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Title: Limited effects of short-term heat acclimation during a rugby league training week

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Running head: "Heat acclimation in rugby league"

ABSTRACT

We investigated the effects of five consecutive days of short-term heat acclimation (STHA), with a 48-h recovery, on simulated rugby league performance, peak oxygen uptake ($\dot{V}O_{2peak}$), physiological and perceptual responses in a temperate environment. Twelve male rugby league players took part in a matched-pairs design, cycling 60-min/day, for five days at 40% $\dot{V}O_{2peak}$, in a control (19 ± 1 °C; 50 ± 3 % RH; n = 6) or STHA group (33 ± 0.5 °C; 70 ± 4 % RH; n = 6). Subjects completed a $\dot{V}O_{2peak}$ test and rugby simulation, followed by a time-to-exhaustion, pre- and post-intervention, with physiological and perceptual responses measured during the interventions. Despite no effect on simulated performance ($P > 0.05$), there were increases in time-to-exhaustion among both groups ($P = 0.016$), without group effects ($P = 0.802$). The STHA group adapted across the intervention, with lower tympanic temperature, perceptual responses and heart rate over time ($P < 0.05$), which were typically higher than the control group ($P < 0.05$). Plasma volume did not increase over time or change between groups ($P = 0.290$) and $\dot{V}O_{2peak}$ reduced pre-to-post ($P < 0.001$). There was a relationship between body mass losses and plasma volume expansion ($r = -0.79$, $P = 0.022$). STHA improved tolerance to the heat but not performance in simulated matches or exhaustive tests in temperate conditions, compared to thermoneutral exercise. The limited effects of STHA might relate to the brief post-intervention recovery period or the lack of fluid ingestion control during acclimation.

Key words: Team sports; heat; endurance performance

INTRODUCTION

Short-term heat acclimation (STHA) describes the process of adaptation to repeated controlled heat exposures, across a period of less than 7 days, sufficient to increase body temperature and induce profuse sweating (19,30,32). The reported physiological changes elicited by heat acclimation include an increase in plasma volume (29) and, thus, stroke volume (34), resulting in less cardiovascular stress at a given exercise intensity (10). The physiological effects induced by STHA are relevant to athletic populations because of the benefits conferred to endurance performance in both hot and thermoneutral conditions (4,6,8,20). These adaptations can occur with as few as four exposures to the correct exercise and environmental stress (35) but are unlikely to significantly change across less than four days (41).

Whilst heat acclimation is popular in individual endurance sports (14,23), there are also reported advantages for team sports athletes (5,6,35). However, there is no published study on the effects of STHA on rugby league performance. Rugby league is a high-speed, collision-based, intermittent team sport, played over a period of 80-min, with players covering between 3,000 m and 8,000 m (39,40,43). To prepare players for this type of competition, training must be multifaceted, concurrently developing endurance, speed, strength, power and repeat sprint ability (16). Indeed, rugby league is an aerobically-biased sport, with players maintaining an average of ~ 81% $\dot{V}O_{2peak}$ during matches (9), alongside ~ 30% anaerobic contributions (11). However, dedicating time to the conditioning of endurance capabilities detracts from that spent on technical and tactical aspects of training, particularly during congested in-season periods. For this reason, heat acclimation has become more popular in practice, with the intention of acutely augmenting the effects of endurance training (7,31). However, the application of STHA is challenging when limited time is available for the necessary adaptation and recovery. Therefore, STHA in team sports should be understood from an applied perspective.

During the competitive season, rugby league matches are usually separated by 5-9 days (26), although this can be as low as 2 days (37). Therefore, the volume of training is typically manipulated (periodised) to account for the recovery required between matches and to attenuate signs of fatigue. With this in mind, a STHA protocol could be effectively incorporated into a rugby league players training regime but must be conducted within a time-frame that permits the minimal amount of stimuli (4-5 days) and subsequent adaptation (super-compensation), without interfering with other forms of training. This might be useful during clustered in-season periods, where detraining has been demonstrated in rugby league players (17) but could be offset by a novel training stimulus, without the need for heavy loading or physical contact between players. The amount of time provided for post-heat acclimation recovery in the literature has varied from 24-h (8,14) to 30 days (24), yet the decay of short-term adaptation can occur in as few as 2 days (18). Therefore, whilst it is feasible that a STHA protocol can be incorporated into a typical rugby league micro-cycle, similar to that of soccer (6), it is unknown whether the provision of minimal STHA doses and recovery periods that are available in practice are sufficient to permit performance improvements. The aim of this study was, therefore, to determine if 5 consecutive days of heat acclimation, with a realistic 48-h recovery period, could elicit a greater physiological response and thus improve simulated rugby league performance and maximal oxygen uptake when compared with a matched training stimulus in a temperate environment. It was hypothesised that the STHA group would adapt to the intervention and, subsequently, show an improved physiological response to exercise, increases in performance on the rugby league simulation, improved $\dot{V}O_{2peak}$ and an expansion in plasma volume levels.

METHODS

Design

The subjects took part in a matched-pairs design, visiting the laboratory on nine occasions in a well-hydrated and fed state. At visit one, the subjects were familiarised to the rugby league simulation (Rugby League Match Simulation Protocol; RLMSP, 36) by completing two repeat cycles (2 x ~130 s). At visit two, capillary blood was drawn at rest for analysis of baseline plasma volume, after which baseline tests of maximal oxygen consumption ($\dot{V}O_{2peak}$) were performed in the laboratory on a cycle ergometer. The subjects were then matched into pairs based on their $\dot{V}O_{2peak}$. At visit 3 (24-h later), subjects performed the RLMSP, followed by a time to exhaustion trial. This was performed outdoors on an athletics track, where physiological and perceptual responses were recorded throughout. During visits 4 – 8, all subjects performed 60-min of ergometer cycling at 40% of their previously determined $\dot{V}O_{2peak}$, power output was used to monitor the intensity, perceptual responses and tympanic temperature were recorded throughout. The acclimation group performed all of their training at the same time each day in a heat chamber, which was controlled at a temperature of 33 ± 0.5 °C and 70 ± 4 % relative humidity. The control group also performed their training together in controlled thermoneutral laboratory conditions (19 ± 1 °C and 50 ± 3 % relative humidity). Plasma volume was measured again on the morning of day 8. The baseline $\dot{V}O_{2peak}$ testing procedures were replicated 48-h after the final heat session at visit 9, with the RLMSP conducted another 24-h later (72-h post-intervention), in general accordance with a typical pre-match taper period in a rugby league micro-cycle. The incremental test was conducted prior to the RLMSP as it was deemed to be less demanding. During the acclimation protocol subjects consumed water *ab-libitum* and were always at least 2-h post prandial during testing or acclimation sessions.

Subjects

Following institutional ethical approval, 12 male collegiate rugby league players provided written informed consent to take part in this study. Subjects were randomly assigned to one of two training protocols; acclimation (*n* = 6; age 21 ± 2 years, stature 175.8 ± 6.7 cm, body mass 81.9 ± 7.3 kg, $\dot{V}O_{2peak}$ 39.0 ± 6.9 ml/kg/min) or control (*n* = 6; age 21 ± 1 years, stature 182.3 ± 4.7 cm, body mass 85.8 ± 9.5 kg, $\dot{V}O_{2peak}$ 38.9 ± 8.7 ml/kg/min). The subjects all trained twice and played one match per week, which was also supplemented with two gym-based sessions as part of the university rugby team. The mean training age of the two groups was 10 ± 3 years. The subjects abstained from other forms of exercise and did not take any other forms of ergogenic aid, such as caffeine or concentrated nitrate containing products, as well as alcohol, during the study period, which took place during January pre-season. All subjects completed a food diary for two days prior to each test, which was replicated in content and volume for the remainder of the study. The average high and low temperatures for the 6 weeks preceding the testing were 7.5 and 3.5°C, respectively (accuweather.com), subjects were therefore not deemed to be acclimatized to exercise the heat. The subjects were instructed not to use saunas or take hot baths during the study period. This study was conducted in accordance with the 1964 Helsinki declaration.

Incremental $\dot{V}O_{2peak}$ test

After a 5-min, self-paced warm-up at an external workload of 102 W, subjects completed a

$\dot{V}O_{2peak}$ test, using a mechanically braked cycle ergometer (Monark Exercise AB, Ergomedc 874E, Varberg, Sweden). The bike was fitted to the participant such that only 5° of knee flexion was achieved at the bottom of the pedal stroke. Subjects cycled to exhaustion, with power output increasing by 24 W/min at a fixed cadence of 60 rev/min. The starting power output for each participant was based upon text-book recommendations (45) and in accordance with their reported fitness level. Rating of perceived exertion (3) (RPE) (Borg scale 6-20) and heart rate (HR) were recorded in the final 15-s of each stage. Gas exchange was measured breath-by-breath using a mask connected to a gas analysis system (Jaeger Oxycon Pro, Viasys Healthcare, Hoechberg, Germany). The gas analyzer and flow turbine were calibrated before each test using a known gas mixture (15% O_2 and 5% CO_2) and a 3-L syringe, respectively (Hans Rudolph, Kansas City, KS). $\dot{V}O_{2peak}$ was determined as the mean value recorded over the final 30-s of the test. The power output achieved at 40% $\dot{V}O_{2peak}$ was set as their intensity for the intervention and was monitored using power output. It was assumed that the ~10 % reductions in $\dot{V}O_{2peak}$ experienced in the heat (22) during the intervention would increase the relative intensity to approximately 50% $\dot{V}O_{2peak}$, which is very similar to the range (45-60% $\dot{V}O_{2peak}$) used in short-term heat acclimation studies (19,25,28).

Rugby League Match Simulation Protocol (RLMSP)

After a standardised warm-up, the subjects performed half (43-min) of the RLMSP (36), comprising 20 x 2-min and 10-s cycles. This was followed by a 5-min half-time period, after which a continuous shuttle running time to exhaustion (TTE) was performed at 3.3 m/s. This speed was selected because it is higher than the average RLMSP jogging speed (2.9 m/s) but lower than the moderate-high speed threshold ~3.8 m/s used in many rugby league match analysis studies (38,40,42,43). The outdoor trials were only performed on dry, calm days, with a mean outside temperature and relative humidity of 9 ± 2 °C and 59 ± 8 %, respectively. The reliability of this protocol has been previously reported as 0.8%, 0.9%, 3.7%, and 6.8% coefficient of variation (CV) for overall distance covered, low-, high-, and very high-intensity activity, respectively (36).

Prior to the simulation, subjects were fitted with a 10 Hz Global positioning system (GPS) (FieldWiz Advanced Sport Instrument Sarl, Paudex), which was fitted between the scapulae in a tightly fitting vest. The GPS units were activated 20-min before exercise to ensure satellite fixes and have an intra-unit reliability of between 1 %, 3.1 %, 4.7 % and 5.9 % CV for walking, jogging, sprinting and linear change of direction activities, respectively, which are all included in the RLMSP. A polar FT1 HR monitor (Polar Electro Oy, Kempele, Finland) was used in conjunction with the GPS to record continuous HR during the trials. All data were downloaded to a csv. file and analysed using a custom-built spreadsheet in Microsoft Excel. RPE was recorded following the second sprint of each cycle. Capillary blood samples were drawn from the finger-tip of the subjects, for measurements of blood lactate concentration (B[la]), at three points during the simulation; at rest (2-min pre-test), 1-min post 43-min simulation and 1-min post continuous time to exhaustion running. The finger-tip was cleaned using an alcohol swab and punctured using an automated lancet. Blood was taken from the site using 20 µl capillary tube (EKF Diagnostics, Barleben, Germany), hemolysed in a pre-filled micro test tube and stored at 0 °C before being analysed using a blood lactate analyser (Biosen C_Line, EKF Diagnostics, Barleben, Germany).

The GPS variables analysed included peak speed (km/h), mean speed (km/h) during the first half of the RLMSP and distance covered (m) during the TTE. Fatigue index (%) was also calculated, based on the change in peak sprint speed across each cycle of the RLMSP, using the equation of Fitzsimons et al. (15).

Plasma volume

On arrival at the laboratory in the morning of the first and final intervention sessions (visits 4 and 8), a urine sample was taken to measure hydration using a portable osmometer (Osmocheck, Perform Better, Warwickshire, UK). The subjects were then rested in an upright seated positon in an air conditioned room (19 °C and 50 % relative humidity) for 15-min. Changes in the concentration of haematocrit [Hct] and haemoglobin [Hb] were subsequently recorded to determine the relative change in plasma volume, based on the equations of Dill and Costill (13). Capillary blood was drawn from the index finger into two 75 mm haematocrit capillary tubes for duplicate measurements. The whole blood was centrifuged (Hawksley Haematospin 1400 Centrifuge, Hawksley & Sons Ltd., Sussex, UK) for 5-min at 13000 g. Post centrifugation, capillary tubes were analysed for [Hct] using a micro-capillary reader (Hawksley & Sons Ltd., Sussex, UK), with the mean of the two measurements reported. All measurements agreed by less than 2%. Capillary blood was taken from the same site for measurement of [Hb] using a Hemocue Hb 201+ (Hemocue Ltd, Viking Court, Derbyshire, UK). Data were reported as Δ% pre-exercise plasma volume between days 1 and 5.

Intervention protocols

After urinary analysis and blood sampling procedures had taken place, the subjects had their body mass recorded, wearing only underwear (MPMS-230, Marsden Weighing Group, Oxfordshire, UK). The subjects then entered the heat chamber wearing shorts, socks and trainers, where they sat upright on the same ergometer used during the ramp test. Tympanic temperature (TH809 Infrared Ear Thermometer, Radiant Innovation IInc., Hsin Chu City, Taiwan), HR, RPE, thermal comfort and thermal sensation were recorded at the start and at 10-min intervals throughout the exercising protocol. Tympanic temperature was selected as an approximation of core temperature, owing to the number of subjects concurrently exercising. Based on analysis conducted in our laboratory, tympanic temperature measured with the current device underestimates rectal temperature by 0.6 ± 0.3 °C but correlates strongly with in (R^2 = 0.95) across a range of exercise intensities and environmental conditions. Therefore, the reported results are likely to underestimate rectal temperature by approximately 1 °C but the changes during the trial provide a valid index of core temperature. Thermal comfort was recorded on the Bedford 7-point analogue scale where -3 = "much too cool", 0 = "comfortable", and 3 = "much too warm" (2). Thermal sensation was recorded on an ASHRAE 7-point analogue sensation scale, where -3 = "very cold", 0 = "neutral", and 3 = "very hot" (46). The subjects cycled at 40% of their thermoneutral $\dot{V}O_{2peak}$ at a fixed cadence of 60 r/min for 60-min. In the control condition, the chamber was controlled at 19 °C and 50 % relative humidity. No fans were used during the exercise trials and *ab-libitum* fluid intake was monitored throughout. Sweat rate was estimated by recording post-exercise body mass of the subjects and subtracting this from their pre-exercise mass, with adjustment for fluid intake.

STATISTICAL ANALYSIS

Pre to post-intervention changes in $\dot{V}O_{2peak}$, TTE and fatigue index were analysed using a two-way (group [2] x time [2]) within and between analysis of variance. Changes in RLMSP performance (peak sprints per cycle, HR, RPE and B[la] – values were taken based on an average of 5 cycles) were analysed using a three-way model in a group (2) x match quartile (4) x time (2) format, while responses to the intervention (tympanic temperature, thermal comfort, thermal sensation) were analysed in a group (2) x time (5) format. Changes in body mass across both day 1 and day 5 of the intervention (Δ%body mass) and plasma volume (Δ%PV) between the morning of day 1 and day 5 were assessed using paired *t*-tests. Assumptions of sphericity were assessed using Mauchly's test, with any violations adjusted by use of the Huynh-Feldt correction. When significant *F*-values were observed, *post-hoc* Bonferroni tests were used to determine differences. Pearson's correlation coefficient was used to determine the relationships between body mass losses during interventions and plasma volume changes. Effect sizes (Cohen's *d*) were also calculated for all pairwise differences. Effect sizes were defined as: trivial = 0.2; small = 0.21–0.6; moderate = 0.61–1.2; large = 1.21–1.99; very large > 2.0. Statistical significance was accepted at $P < 0.05$ and all analyses were performed on IBM SPSS Statistics (Version 21, IBM Corp., Armonk, NY, USA).

RESULTS

Perceptual and physiological responses to the intervention

There was an effect of time on maximum HR during the acclimation protocol ($F_{(4,40)}$ = 11.483, $P < 0.001$). *Post-hoc* tests revealed differences between day 1 and day 4 (157 ± 4 *vs.* 147 ± 3.8 b/min, respectively; $P = 0.022$; $d = 2.63$), day 1 and day 5 (157 ± 4 *vs.* 139 ± 4 b/min, respectively; $P < 0.001$; $d = 4.63$) and day 2 and day 5 (152 ± 4 *vs.* 139 ± 4 b/min, respectively; $P < 0.001$; $d = 3.34$). There was also a main effect of the intervention on maximum HR ($F_{(1,10)}$ = 18.295, $P = 0.02$). However, there was no interaction between time and group ($F_{(4,40)}$ = 2.370, $P = 0.069$) (Figure 1A).

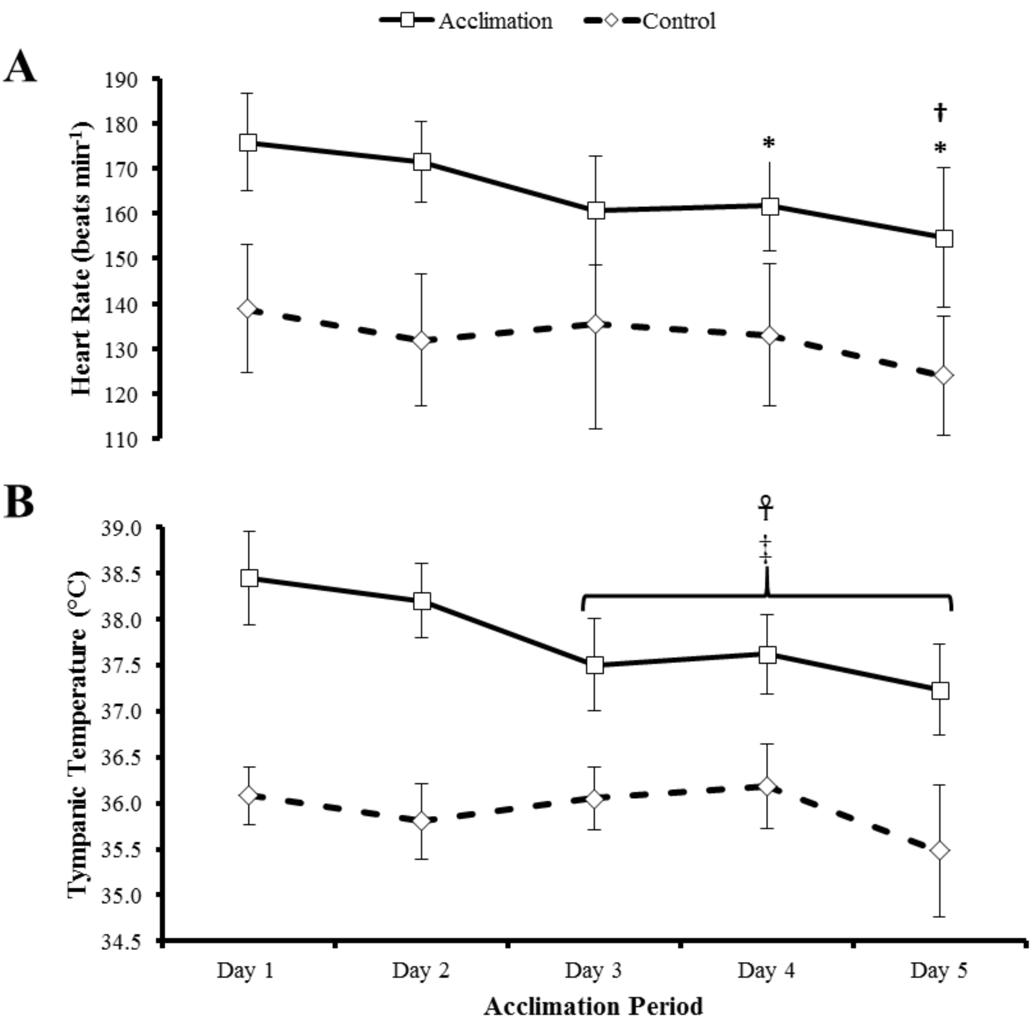

Figure 1. Changes (mean ± SD) in maximum heart rate (panel A; HR; beats·min^{-1}) and tympanic temperature (panel B; TT; ˚C) across a 5-day acclimation protocol among rugby league players (n = 12). * = significantly different (P < 0.05) from day 1 for both groups; † = significantly different (P < 0.05) from day 2 for both groups; ♀ = significantly different (P < 0.05) from day 1 for the acclimation group; ‡ = significantly different (P < 0.05) from day 2 for the acclimation group

There was a main effect of the intervention on tympanic temperature (Figure 1B) during the acclimation protocol ($F_{(1,10)}$ = 86.345, P < 0.001). There was also an effect of time on tympanic temperature during the acclimation protocol ($F_{(4,40)}$ = 10.962, P < 0.001). *Post-hoc* tests revealed differences between day 1 and day 3 (P = 0.029; d = 5.22), day 1 and day 4 (P = 0.013; d = 4.18), day 1 and day 5 (P = 0.005; d = 9.4) and day 2 and day 5 (P = 0.003; d = 3.96). There was an interaction between time and group for tympanic temperature during the acclimation protocol ($F_{(4,40)}$ = 5.589, P = 0.002), with *post-hoc* tests showing differences between groups at day 1 (P < 0.001; d = 10.85), day 2 (P < 0.001; d = 12.53), day 3, (P< 0.001; d = 7.31), day 4 (P < 0.001; d = 7.3) and day 5 (P < 0.001; d = 6.09). There were no systematic changes in environmental temperature across days (P < 0.05), with a CV of 1.5%.

There was an effect of time on thermal comfort (Figure 2B) during the acclimation protocol ($F_{(4,40)}$ = 9.181, P < 0.001). *Post-hoc* tests revealed differences between day 1 and day 2 (P = 0.002; d = 7.88), day 1 and day 3 (P = 0.022; d = 3.02), day 1 and day 4 (P = 0.004; d = 5.65) and day 1 and day 5 (P = 0.033; d = 3.32). There was also a main effect of the intervention ($F_{(1,10)}$ = 39.126, P < 0.001) but no interaction between time and group ($F_{(4,40)}$ = 0.972, P = 0.423). There was an effect of time on thermal sensation (Figure 2A) during the acclimation protocol ($F_{(4,40)}$ = 9.893, P < 0.001). *Post-hoc* tests revealed differences between day 1 and day 2 (P = 0.004; d = 8.10), day 1 and day 4 (P = 0.014; d = 4.51) and day 1 and day 5 (P = 0.02; d = 3.4). There was also a main effect of group ($F_{(1,10)}$ = 34.606, P < 0.001) but no interaction between time and group ($F_{(4,40)}$ = 0.398, P = 0.809).

There was an effect of the intervention ($F_{(1,10)}$ = 24.006, P < 0.001) and time on RPE (Figure 2C) during the acclimation protocol ($F_{(4,40)}$ = 9.740, P < 0.001). There was an interaction between time and group ($F_{(4,40)}$ = 26.745, P < 0.001), with *post-hoc* tests demonstrating differences between the acclimation and non-acclimation groups at day 1 (P < 0.001; d = 6.61), day 2 (P < 0.001; d = 5.28), day 3 (P = 0.005; d = 4.22) and day 4 (P = 0.012; d = 3.10).

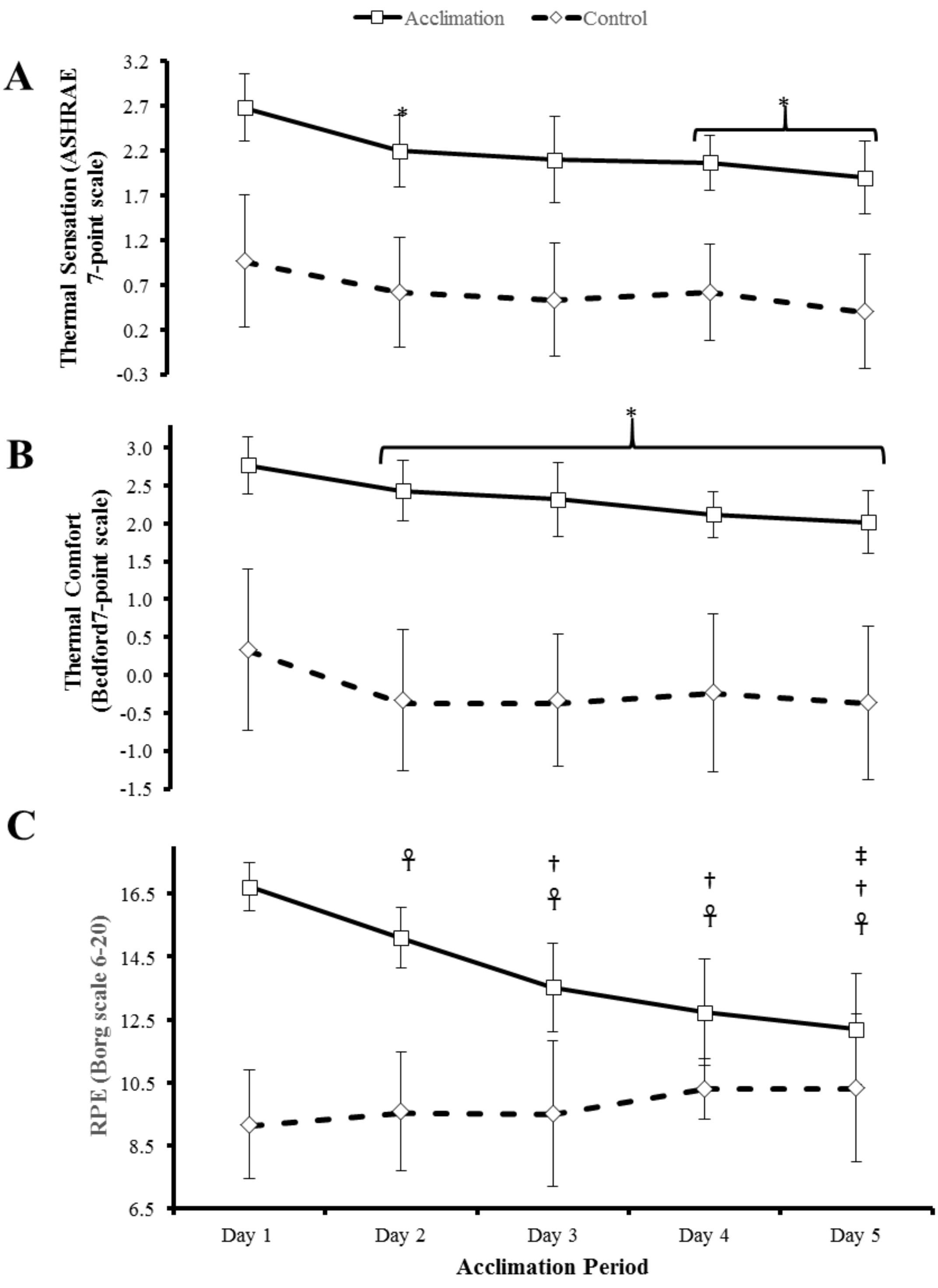

Figure 2. Changes (mean ± SD) in thermal sensation (panel A; TS; ASHRAE 7-point scale), thermal comfort (panel B; TC; Bedford 7-point scale) and RPE (C; Borg scale 6-20) across a 5-day acclimation protocol among rugby league players (n = 12). * = significantly different ($P < 0.05$) from day 1 for both groups; ☥ = significantly different ($P < 0.05$) from day one for

the acclimation group; † = significantly different ($P < 0.05$) from day 2 for the acclimation groups; ‡ = significantly different ($P < 0.05$) from day 3 for the acclimation groups.

There were no baseline differences in PV ($P = 0.786$; $d = 1.1$). Δ%PV between day 1 and 5 of the intervention was not different between groups ($P = 0.290$; $d = 0.91$). There were non-significant, yet descriptively larger Δ% body mass at day 1 in the STHA group (2.2 ± 1.3 % *vs.* 1.5 ± 0.8 %; $P = 0.280$; $d = 0.68$) but, by day 5, there were significantly larger losses in the STHA group (2.6 ± 0.9 % *vs.* 1.2 ± 0.6 %; $P = 0.011$; $d = 1.91$). There was an inverse correlation between plasma volume expansion and body mass losses ($r = -0.79$, $P = 0.0481$), yet weaker correlations between plasma volume expansion and the change in $\dot{V}O_{2peak}$ pre-to-post intervention ($r = 0.29$, $P = 0.577$).

Maximal oxygen uptake testing

There was no difference ($P = 0.898$) in $\dot{V}O_{2peak}$ at baseline between groups. There was an effect of time on $\dot{V}O_{2peak}$ (Figure 3A) following the intervention, indicating a decrease in $\dot{V}O_{2peak}$ ($F_{(1,10)} = 25.954$, $P < 0.001$) but there was no effect of the intervention ($F_{(1,10)} = 3.643$, $P = 0.339$).

There was no effect of time ($F_{(1,10)} = 2.771$, $P = 0.127$) or group ($F_{(1,10)} = 0.745$, $P = 0.408$) on maximal heart rate during $\dot{V}O_{2peak}$ testing (Figure 3B).

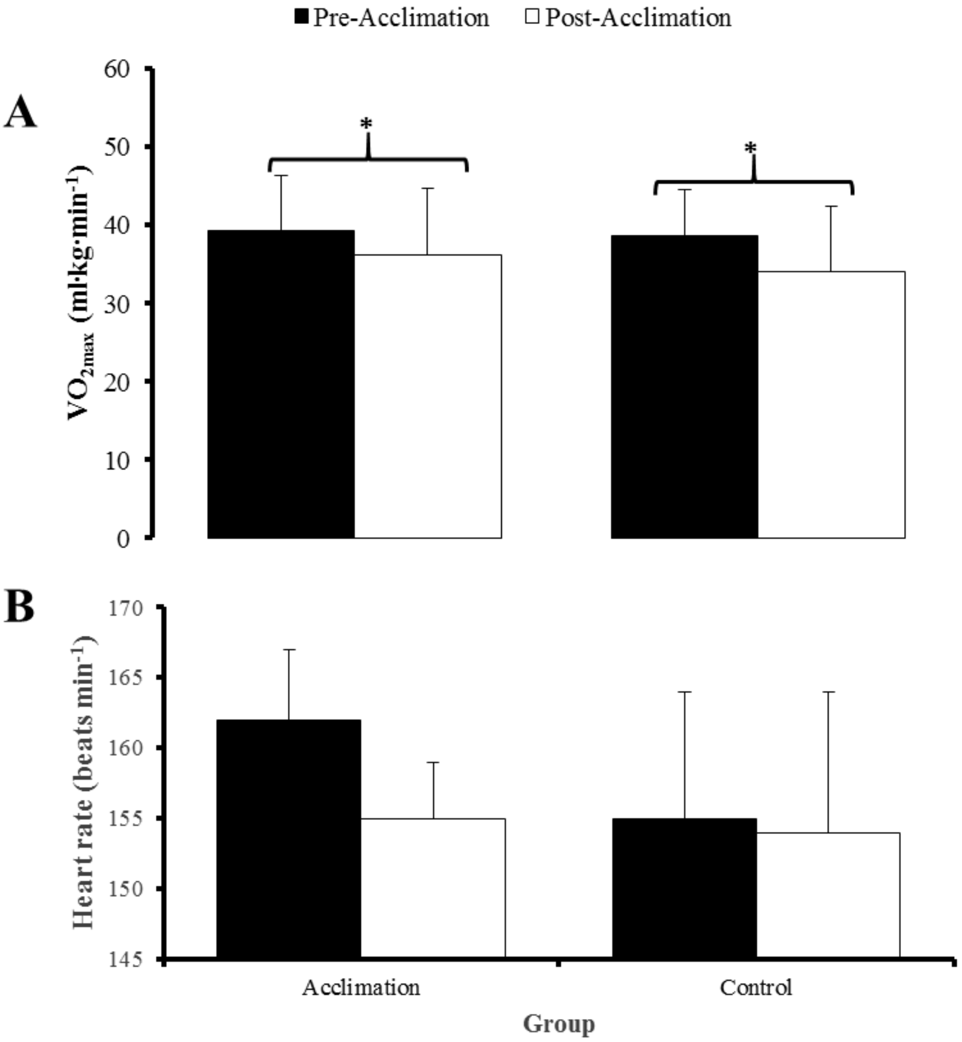

Figure 3. Changes (mean ± SD) in $\dot{V}O_{2peak}$ (ml·kg·min^{-1}) and heart rate (HR; beats·min^{-1}) from pre to post-acclimation among rugby league players (*n* = 12). * = significantly different from pre-acclimation test.

Rugby league movement simulation protocol testing

There was a significant effect of time on TTE (Figure 4B) following the intervention ($F_{(1,8)}$ = 0.148, P = 0.016), with differences pre-to-post acclimation (266 ± 70 s *vs.* 331 ± 89 s, respectively; d = 0.85). There was no significant interaction with group ($F_{(1,8)}$ = 0.067, P = 0.802). There was no significant effect of time ($F_{(1,9)}$ = 1.237, P = 0.295) or group ($F_{(1,9)}$ = 0.077, P = 0.788) on B[la] following the RLMSP (Figure 4A).

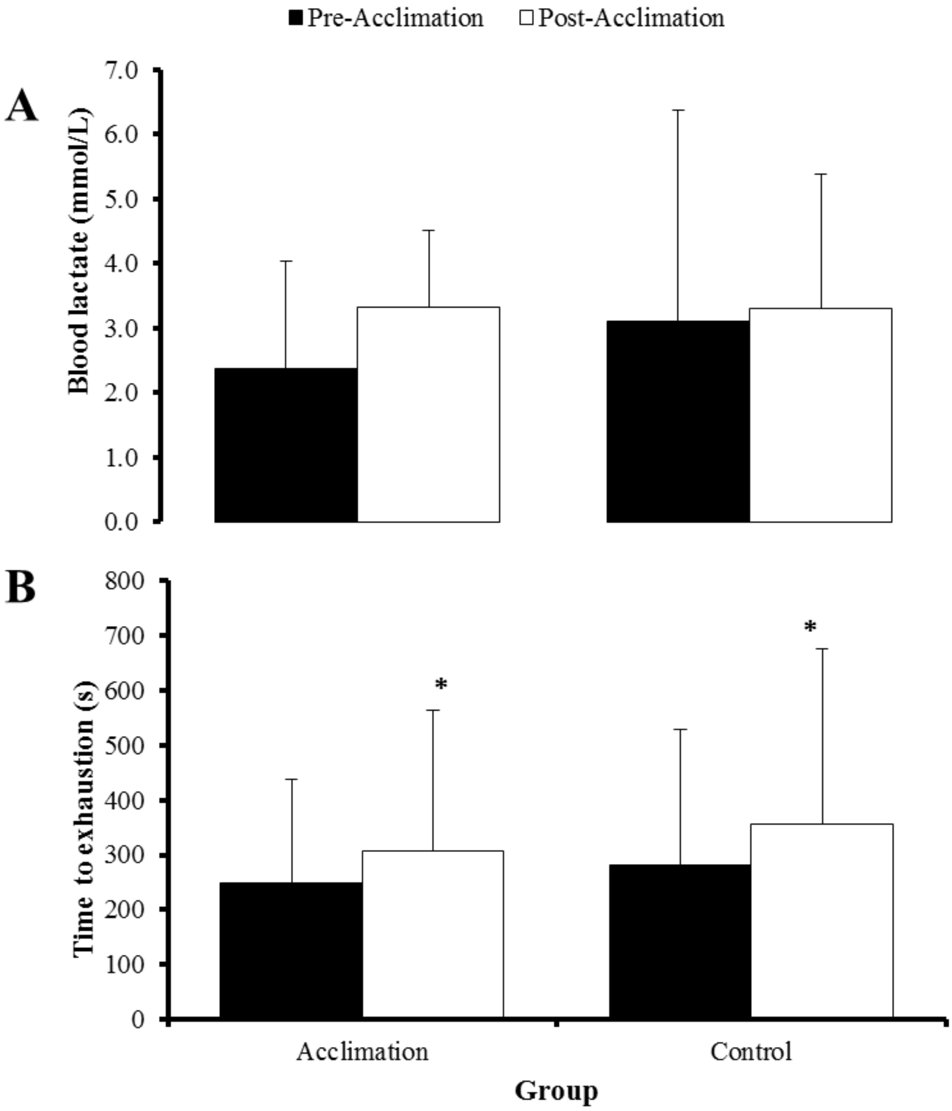

Figure 4. Changes (mean ± SD) blood lactate (B[la]; mmol/l) and in time to exhaustion (TTE; s) from pre to post-acclimation among rugby league players (*n* = 12). * = significantly different from pre-acclimation test.

Data for the RLMSP performance and physiological response are shown in Table 1. There was no three-way interaction between group, time and match quartile for high speed running (HSR) during the RLMSP ($F_{(3,7)} = 0.838$, $P = 0.108$). There were also no effects of time (pre-post acclimation) ($F_{(3,27)} = 0.799$, $P = 0.505$) or group ($F_{(1,9)} = 1.762$, $P = 0.217$). No three-way interaction was found for HR ($F_{(3,7)} = 0.843$, $P = 0.512$). There was also no main effect between groups ($F_{(1,9)} = 0.953$, $P = 0.353$); however, there was an effect of time ($F_{(1,9)}$

= 7.939, P = 0.020), without two-way interactions with group (P = 0.236) or quartile (P = 0.453). The same results were observed for RPE, where only effects of time ($F_{(1,9)}$ = 6.562, P = 0.031) were found. There was no significant three-way interaction between group, time and match quartile for fatigue index during the RLMSP ($F_{(3,7)}$ = 1.111, P = 0.407). There were also no effects of time (pre-post acclimation) ($F_{(3,27)}$ = 0.610, P = 0.575) or group ($F_{(1,9)}$ = 0.148, P = 0.709).

Table 1. RPE, HR (beats·min^{-1}), high speed running (HSR; km/h) and fatigue index (FI; %) across four quartiles of the RLMSP (mean ± SD) (n = 12).

		Pre-Acclimation				Post-Acclimation			
		Quartile 1	**Quartile 2**	**Quartile 3**	**Quartile 4**	**Quartile 1**	**Quartile 2**	**Quartile 3**	**Quartile 4**
RPE (6-20)	Acclimation	9 ± 1	12 ± 2	13 ± 3	14 ± 2	10 ± 2	12 ± 3	13 ± 3	14 ± 3
	Control	8 ± 0	11 ± 1	12 ± 2	12 ± 2	9 ± 1	10 ± 2	12 ± 3	12 ± 3
HR (beats·min^{-1})	Acclimation	166 ± 8	171 ± 7	170 ± 11	172 ± 8	161 ± 12	162 ± 10	162 ± 9	162 ± 10
	Control	172 ± 12	176 ± 12	175 ± 14	176 ± 11	162 ± 17	160 ± 16	158 ± 16	158 ± 16
HSR (m min^{-1})	Acclimation	24 ± 1	25 ± 2	24 ± 3	23 ± 2	26 ± 2	25 ± 3	24 ± 2	24 ± 2
	Control	24 ± 1	24 ± 2	23 ± 3	23 ± 2	24 ± 2	23 ± 2	22 ± 1	23 ± 2
FI (%)	Acclimation	76.3 ± 0.6	75.9 ± 0.5	75.9 ± 0.3	76.2 ± 0.9	76.1 ± 0.5	76.3 ± 0.7	76.6 ± 1.1	76.3 ± 0.4
	Control	75.8 ± 0.5	76.1 ± 0.6	75.9 ± 0.3	76.1 ± 0.5	76.4 ± 0.6	76.2 ± 0.5	76.5 ± 0.9	76.6 ± 1.1

DISCUSSION

The main findings of this study were that five consecutive days of continuous sub-maximal aerobic training on a cycle ergometer was sufficient to improve time-to-exhaustion at the end of a rugby league specific protocol in temperate conditions. Given the early stage of the rugby league season, changes of this magnitude (ES = *moderate*) might be expected. However, both forms of training did not improve performance on the RLMSP (as inferred from self-paced episodes) but did change the heart rate and perceptual responses to the predominantly fixed movement demands. Furthermore, there were no group differences for any performance variable, indicating that the STHA intervention did not provide any benefit to simulated rugby league running performance, above that elicited by thermoneutral cycling exercise. Therefore, the STHA administered in the current study appears to have limited benefit for rugby league performance.

The STHA group acutely adapted during the intervention, as indicated by an improved physiological and perceptual response to cycling at ~ 33 °C environmental temperatures across the trial (Figure 1). For example, tympanic temperature, HR, RPE, TC and TS were all reduced at days 4 or 5 compared to day 1. The changes found herein are consistent with those reported elsewhere after 4 or 5 days of heat acclimation (1,19). Indeed, the larger changes in body mass during the trial in the STHA group by day 5 provide evidence of the increase in the rate of sweat production among these subjects, which is a common response to heat adaptation (7). These findings show that the ability to thermoregulate in the heat was improved in accordance with previously suggested time-scales (19). On this basis, it is possible that performance in a hot environment could have been improved; however, this was not investigated herein.

The non-acclimation group also reduced their HR response to the training intervention across the days of the trial, inferring an acute training effect. The higher overall HR, RPE and thermal stress in the acclimation group demonstrates the greater stimulus provided to the STHA group, which was intended by the research design. However, the hypothesized increases in plasma volume and $\dot{V}O_{2peak}$ were not found from pre- to post-trial. In fact, there were small mean decreases in $\dot{V}O_{2peak}$ from pre-to-post intervention but no group or interaction effects. The concurrent changes in plasma volume and $\dot{V}O_{2peak}$ were anticipated, based on their established relationship (44), but we are unsure why the adaptive responses exhibited during the acclimation protocol did not manifest during thermoneutral performance. For example, the TTE at the end of the RLMSP improved in both groups, without differences between groups, which would be expected if the stress induced by STHA caused physiological changes to support endurance performance. We propose that this might be related to the intentional imbalance between stress and recovery in this study, which was designed to replicate a real-world scenario.

A possible explanation for unanticipated reductions in $\dot{V}O_{2peak}$ across groups is related to the realistic micro-cycle of a rugby league player that was incorporated into the design of the current study. During in-season periods, players will typically have 5 days of a calendar week to prepare for a weekend match and periodise their training, such that the 24-to-48-h before match day comprises lighter loads (37). This tapering strategy permits recovery and short-term adaptation. Adaptation to the heat follows a similar pattern, whereby a post-acclimation period of recovery is needed to facilitate supercompensation. Indeed, Daanen and colleagues (12) investigated this phenomenon and showed that a period of 70-h without exercise augmented the response to heat acclimation, conducted in the preceding 12 days. In agreement with our observations, with shorter post-acclimation recovery periods (24-h), $\dot{V}O_{2peak}$ is often unchanged (27) or lower than baseline (14). Others have reported that a period of 30 days post-acclimation is necessary to achieve a performance benefit (24). However, this theory is at odds with the suggested decay in the effects of STHA after only a few days (18). Our data, which showed no difference between hot and temperate training, lends support to the theory that a period of 48-h is insufficient to permit full adaptation from a 5 day STHA protocol. Interestingly, the RLMSP was performed the day after the $\dot{V}O_{2peak}$ test, where an improvement in TTE was observed, albeit in both groups. This perhaps indicates that a longer period of recovery (~ 70-h) is necessary to allow adaptation to occur but we are unable to confirm this, owing to the design of the study, whereby the $\dot{V}O_{2peak}$ test could not be performed on the same day as the RLMSP.

Unlimited *ab-libitum* water intake was permitted during the acclimation sessions. Minus fluid intake, body mass losses (i.e. sweat rates) varied during the intervention, ranging from 0.8% to 4.0%. However, some of the subjects replaced fluid successfully during this time, whilst some did not. This means that some subjects permitted dehydration to occur. It has been suggested than restricted fluid intake during heat acclimation can augment the expansion in plasma volume, as well as cardiovascular responses (21,25). In an attempt to ascertain the importance of hydration practices during the intervention, we followed up our main analysis by investigating the relationships between, firstly, the absolute losses in body mass among the subjects during the interventions (mean of day 1 and day 5) and, secondly, the change in $\dot{V}O_{2peak}$ from pre-post intervention, with the change in plasma volume (%) between the first and final day of acclimation. There was a negative relationship between plasma volume expansion and body mass losses (r = -0.79, P = 0.022) and weak correlations between plasma volume expansion and the change in $\dot{V}O_{2peak}$ (r = 0.29, P = 0.065), inferring that the subjects who experienced greater fluid loss during the acclimation protocols on day 1 and day 5 were the most likely to exhibit plasma volume expansions and possibly increases (or less decrease) in $\dot{V}O_{2peak}$. These findings highlight a limitation in the current STHA protocol, where the fluid balance of subjects was uncontrolled and might explain the limited changes in performance observed herein. Future studies should consider the control of fluid intake during STHA protocols.

Whilst further research is needed to fully explore the correct tapering of STHA for athletes, rugby league practitioners should be aware that incorporating this type of intervention into a training week is unlikely to improve physical performance of players, assuming that a match is to be played in the following 48-h. Of course, the reductions in core temperature, perceptual responses and increased sweat production indicate a physiological response, which doesn't confer the anticipated acute ergogenic effect. The apparent discord in the current literature regarding the required recovery periods has further implications for team sports practitioners who are considering using STHA. This is because it is likely to be most useful when 'detraining' has occurred, which can appear during clustered in-season periods (17). In these instances, a period of 5 days without heavy loading or contact might be available to provide an additional or novel training stimulus. However, the current study is consistent with the thought that the stress caused by STHA requires a period longer than that typically available (24-48-h) for adaptation before competition. Of course, pre-season periods or prolonged turnaround times would allow a greater recovery period but short-term gains are normally unnecessary during these phases of training and can be achieved through other means.

In conclusion, a STHA intervention was able to improve the tolerance of well-trained rugby league players to the heat, indicated by a lowered thermal, cardiovascular and perceptual response; however, these changes did not improve performance in a simulated rugby league match or subsequent TTE in temperate conditions, above that of thermoneutral cycling exercise. We propose that the null effects of the STHA intervention are related to the realistic micro-cycle used in the current study and are unlikely to permit sufficient time for adaptations to influence exercise performance. The link between plasma volume expansion and body mass losses or $\dot{V}O_{2peak}$ highlight the importance of controlling fluid balance during acclimation, which should be considered in future research.

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