2,18)}$=0.82, p=0.46, n_p^2=0.1) or NUFL ($F_{(2,18)}$=0.20, p=0.82, n_p^2=0.1) (Table 5).**

4. Discussion

4.1 Overview

The aim of this study was to investigate the physiological and perceptual adaptions, and 3km running performance, after four days of single session HA, two days of twice daily HA and four days of single session normothermic training. The prescribed method of HA and temperate training (four 45 min sessions of cycling at 50% $\dot{V}O_{2peak}$) did not facilitate statistically significant pre-to-post alterations in resting T_{re} or HR, within either group, although moderate to large effects were observed after TDHA and SDHA compared to small effects in the CON group (Table 3 and Figure 1). Exercising T_{re}, HR, perceptual scales and fluid losses across the four training sessions did not significantly alter, although, meaningful changes were observed. Likewise, during the initial 2 km time trial both HA groups lowered thermoregulatory and cardiovascular strain, and improved perceived comfort within the heat, however, surprisingly these results did not significantly differ within- or between-groups pre-to-post interventions, suggesting partial HA adaptation. 3 kmTT performances were improved above the predefined TEM (2.1%), albeit non-significantly, within the TDHA (+3.5%) and SDHA (+2.8%) group, but not CON (+0.6%).

4.2 Single **session per** day and twice daily heat acclimation

STHA studies typically consist of ≤7 consecutive daily exposures within hot (30-40°C) and humid (20-60% RH) conditions, for 90 min fixed intensity or controlled hyperthermia (Cotter et al., 1997; Patterson et al., 2004; Garrett et al., 2009, 2011; Gibson et al., 2015, Mee et al., 2015, Neal et al., 2015). Further comparable studies which have investigated a shorter 4 to 5 day protocol, sport specific or high intensity exercise, either over consecutive or non-consecutive days, have primarily reported "partial" heat acclimation adaptions and performance improvements (Sunderland et al., 2008; Petersen et al., 2010; Brade et al., 2012). However, Marshall et al. (2007) observed reductions in physiological strain and cellular stress, after just two days of prolonged, low-intensity cycling exercise.

Within this study an alternate and rapid STHA method, which may be more applicable to athletes and military personnel was selected. HA prescription included cycling at 50% $\dot{V}O_{2peak}$ for 45 min within conditions hotter (35°C) than the performance trials (30°C), over a shorter timeframe consisting single exposures on four consecutive days and doubling the daily exposure in two days. Although the authors acknowledge methodology limitations, reducing the number of days exposed to thermal stress but maintaining exposure duration and exercise intensity, may provide a new concept for HA, especially when investigating the enhanced benefits for those required to compete or serve optimally when short periods of notice are provided, or if practitioners consider the method less beneficial, as opposed to extending the quality of training prior to competition.

Although a key adaptation associated with STHA is indicated by a ~6-7% improvement in cardiovascular stability (Garrett et al., 2011), no statistically significant changes were evident in resting or exercising HR after interventions. However, using our predefined analytical limits (>5 $b.min^{-1}$), meaningful reductions of 4% (TDHA) and 6% (SDHA) were observed in exercising HR from session 1 to 4 with moderate effect, though CON group did not change (1%). This adaptation is typically concurrent with hypervolemia and is suggested to reduce HR 1 $b.min^{-1}$, per 1% ΔPV (Convertino, 1991), which was present in HA interventions (4.8% TDHA and 5.2% SDHA), consistent with our analytical limits (>5%) and previous research (Nielsen, et al., 1993; Aoyagi, et al., 1994; Patterson, et al., 2004; Garrett, et al., 2009; Lorenzo, et al., 2010). Another key adaptation is reductions in resting and exercising T_{re} of ~0.6% (Garrett et al., 2011), which significantly altered during TDHA, although this was solely contributed by circadian rhythm variations from morning to afternoon sessions. No other statistically significant changes were evident. Nonetheless, when comparing our predefined analytical limits (>0.2°C) and effect size between session 1 and 4, meaningful reductions of 0.4% were present in the SDHA group similar to that reported elsewhere (Sunderland et al., 2008; Petersen et al., 2010). Similar results were present in T_{repeak} reductions of 0.7% within the SDHA but not TDHA group, as comparison between sessions 1 to 4 are contributed by circadian fluctuations in T_{re} (Waterhouse et al., 2005). No change in T_{re} occurred in the CON group. RPE_{peak} reductions within SDHA and TDHA groups presented large effect and meaningful change. Similarly, large effects were present in the SDHA group for TSS_{peak} reductions, in addition to superior non-urine fluid losses, although no change in TDHA or CON were observed.

One constituent of obtaining HA adaptations depends on attainment and maintenance of elevated T_{re} throughout the session, typically targeted at 38.5°C (Taylor, 2014). During this study, T_{re} rose linearly with time during HA, however, lower T_{repeak} were evident in all groups across the four sessions, suggesting the magnitude of physiological strain was not sufficiently elevated compared to other studies (Patterson, et al., 2004; Garrett, et al., 2009, 2011; Gibson et al., 2015). This may have led to reduced and partial adaptive responses, whereas, if controlled hyperthermia was administrated, greater adaptation may have been achieved (Fox, et al., 1973). Although, contrary to less work completed during controlled hyperthermia methods, fixed exercise intensity may be optimal for larger cohorts of military personnel, due to lower exercise intensity and simplified administration (Gibson et al., 2015). Moreover, although guidelines suggest >60 min exposure time for HA (Taylor, 2014; Racinais et al., 2015), in line with the nature of this study's aim of prompt STHA, 45 min sessions may be more attractive to the busy, modern-day athlete or called upon military soldier, especially if they are adopted twice daily. Future studies must investigate the method prescribed to achieve the target level of physiological strain by increasing T_{re} to a fixed absolute temperature, or change from baseline, which may be performed prior to the exposure within a hot bath or the selected exercise intensity may have to be more rigorous, as a means of thermal stimuli.

Surrounding problems which may hinder TDHA efficacy include lack of recovery time, circadian rhythm alterations and in particular hydration status differing between morning and afternoon sessions. However, recent studies suggest the use of permissive dehydration during HA (Garrett, et al., 2009; Neal et al., 2015), to induce PV expansion and cutaneous blood flow, and decrease cardiac frequency. This method also increases aldosterone, a fluid-regulatory hormone which effects sodium and urine water retention, therefore, facilitating fluid-regulatory efficiency and mediating hypervolemia (Garrett, et al., 2009). In this study no practical or statistical significant improvements in sweat rate occurred, suggesting no heat dissipation improvements, as sudomotor function adaptation typically occurs in the latter stages of HA (Armstrong and Maresh, 1991; Racinais et al., 2015).

4.3 Heat strain alleviations during pre-loaded 2 km trial

The most prominent indicators of HA effectively alleviating physiological strain under heat stress were identified in the pre-loaded 2 km, where fixed intensities were repeated within the same environmental conditions pre-to-post interventions. During this pre-loaded trial, treadmill speed was fixed to elicit 40% $\dot{V}O_{2peak}$, similar to speeds and aerobic demands of a military march (van Dijk, 2009). Although limitations surround the use of prescribing relative intensity in populations of varied aerobic fitness and biophysical characteristic (Willmott et al., 2015), $\dot{H}_{prod}$ did not differ between-groups. Unfortunately no statistically significant interaction effects were evident in the thermoregulatory, cardiovascular or perceptual measures between-groups, pre-to-post intervention. However, when assessing the individual-group differences during exercise and using our predefined analytical limits, meaningful reductions with moderate-large effect were presented post TDHA. Improvements were observed in mean HR (6%), HR_{peak} (9%), T_{re} (0.3%) and T_{repeak} (0.4%). This was similarly observed in the SDHA group for mean HR (5%), HR_{peak} (7%), T_{re} (0.5%) and T_{repeak} (0.4%). Yet no meaningful changes were observed in the CON group. A lowered HR post HA during the same exercise intensity suggests the increased demand to maintain cardiac output was not required to compensate for stroke volume reductions, as blood flow shifted towards the periphery to reduce T_{re} (Crandell and González-Alonso, 2010). The increased cardiovascular stability and reduced thermoregulatory strain is partially demonstrated within both HA groups and provides further support to recent STHA studies reporting similar incomplete adaptations (Sunderland et al., 2008; Petersen et al., 2010; Brade et al., 2012). Perceived exertion did not significantly change pre-to-post interventions within either group during the pre-loaded fixed intensity trial. Although thermal comfort did not statistically change, a large effect was evident in the reductions within TDHA and SDHA groups, in compliance with 95% of perceptual measures decreasing within 3-6 days of HA (Armstrong and Maresh, 1991). This adaptation may have been due to post-trial familiarity, but could have also been contributed by the higher level of heat stress experienced during HA (35ºC) compared to the pre and post-tests (30ºC). This may contribute to future HA protocols increasing the magnitude of heat stress required during HA, above that which may be experienced during competition or geographical area of deployment.

4.4 3 km performance time trials

3 km time trial performances did not significantly improve post interventions, although, improvements of 36 s (3.5%,TDHA) and 26 s (2.8% SDHA), were observed, with little change in the CON group (6 s, 0.6%). These results post HA are above the predefined TEM of 2.1% (Rodriguez et al., 2007), which may present meaningful improvements associated with the intervention and practitioners may be confident in true interpretations of at least 1.4% (15 s) and 0.7% (7 s) improvement. Consequently, for moderately trained individuals used within the study, there remains a high probability that both HA group's minor performance improvements merely reflect a reduction in perceived exertion and improved thermal comfort under heat stress. Previous studies investigating various interventional strategies on 3 km performance, include intermittent hypoxic (Stray-Gundersen, et al., 2001; Katayama, et al., 2003, 2004; Rodriguez et al., 2007), plyometric (Spurrs et al., 2003; Pellegrino et al., 2015) and intense training periods (Smith et al., 1999; Coutts, et al., 2007; Esfarjani, et al., 2007), reporting a vast range in improvements from 0.8 to 7.3%. However, the level of athlete's aerobic fitness level, running experience, length and method of intervention vary considerably and so comparisons to this study must be interpreted lightly. Improvements in 3 km TT performances, determined by time reductions, may reflect increased cardiovascular stability and reduced thermoregulatory strain, or improved thermal comfort during the self-paced exercise.

As no statistically significant alterations in performance, physiological or perceptual measures occurred within- or between-groups, confident conclusions regarding the potency of TDHA cannot be drawn. However, during the 3 kmTT, trivial reductions in T_{repeak} were observed in the SDHA (0.4%) group. Reductions were also found in perceived thermal comfort in the SDHA (7.9%), but not for TDHA (2.4%) group. There was main effect in HR within the CON group, which presented a reduction of 3.3%, as opposed to no change in the TDHA (2.2%) or SDHA (1.3%) groups. No apparent changes in RPE_{peak} were observed for either group, although the SDHA group reported a slight increase in perceived exertion (5.4%) post HA.

4.7 Study limitations

Limitations associated with this study surround the HA method and prescription of exercise, in addition to the 3 km TT, which must be viewed with an air of caution due to ecological validity boundaries associated with lab based TT performances (Stevens and Dascombe, 2015). Fixed work rates relative to $\dot{V}O_{2peak}$ may evoke large inter-individual physiological strains, as higher aerobically trained individuals will exercise at greater absolute intensities compared to untrained, thus, generating greater metabolic heat due to superior absolute O_2 uptake (Jay et al., 2011). Moreover, although the study aimed at an prompt STHA protocol, the choice of shortening the HA sessions to just 45 min over two to four days, did not allow T_{re} to reach the desired 38.5ºC and enough time needed for optimal adaptations. This is clearly identified within Table 3, where the time above 38ºC in the TDHA group varies considerably between morning and afternoon sessions. Therefore, future investigations are warranted before comprehensive conclusions between twice daily HA and typical STHA can be drawn.

4.8 Practical applications and future studies

We assessed the efficacy of a twice daily HA protocol compared with traditional single session per day STHA, where exposure time, ambient conditions and exercise intensity across HA groups were controlled. This study has profound practical implications that may enable athletic or physically active personnel (i.e. soldiers) to acclimate to hot and humid environments within a prompt period of time. The new concept of training under heat stress twice daily may begin a new strategy for rapid HA, although changes to the methodology are required, as highlighted by the limitations. Nonetheless, practitioners may choose TDHA over a period of 2 to 4 days, to evoke partial physiological adaptations prior to competing or performing military duties, without cost implications and prolonged training disturbances. Future studies should incorporate the controlled hyperthermia method into a longer 4 to 6 day, twice daily protocol, which may offer principal adaptations (Taylor, 2014) within the initial few days of HA (Marshall et al., 2007) and confer acquired cellular thermotolerance with changes in heat shock proteins (Gibson et al., 2015). Furthermore, establishing sufficient recovery time between heat exposures and adequately rehydrating participants is necessary.

5 Conclusion

In conclusion, this is the first study to investigate the effects of HA twice daily and compare it with traditional single session per day STHA. On the whole, regardless of heat exposure volume per day, physiological and perceptual measures, and performance improvements did not differentiate between groups, therefore suggesting TDHA over two consecutive days is as effective as traditional single session per day STHA. Nevertheless, with the added control group comprehensive conclusions cannot be completed as HA interventions did not confer practically significant interaction effects. It may appear that SDHA evoked greater partial cardiovascular, thermoregulatory, sudomotor and perceptual alterations compared with TDHA, as highlighted by moderate effects between session 1 and 4 of HA. Although, this did not translate to heat strain alleviations during the post fixed intensity trial. Irrespective of the number of HA daily sessions these STHA protocols may have the ability to induce partial adaptive responses to heat stress and possibly enhance future performances. The results are encouraging, however more research is warranted, which could enable athletes and military personnel to effectively fast track acclimation to hot conditions within two to four days. The minor performance improvements suggests athletes who may be required to compete in hot, humid environments in less than 4 days could adopt either protocol, or adapt TDHA by enlarging physiological strain and lengthening the time scale. Unfortunately the magnitudes of adaptive responses were smaller than expected, suggesting only partial acclimation transpired. Future development of this unique concept of rapid twice daily, short term heat acclimation is warranted.

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Vitae

Mr Ashley Willmott

Ash completed his B.Sc. (Hons.) undergraduate degree in Sport and Exercise Science in 2012 at the University of Brighton. He continued his academic studies at the University by starting an M. Phil. in October 2012, which is examining the effects of short and long term heat acclimation protocols on the interplay between heat acclimation state, training status and inflammatory markers in hot and humid conditions. Ash is currently working within the Sport and Exercise Science Consultancy Unit (SESCU), undertaking physiology support to athletes and is an active member of British Association of Sport and Exercise Science (BASES).

Dr. Oliver Gibson

Oliver Gibson began his Ph.D. at the University of Brighton in 2010 after receiving his master's degree in Applied Exercise Physiology in 2009 and undergraduate degree in Sport and Exercise Science in 2007. Oliver's Ph. D. examined the cellular stress response to acute and chronic exercise heat stress and combines his thermoregulatory doctoral research with research allied to intermittent sprint performance and training in hypoxia, and ergogenic aids to improve endurance performance. Oliver is now lecturer at the University of Brunel after completing his Ph. D. and technical instructor role at the University of Brighton.

Dr Mark Hayes

Mark studied a B.Sc. (Hons.) Sport and Exercise Science Degree at the University of Brighton, before moving to a full-time lecturing position at Sussex Downs College, Eastbourne. Mark then returned to the University of Brighton as a lecturer in sport and exercise science in 2011, where he completed his Ph.D., entitled "The effect of progressive heat acclimation on games players performing intermittent-sprint exercise in the heat", in 2014. He teaches in the areas of sport and exercise physiology, environmental and expedition physiology, and was awarded one of the University of Brighton's Excellence in Facilitating and Empowering Learning Awards in 2013.

Dr Neil S. Maxwell

Neil joined the University of Brighton as a lecturer in sport and exercise science in 1997, where he lectures undergraduate and postgraduate students, predominantly in the areas of exercise and environmental physiology and research methods. Neil is research active, an approved higher degrees supervisor with M.Phil./Ph.D. completions and a bank of existing postgraduate research students. He has published extensively in the international, scientific literature in areas allied to thermal and hypoxic stress and how the body tolerates each, particularly during exercise. He is now Reader and leads the Environmental Extremes Laboratory which sits within the Centre for Sport and Exercise Science and Medicine.

7. Figures

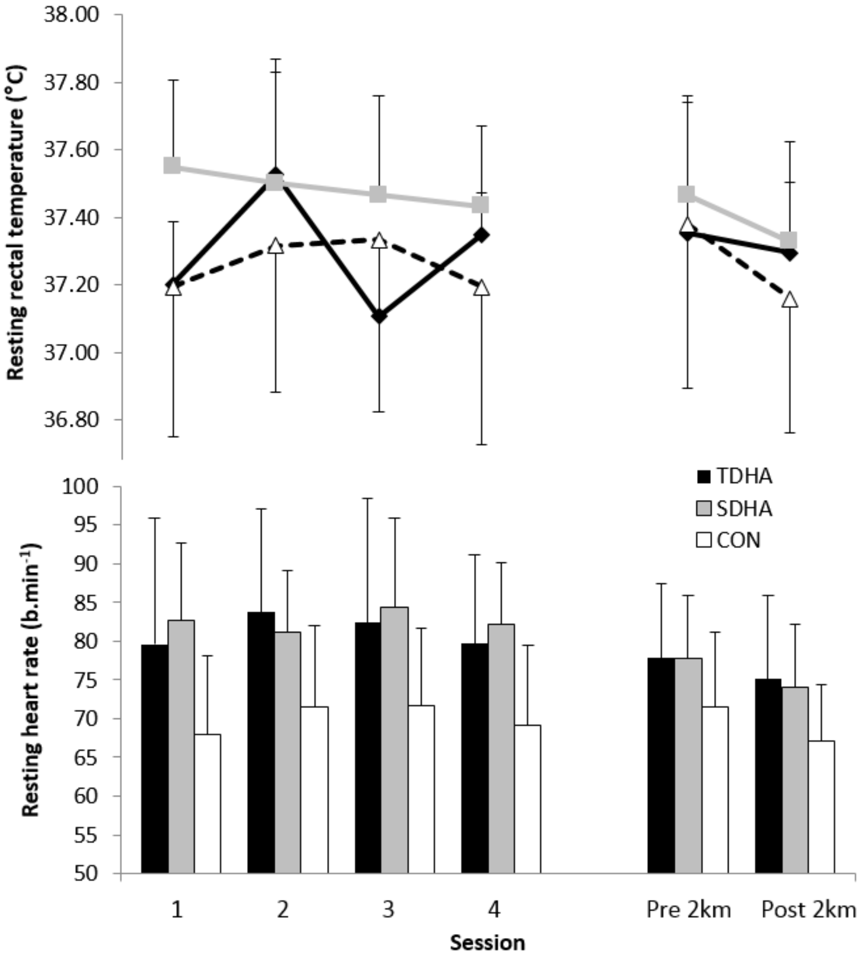

Figure 1. Resting rectal temperature and heart rate across sessions 1 to 4 and pre-to-post 2 km trials within the twice daily heat acclimation (TDHA), single session per day heat acclimation (SDHA) and temperate control (CON) training groups. No statistical significance were found between groups. Error bars are displayed for both resting rectal temperature (°C) and heart rate ($b.min^{-1}$).

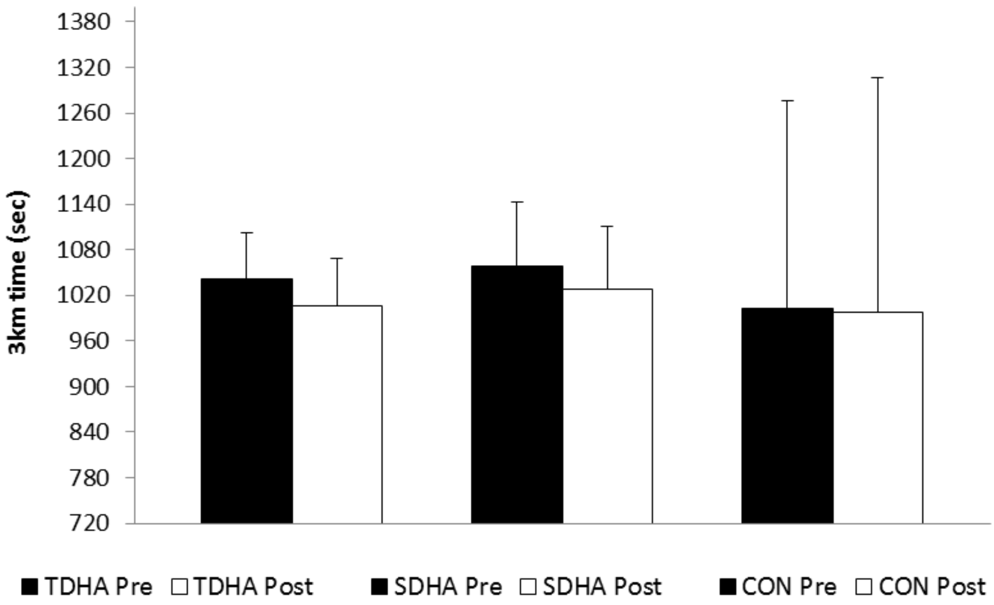

Figure 2. Pre and post 3 km time trial performance times in 30°C and 60% relative humidity for twice daily heat acclimation (TDHA), single session per day heat acclimation (SDHA) and control (CON) groups. No statistical significance were found between groups. Performance improvements (mean ± SD [CI]) were 36±34 (11, 61) s for TDHA, 25±29 (4, 46) s for SDHA and 6±44 (-27, 39) s for CON. Meaningful changes were accepted above the predefined 2.1% CV (Rodriguez et al., 2007).

Title:

The effects of single versus twice daily short term heat acclimation on heat strain and 3000m running performance in hot, humid conditions

Running Title:

Single versus twice daily short term heat acclimation

Authors:

A.G.B. Willmott[1], O.R. Gibson[12], M. Hayes[1], N.S. Maxwell[1]

Highlights

The study investigated and evaluated a new concept for prompt short term heat acclimation, by comparing once versus twice daily heat exposures.

Comparisons are drawn between a short term traditional protocol, a twice daily protocol and a control group who underwent normothermic training

Heat strain alleviations were investigated within a 2 km trial set at similar speeds and aerobic demands as a military march

3000m running performance were improved and partial adaptations in physiological and perceptual measures were reported post heat acclimation protocols, but not for temperate training interventions

This new concept of short term twice daily heat acclimation may offer athletic and military personnel an opportunity to acclimate to heat stress effectively with 2 to 4 days

Further research is warranted to refine heat acclimation methods within twice daily protocols to ensure optimal adaptations are achieved

8. Tables

Table 1. Mean ± SD physiological characteristics and prescribed running and cycling intensities

	TDHA	SDHA	CON	*p*
Age (years)	25±1	23±1	27±3	0.12
Stature (cm)	178±4	182±3	178±4	0.21
Body mass (kg)	80.1±11.9	81.3±16.0	78.6±16.7	0.47
BSA (m^2)	1.98±0.13	2.09±0.16	1.96±0.19	0.31
Running $\dot{V}O_{2peak}$ ($mL.kg^{-1}.min^{-1}$)	46.1±7.0	45.8±6.1	47.1±3.5	0.90
Cycling $\dot{V}O_{2peak}$ ($L.min^{-1}$)	3.9±0.4	3.9±0.5	3.5±0.6	0.33

TDHA = twice daily heat acclimation group, SDHA = single session per day heat acclimation group and CON = control group, BSA = body surface area, $\dot{V}O_{2peak}$ = peak oxygen uptake.

Table 2. Mean ± SD prescribed exercise intensities and data across intervention for training

	TDHA	SDHA	CON	*p*
Pre-loaded 2km fixed running speed at 40% running $\dot{V}O_{2peak}$				
Speed ($km.hr^{-1}$)	6.1±1.1	6.1±0.3	6.2±0.4	0.80
Absolute $\dot{H}_{prod}$ (W)	454±42	451±32	423±48	0.34
Relative $\dot{H}_{prod}$ ($W.kg^{-1}$)	5.2±0.7	5.7±0.5	5.5±0.5	0.32
Fixed cycling intensity at 50% $\dot{V}O_{2peak}$				
Power output (W)	144±32	140±35	146±39	0.87
Total work done (kJ)	389±86	396±97	410±107	0.92
Exercise time (mins)	135±0	135±0	135±0	1.00
Training				
HR ($b.min^{-1}$)	152±12*	151±19†	128±15	0.02
HR_{peak} ($b.min^{-1}$)	172±9*	167±22†	136±16	0.01
T_{re} (°C)	37.72±0.18	37.88±0.17	37.79±0.32	0.48
T_{repeak} (°C)	38.12±0.21	38.18±0.21	38.14±0.32	0.97
RPE_{peak}	16±1	15±2	14±2	0.25
TSS_{peak}	7±0*	7±1†	5±0	0.00
NUFL ($L.hr^{-1}$)	0.94±0.26	0.82±0.06	0.72±0.17	0.11

***represents a significant difference between TDHA and CON, and † represents a difference between SDHA and CON ($p<0.05$). TDHA = twice daily heat acclimation group, SDHA = single session per day heat acclimation group and CON = control group, $\dot{V}O_{2peak}$ = peak oxygen uptake, $\dot{H}_{prod}$ = metabolic heat production.**

Table 3. Mean ± SD exercising physiological and perceptual data during four sessions of heat acclimation or temperate training

Group	Session 1	Session 2	Session 3	Session 4	Session 1 to 4	
HR ($b.min^{-1}$)					**Δ**	**Cohen's *d* (CI)**
TDHA	155±13	155±14	150±12	149±14	7±6	0.4 (0.1, 0.9)
SDHA	157±24	146±21	152±21	147±23	10±7	0.4 (0.1, 0.8)
CON	126±20	127±22	129±15	126±10	0±11	0.0 (-0.6, 0.5)
HR_{peak} ($b.min^{-1}$)						
TDHA	173±10	172±11	171±9	171±10	3±9	0.2 (-0.9, 0.5)
SDHA	170±25	169±24	170±26	162±25	8±4	0.3 (0.0, 0.6)
CON	132±22	136±24	137±15	134±12	1±13	0.1 (-0.6, 0.6)
T_{re} (°C)						
TDHA	37.64±0.10	37.96±0.35*	37.55±0.25	37.75±0.22	0.11±0.22	0.6 (0.4, 1.0)
SDHA	37.97±0.23†	37.79±0.29*	37.86±0.23	37.82±0.15	0.15±0.28	0.7 (0.4, 1.0)
CON	37.70±0.28	37.78±0.39	37.71±0.30	37.77±0.26	0.02±0.10	0.2 (0.1, 0.3)
ΔT_{re} (°C)						
TDHA	0.80±0.38	0.84±0.34	0.91±0.33	0.73±0.30	0.07±0.36	0.2 (0.0, 0.4)
SDHA	0.82±0.29	0.57±0.21	0.73±0.37	0.67±0.16	0.15±0.34	0.6 (0.2, 1.0)
CON	0.75±0.29	0.77±0.26	0.87±0.26	0.93±0.37	0.18±0.18	0.5 (0.3, 1.0)
T_{repeak} (°C)						
TDHA	38.01±0.17	38.37±0.35*	38.01±0.29^{+}	38.08±0.28^{+}	0.07±0.36	0.3 (0.1, 1.0)
SDHA	38.34±0.33	38.04±0.32*	38.14±0.30	38.07±0.21	0.27±0.48	1.0 (0.1, 1.0)
CON	37.97±0.26	38.06±0.38	38.18±0.35	38.11±0.36	0.09±0.26	0.4 (0.1, 1.0)
Time T_{re} > 38.0°C (mins)						
TDHA	12±7*†	29±15^{+}	10±9	20±11	8±13	0.9 (0.4, 1.0)
SDHA	32±13	28±8	27±17	23±15	9±21	0.6 (0.2, 1.0)
CON	18±17	21±18	22±19	14±17	4±12	0.2 (-0.8, 0.9)
RPE_{peak}						
TDHA	17±1	16±2	16±1	15±2	1±2	1.0 (0.3, 1.0)
SDHA	15±2	16±1	15±2	13±3	2±3	0.8 (0.1, 1.0)
CON	15±2	14±2	14±3	14±2	0±2	0.5 (0.3, 0.8)
TSS_{peak}						
TDHA	7.2±0.3	7.1±0.4	7.1±0.3	7.1±0.4	0.1±0.6	0.3 (-0.9, 0.5)
SDHA	7.3±0.5	6.8±0.8	7.1±0.7	6.8±0.9	0.5±0.6	0.7 (0.2, 1.0)
CON	5.1±0.8	5.2±0.3	5.2±0.5	5.2±0.4	0.1±0.8	0.1 (-0.7, 0.8)
NUFL ($L.hr^{-1}$)						
TDHA	0.86±0.24	0.93±0.39	1.03±0.32	0.95±0.20	0.10±0.23	0.4 (0.3, 0.7)
SDHA	0.78±0.13	0.84±0.14	0.93±0.19	0.91±0.16	0.14±0.20	0.9 (0.2, 1.0)
CON	0.60±0.16	0.75±0.15	0.69±0.18	0.65±0.13	0.07±0.09	0.3 (-0.7, 0.5)

***represents a significant difference within group between session 1 and 2, and $^{+}$ represents a significant difference within group between session 2 and 3, and 3 and 4, and † represents a difference between SDHA and TDHA in session 1 ($p<0.05$). Environmental conditions during 45 minutes of training for SDHA and TDHA were 35°C and 60% relative humidity, while they were 22°C and 39% for the CON group.**

Table 4. Mean ± SD exercising physiological and perceptual data during pre-loaded 2km trial.

Group	Pre 2 km	Post 2 km	Δ	Cohen's *d* (CI)
HR (b.min^{-1})				
TDHA	118±15	112±11	6±8	0.5 (0.3, 1.0)
SDHA	117±16	111±14	8±6	0.4 (0.2, 0.8)
CON	107±17	104±15	3±8	0.2 (-0.6, 0.4)
HR_{peak} (b.min^{-1})				
TDHA	131±20	122±14	10±24	0.5 (0.0, 0.8)
SDHA	128±22	121±19	8±20	0.3 (0.2, 1.0)
CON	109±13	106±11	3±9	0.2 (-0.6, 0.5)
T_{re} (°C)				
TDHA	37.54±0.31	37.42±0.28	0.12±0.08	0.4 (0.1, 0.9)
SDHA	37.66±0.36	37.48±0.24	0.18±0.21	0.6 (0.4, 1.0)
CON	37.47±0.42	37.49±0.43	0.02±0.50	0.1 (-0.7, 0.3)
T_{repeak} (°C)				
TDHA	37.64±0.32	37.49±0.29	0.15±0.09	0.5 (0.3, 1.0)
SDHA	37.74±0.37	37.57±0.29	0.17±0.20	0.5 (0.3, 0.8)
CON	37.56±0.36	37.53±0.32	0.04±0.32	0.1 (-0.7, 0.4)
RPE_{peak}				
TDHA	10±2	10±2	1±2	0.0 (-0.7, 0.7)
SDHA	10±2	10±2	0±2	0.0 (-0.7, 0.7)
CON	10±3	11±1	1±2	0.4 (-0.4, 1.0)
TSS_{peak}				
TDHA	6.0±0.5	5.4±0.3	0.6±0.6	1.0 (0.4, 1.0)
SDHA	5.4±0.7	4.9±0.7	0.4±0.9	0.7 (0.0, 0.9)
CON	4.9±0.4	5.2±0.4	0.3±0.6	0.7 (0.2, 1.0)

TDHA = twice daily heat acclimation group, SDHA = single session per day heat acclimation group and CON = control group. Environmental conditions for the time trial were 30°C and 60% relative humidity.

Table 5. Mean ± SD exercising physiological and perceptual data during the 3 km time trial

Group	Pre 3 kmTT	Post 3 kmTT	Δ	Cohen's *d* (CI)
HR_{peak} (b.min^{-1})				
TDHA	185±13	188±9	4±6	0.3 (0.2, 0.7)
SDHA	180±11	183±10	2±6	0.3 (0.1, 0.7)
CON	190±8	184±9*	6±8*	0.7 (0.4, 1.0)
T_{repeak} (°C)				
TDHA	38.59±0.37	38.52±0.50	0.09±0.25	0.2 (0.6, 0.9)
SDHA	38.69±0.38	38.53±0.45	0.16±0.31	0.4 (0.2, 0.8)
CON	38.58±0.27	38.47±0.23	0.11±0.14	0.4 (0.5, 1.0)
RPE_{peak}				
TDHA	16±2	16±2	1±1	0.0 (-0.7, 0.7)
SDHA	16±2	17±2	1±2	0.5 (0.3, 1.0)
CON	18±0	18±1	0±1	0.0 (-0.7, 0.7)
TSS_{peak}				
TDHA	7.1±0.7	6.9±0.7	0.2±0.5	0.3 (0.1, 1.1)
SDHA	7.2±0.6	6.6±0.6	0.6±0.7	1.0 (0.5, 1.0)
CON	7.0±0.4	6.7±0.4	0.3±0.7	0.7 (-0.2, 0.8)
NUFL (L.hr^{-1})				
TDHA	0.74±0.28	0.73±0.15	0.02±0.36	0.0 (0.0, 0.5)
SDHA	0.70±0.19	0.70±0.14	0.00±0.10	0.0 (-0.1, 0.3)
CON	0.53±0.26	0.58±0.32	0.04±0.07	0.2 (0.0, 0.6)

***represents a significant difference pre and post 3 kmTT ($p<0.05$). TDHA = twice daily heat acclimation group, SDHA = single session per day heat acclimation group and CON = control group.**