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The repeatability of a cycling exercise-heat stress test in a male population

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A B S T R A C T

Introduction: There is considerable inter-individual variability in the physiological responses to environmental stressors and so to accurately assess and monitor changes in an individual's ability to cope with exercise-heat stress, a reliable protocol is required. The aim of this study was to examine the repeatability of a 90-min steady-state heat exercise bout with physiological and subjective variables, and performance during an incremental test to exhaustion post 90-min steady-state exercise.

Method: Sixteen mixed ability males (Age: 39 ± 15yrs; Height: 176.5 ± 4.8 cm; BM: 79.7 ± 10.3 kg; $\dot{V}O_{2peak}$: 46.2 ± 8.6 ml/kg/min; PPO: 309 ± 39W) who trained at least three times a week undertook two 90-min steady-state – followed by an incremental protocol to exhaustion – cycling heat stress tests (HSTs) in a hot-humid environment (35 °C, 60%RH). Heart rate (HR), rectal ($\overline{T}\ r_e$) and skin temperature ($\overline{T}_{sk}$), rating of perceived exertion (RPE), thermal sensation (TS), and thermal comfort (TC) were measured throughout. Data was analysed using Intraclass Correlation Coefficients (ICC), technical error of measurement (TEM), Bland-Altman plots, *t*-tests, and Cohen's *d* to indicate magnitude of change.

Results: Physiological variables indicated good repeatability evident through moderate to strong ICC ratings, low magnitudes of change (*d*), lower mean biases compared to their respective calculated TEMs, and statistical non-significance, except HR90, $\overline{T}_{sk}$90, and $\overline{T}_{sk}$. Hydration status showed good repeatability except for urine osmolality (osm_u90) and resting urine colour (col_u). Perceptual variables showed encouraging repeatability apart from resting TS and mean TS. Performance data showed good repeatability overall, however 11 participants progressed to the incremental test to exhaustion in the second visit compared to 7 in the first.

Conclusion: Current data demonstrated favourable physiological, perceptual, and performance repeatability during repeated cycling HSTs in hot-humid conditions. However, given more participants progressed to the incremental trial to exhaustion protocol in the second visit, at least one familiarisation trial may improve the reliability of exercise capacity assessment.

Data accessiblity statement

Data is available at https://doi.org/10.6084/m9.figshare.24994242 in its unedited form.

1. Introduction

For amateur or recreational athletes, heat acclimation (HA) can be a useful alternative where financial restraints or professional commitments prevent undergoing natural acclimatization prior to competition in thermally stressing conditions. Heat acclimation is a process intended to encourage adaptations in an artificial environment to reduce physiological strain thereby improving endurance performance in hot conditions and alleviating the risk of adverse heat-related effects (Armstrong and Maresh, 1991; Brokenshire et al., 2009). When exercising, actively performing muscles contribute to increasing metabolic heat production, which increases faster than total body loss, therefore raising core body temperature (T_c) (Gonzalez-Alonso et al., 2000; Webb, 1995). The increase in core temperature can then be exacerbated further when exercising in hot conditions where body heat loss is limited and, depending on temperature gradients, heat can even be absorbed from the environment (Jay and Kenny, 2007; Mee et al., 2015). However, responses and ability to endure such stressors vary between individuals due to a wide range of factors such as insufficient HA, dehydration, or infection (Epstein, 1990; Mee et al., 2015). Likewise, varied gene expression (Mee et al., 2015; Moran et al., 2006), training status (Garrett et al., 2012, 2014; Moss et al., 2020; Neal et al., 2016; Shaw et al., 2022); cardiac disease or sweat gland impairment (Epstein, 1990; Mee et al., 2015); and genetic conditions can contribute to variation between individuals. Typically, heat adaptation is assessed using pre-post trials although the test re-test repeatability is not always known.

Variables such as T_c and heart rate (HR) are commonly used to quantify heat adaptation (Garrett et al., 2012, 2014; Moss et al., 2020; Shaw et al., 2022; Tyler et al., 2016) and so establishing the repeatability of such variables during exercise-heat stress would increase confidence in changes produced post heat interventions. Such data are

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limited (Mee et al., 2015) but would allow for accurate determination of physiological adaptations and, consequently, benefit future heat acclimation research (Brokenshire et al., 2009). Limited research has examined the test re-test responses during exercise in elevated ambient conditions although data from running and cycling studies do exist. Prolonged (90-min) preloaded running time trial performance (Tyler and Sunderland, 2008) and the physiological and perceptual responses to running appear reproducible for "well-trained, familiarised male runners" in hot conditions (30.4 ± 0.1 °C; 53 ± 2%RH) (Tyler and Sunderland, 2008) and in healthy participants in very hot conditions (40.0 ± 0.5 °C; 39.9 ± 1.3%RH) (Mee et al., 2015). Similar data have been observed during cycling trials in elevated thermal conditions with cycling performance and the physiological responses to cycling (Che Jusoh et al., 2015; Marino et al., 2002) deemed reproducible in cyclists of varying abilities in trials of ≤60-min. However, prolonged trials (i.e. time to exhaustion trials) have, in the past, produced higher variation compared to time-trials with a coefficient of variation of 5.2, 26.6, and 55% (Currell et al., 2006; Krebs and Powers, 1989; Maughan et al., 1989); and 1 and 3.4, %; (Currell et al., 2006; Laursen et al., 2003; Palmer et al., 1996; Smith et al., 2001), respectively.

Therefore, the aim of this study was to contribute to the limited data available by examining the repeatability of a prolonged 90-min steady-state heat exercise bout followed by an incremental protocol to exhaustion repeated one week apart – to limit any adaptive response – in a hot humid environment. The hypothesis was that data from the second exercise-heat stress bout would show limited variance compared to the first, thereby contributing to future research decisions and designs as well as the methodology of exercise-heat stress bouts.

2. Method

2.1. Participants

Sixteen recreationally active (training 3+ times/week) non-heat acclimated males participated (Age: 39 ± 15yrs; Height: 176.5 ± 4.8 cm; body mass (BM): 79.7 ± 10.3 kg; $\dot{V}O_{2peak}$: 46.2 ± 8.6 ml/kg/min; peak power output (PPO): 309 ± 39W). Conditioning was determined via peak oxygen uptake ($\dot{V}O_{2peak}$) and categorised into performance levels (PL) according to De Pauw et al. (2013) (PL1=<45; PL2 = 45–54.9; PL3 = 55–64.9; PL4 = 65–71; PL5 >71 ml/kg/min). Eight of the sixteen were classed as PL1, 8 were classed as PL2, and 2 were classed as PL3. Written and verbal informed consent was provided – which included making participants aware of the ethical cut off point for T_c being 39.5 °C – and all participants completed a pre-exercise medical questionnaire. All were in good health and free from underlying cardiovascular conditions.

2.2. Experimental procedures

2.2.1. Aerobic fitness test

Participants performed an incremental ramp protocol to exhaustion on a cycle ergometer (Daum Electronic Gmbh, Furth, Germany) to determine $\dot{V}O_{2peak}$ and PPO (W) beginning at 50W. Resistance increased by 25W every minute until volitional exhaustion. Breath by breath expired gas was collected via metabolic cart system (Cortex Metalyzer 3B, Cortex Biophysic, Leipzig, Germany) calibrated by a 3 L calibration syringe (Hans Rudolph 3 L, Cranlea & Co., Birmingham, UK) and calibration gas (5% CO_2, 15% O_2, Cranlea & Co., Birmingham, UK).

Participants' RPE and HR were recorded every minute. Peak oxygen uptake was determined via a rolling 30 s average, therefore the final $\dot{V}O_{2peak}$ value was the final 30 s before exhaustion. Peak power output was determined by the power the participant achieved prior to exhaustion. All participants received verbal encouragement in the waning stages of the test. Termination occurred when either the participants voluntarily ended the test, or the participant could no maintain >60 rpm.

2.2.2. Repeated trials

All participants performed the same exercise-heat stress test one week apart with no intervention (Fig. 1) at the same time of day as the prior trial to control for circadian rhythm. Participants were instructed to avoid strenuous exercise at least 24 h as well as caffeine and alcohol at least 12h prior to each trial, and to wear the same – if not similar – attire to each visit. The study design was a dual-centre, retrospective analysis of HST data from two higher education institutions based in the UK. Ethical approval was provided by The University of Hull's Ethics Committee and the Ethical Advisory Committee of the University of Roehampton (LSC 20/305).

Repeated HSTs were conducted in environmental chambers at two higher education institutions in the UK (Design Environmental Ltd, Gwent, Wales) set to 35 °C, 60%RH (wet-bulb ambient temperature: 28.5 °C). The HST consisted of 90 min continuous exercise on a cycle ergometer (Daum, Electronic Gmbh, Furth, Germany; Monark 824E, Monark Exercise AB, Varberg, Sweden) using individualised workloads at 40% PPO (124 ± 15 W) achieved in the $\dot{V}O_{2peak}$ trial. Upon completion, the participant rested passively for 10 min – within the environmental chamber - before performing an incremental ramp protocol to exhaustion at 2% of PPO applied every 30 s, commencing from the initial 40%PPO workload. Recorded measures from the performance trial were end $\overline{T}_{re}$ (°C), end HR (b·min^{-1}), time to exhaustion (TTE) (s), and PPO (W). Urine and capillary blood samples, and nude body mass (BM_{nude}) were obtained pre- and post-exercise on both visits.

2.2.3. Hydration status and blood samples

Participants were asked to follow a specific order when providing pre-exercise measurements of hydration status prior to entering the environmental chamber. A urine sample (Kern & Sohn GmbH, Balingen, Germany) was provided prior to providing nude body mass (BM_{nude}), both in private. Urine colour ($colour_u$) was determined using $colour_u$ chart (Armstrong et al., 1994) while urine osmolality (osm_u) and urine specific gravity (SG_u) were analysed using an osmometer (Model 3320, Advanced Instruments Inc., Massachusetts, USA) and a refractometer (Unicron-N, Urine specific gravity refractometer, Atago CO., Tokyo, Japan) (Armstrong et al., 1998), respectively.

Capillary blood samples were collected to be analysed for Haemoglobin (Hb) using a Hb analyser (Hemocue 201+, Radiometer Ltd, Crawley, UK), and Haematocrit (Hct) using a microhematocrit centrifuge (Hawksley & Sons, Lancing, UK) to be used to calculate plasma volume (PV) as defined by (Dill and Costill, 1974). For post-exercise hydration measures, capillary blood samples immediately upon exiting the environmental chamber followed by BM_{nude} prior to providing a urine sample. All measures were analysed in duplicate and mean values were reported.

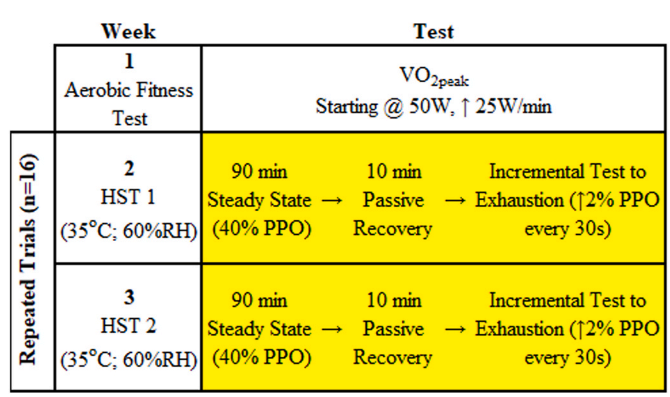

Fig. 1. Experimental design of repeated cycling trials in hot-humid conditions (35 °C, 60%RH).

2.2.4. Body temperature

Core body temperature was measured using a rectal thermistor (U Thermistor, Grant Instruments Ltd, Cambridge, UK) inserted 10 cm past the anal sphincter. Skin thermistors (Type EUS-U-V5-V2, Grant Instruments Ltd, Cambridge, UK) were applied to the participant in four sites as outlined by Ramanathan (1964), allowing for calculation of mean skin ($\overline{T}_{sk}$) and mean body ($\overline{T}_b$) temperatures (Ramanathan, 1964). Temperature measures were recorded at rest and every 10 min throughout the continuous exercise trial using a portable data logger (2020 series data logger, Grant Instruments Ltd, Cambridge, UK). end exercise $\overline{T}_{re}$ was recorded at exhaustion of the incremental performance protocol.

2.2.5. Heart rate

Heart rate was measured at rest and every 10 min throughout the steady-state exercise trial using a HR monitor (Polar FS1, Polar Electro, OY, Finland). End exercise HR was recorded at exhaustion of the incremental performance protocol.

2.2.6. Perceptual measures

Participants were asked to provide their rating of perceived exertion (RPE) (6–20) (Borg, 1982), thermal comfort (TC) (1–5) and thermal sensation (TS) (1–13) adapted from Gagge et al. (1967) (van den Heuvel et al., 2020) at rest and every 10 min until the completion of the 90 min continuous exercise trial.

2.2.7. Performance

Time to exhaustion and PPO were recorded at exhaustion of the incremental performance protocol via a timer and digital display from the cycle ergometer, respectively.

2.2.8. Data analysis

Outcome measures indicative of heat adaptation in two HSTs with no intervention were analysed through JASP (Version 0.17.1, University of Amsterdam, Amsterdam, Netherlands). Data are presented as mean (95%CI). Technical error of measurement (TEM) was calculated as the standard deviation of within-subject differences divided by the square root of 2. Pearson's *r* and Spearman's *rho* were used to determine correlations for normally and non-normally distributed data, respectively. Coefficient of Variation (CoV) was calculated as the percentage representative of the TEM to grand mean of all measures. Magnitude of change was determined via Cohen's *d* effect sizes (<0.2 trivial; 0.2–0.49 small; 0.5–0.79 medium; >0.8 large) (Cumming, 2013). Lines of equality were plotted for each outcome variable to gauge degree of agreement between measures. Bland-Altman plots were used to determine bias and limits of agreement with mean biases and limits of agreement (LoA) reported to three decimal places as provided by JASP. Intraclass Correlation Coefficient (ICC) estimates and their 95% confidence intervals were reported based on a 2-way mixed effects, single measurement (3,1) (Koo and Li, 2016), absolute agreement model categorised as poor (<0.5), moderate (0.5–0.75), good (0.75–0.9), and excellent (>0.9) (Koo and Li, 2016). A paired-samples *t*-test was used to determine if any significant differences were present between repeated trials ($P < 0.05$).

3. Results

Correlations between change in variables and fitness ($\dot{V}O_{2peak}$) did not produce any meaningful significant correlations ($P > 0.05$).

3.1. Physiology

Physiological data are presented in Table 1. Intraclass correlation coefficients indicated good correlations in HR_{mean} (ICC = 0.887) and resting HR (ICC = 0.779), as well as moderate correlations in heart rate at 90-min (HR_{90}) (ICC = 0.697) and $\overline{T}_{sk}$ (ICC = 0.596). Remaining variables produced poor correlations (ICC <0.5), however mean biases for all variables were within calculated TEM (Table 1). Bland-Altman plots for resting HR and resting $\overline{T}_{re}$ (Fig. 2) showed low mean biases, where Bland-Altman plots for HR_{90} and mean skin temperature at 90-min ($\overline{T}_{sk}90$) (Fig. 3) showed higher mean biases. Magnitudes of change (Fig. 3) showed majority trivial and small changes between HST1 and HST2 for physiological variables, except for HR_{90} (0.54: medium), $\overline{T}_{sk}90$ (−0.71: medium) and $\overline{T}_{sk}$ (−0.71: medium). Subsequently, significant differences were detected between HST1 and HST2 in HR90 ($t[15] = -2.162$; $p = 0.047$), $\overline{T}_{sk}90$ ($t[15] = 2.852$; $p = 0.012$), and $\overline{T}_{sk}$ ($t[15] = 2.832$; $p = 0.013$) (Table 1).

3.2. Hydration status

Indicators of hydration status are presented in Table 2. Intraclass correlation coefficient indicated excellent correlations in resting BM (ICC = 0.998) and end BM (ICC = 0.997) (Table 2), while moderate correlations were present for resting osm_u (ICC = 0.541), end osm_u (ICC = 0.689), resting SG_u (ICC = 0.530), change in body mass (ΔBM) (ICC =

Table 1
Mean physiological measures of HR and $\overline{T}_{re}$ pre-to-post repeated cycling HSTs in hot-humid conditions (35 °C, 60%RH) ($n = 16$).

Variable	HST 1		HST2		Statistics					
					p (*t* (*df*))	ICC		TEM (CoV %)	Bland-Altman	
	Mean	95%CI	Mean	95%CI		PE	95%CI		Bias	LoA
HR R ($b{\cdot}min^{-1}$)	79	70–88	82	71–92	0.415 (−0.838 (15))	0.799ˆ	0.515–0.925	8.43 (10.51)	2.500	−25.878–20.878
HR 90 (b.min-1)	150	140–160	158	148–168	**0.047** (−2.162 (15))	0.697<	0.324–0.883	10.14 (6.58)	−7.750	−35.849–20.349
HR_{mean} (b.min-1)	140	130–150	142	132–152	0.483 (−0.720 (15))	0.887ˆ	0.708–0.959	6.30 (4.47)	−1.625	−19.328–16.078
$\overline{T}_{sk}$ R (°C)	34.24	33.77–34.71	33.80	33.36–34.24	0.074 (1.922 (15))	0.416	−0.083–0.748	0.65 (1.92)	0.443	−1.365–2.251
$\overline{T}_{sk}$ 90 (°C)	35.94	35.57–36.3	35.44	35.08–35.79	**0.012** (2.852 (15))	0.462	−0.026–0.772	0.49 (1.39)	0.499	−0.872–1.87
$\overline{T}_{sk}$ (°C)	35.62	35.41–35.84	35.33	35.07–35.6	**0.013** (2.832 (15))	0.596<	0.159–0.837	0.39 (1.12)	0.291	−0.514–1.095
$\overline{T}_b$ R (°C)	36.73	36.46–37	36.73	36.51–36.95	0.983 (0.021 (15))	0.499	0.022–0.791	0.33 (0.90)	0.002	−0.918–0.923
$\overline{T}_b$ 90 (°C)	38.46	38.19–38.74	38.37	38.11–38.62	0.586 (0.557 (15))	0.058	−0.437–0.525	0.49 (1.27)	0.096	−1.258–1.451
$\overline{T}_b$ (°C)	37.72	37.48–37.96	37.66	37.45–37.87	0.667 (0.439 (15))	0.213	−0.300–0.630	0.39 (1.04)	0.059	−0.991–1.108
$\overline{T}_{re}$ R (°C)	37.01	36.73–37.29	37.06	36.83–37.28	0.730 (−0.352 (15))	0.391	−0.112–0.735	0.38 (1.03)	−0.048	−1.105–1.01
$\overline{T}_{re}$ 90 (°C)	38.74	38.44–39.04	38.90	38.6–39.19	0.306 (−1.060 (15))	0.457	−0.032–0.769	0.41 (1.06)	−0.154	−1.296–0.987
$\overline{T}_{re}$ (°C)	37.95	37.69–38.21	38.04	37.8–38.28	0.516 (−0.665 (15))	0.433	−0.062–0.757	0.36 (0.96)	−0.084	−1.079–0.911

Notes: HST = heat stress test; ICC = intraclass correlation; n = number of participants; 95%CI = 95% confidence intervals; TEM = technical error of measurement; LoA = limits of agreement; PE = point estimate; HR = heart rate; R = rest; 90 =* 90 min *measure; HR_{mean} = mean HR; $\overline{T}_{sk}$ = mean skin temperature; $\overline{T}_b$ = mean body temperature; $\overline{T}_{re}$ = mean rectal temperature; ˆ = good ICC rating; ˆ = moderate ICC rating;* ***significance level P < 0.05.*

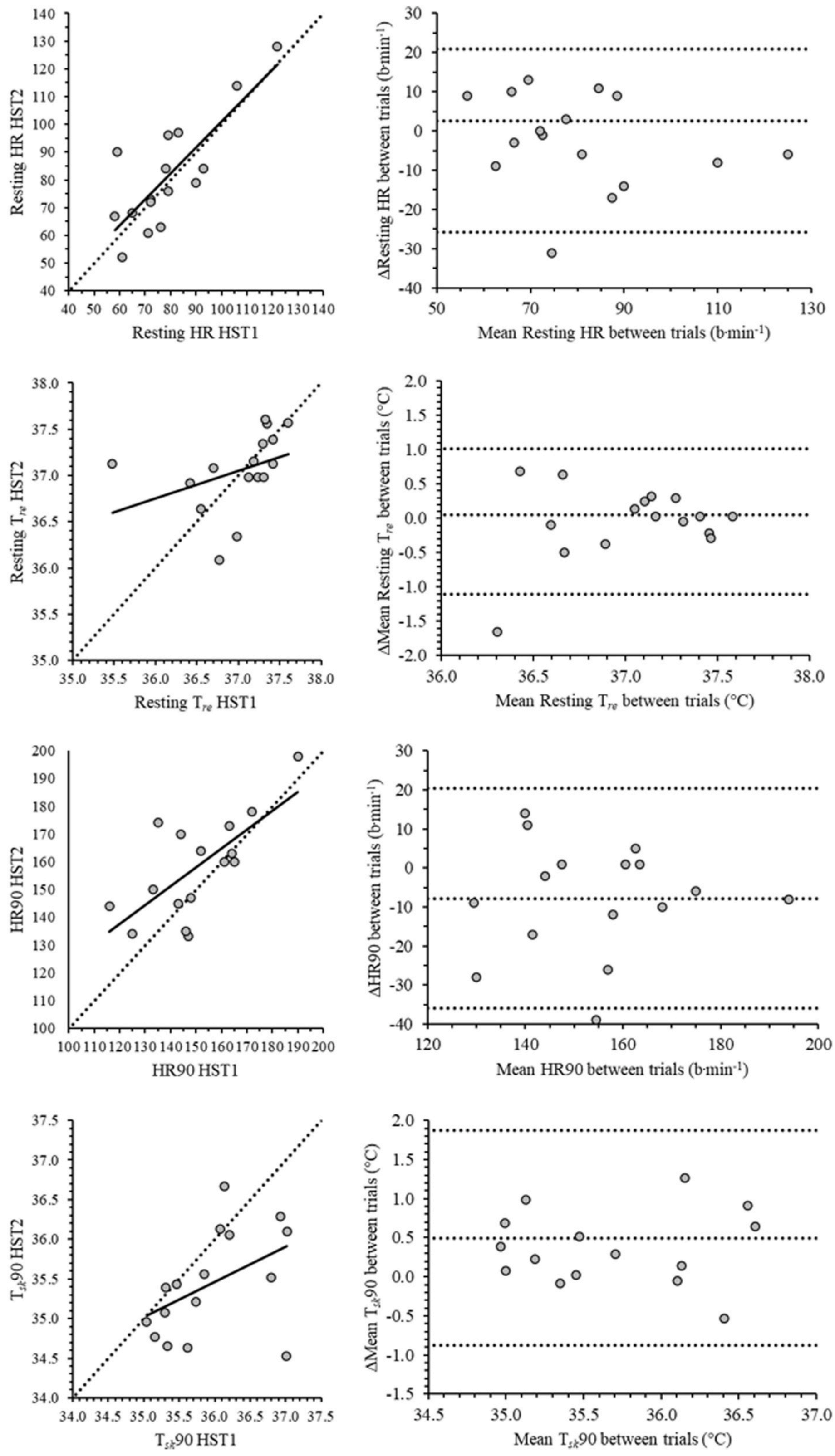

Fig. 2. Scatter plots (left) post HST 1 (x-axis) and HST 2 (y-axis), and Bland-Altman plots (right) with mean bias (middle dotted line) and 95% limits of agreement (outer dotted lines) for resting HR (upper), resting T_{re} (upper middle), HR90 (lower middle), and Tsk90 (lower). $N = 16$.

0.500), resting Hb (ICC = 0.671), end Hb (ICC = 0.584), and change in percent of plasma volume (Δ%PV) (ICC = 0.525). Remaining variables produced poor correlations (ICC <0.5) (Table 2). All variables presented a mean bias lower than the TEM – except for end osm_u (mean bias = 180.3mOsm/kg; TEM (CoV) = 154 (29.16%) and end Hct (mean bias = −3.2%; TEM (CoV) = 1.80 (4.08)) (Table 2). Both variables with an "excellent" ICC rating – resting and end BM – produced mean biases of −0.33 kg and −0.016 kg, respectively (Table 2). All variables showed

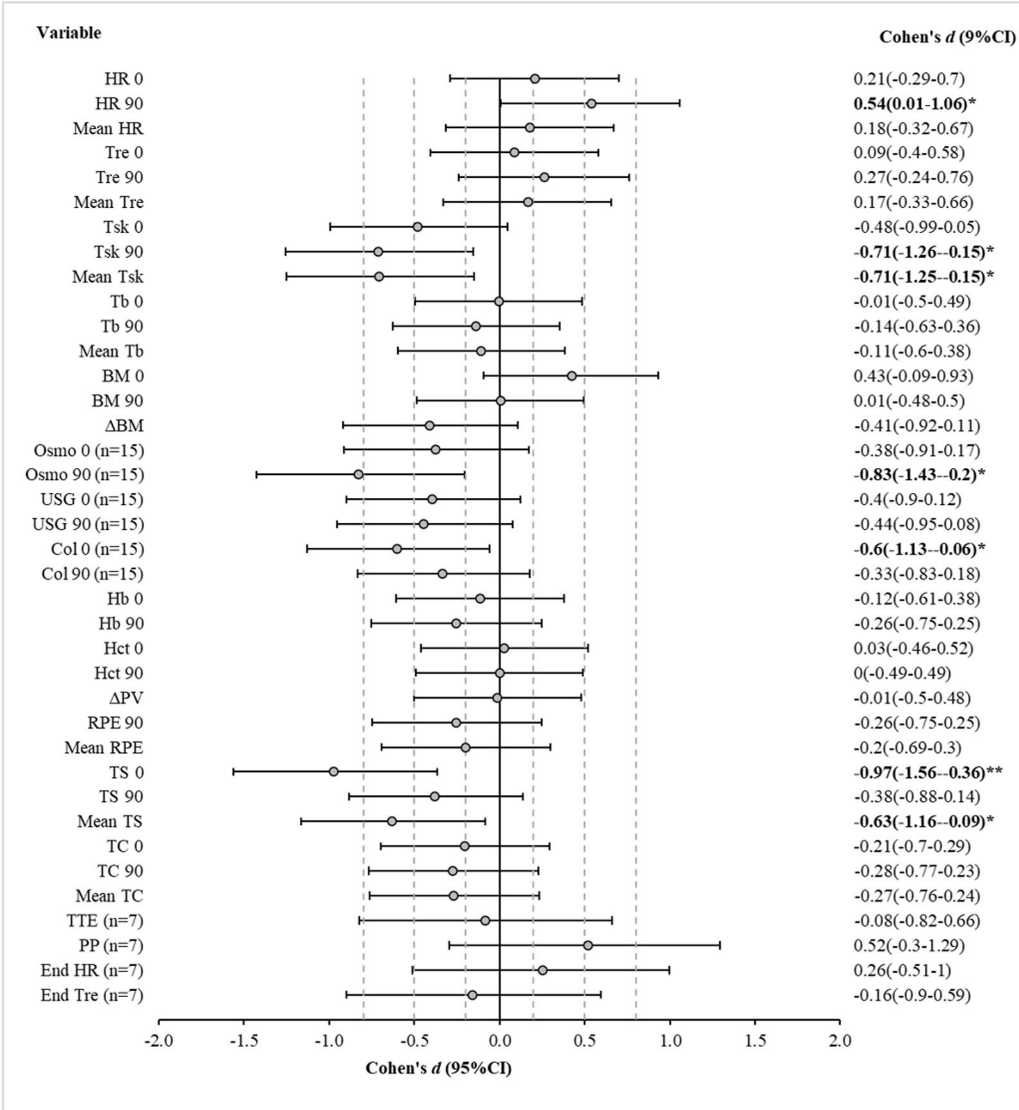

Fig. 3. Magnitude of change (Cohen's *d*) and 95%CI for all variables taken in HST1 and HST2 in a male population in hot and humid conditions (35 °C; 60%RH).

trivial or small magnitudes of change (Fig. 3), except for end osm_u (−0.83: large) and resting col_u (−0.6: medium). Consequently, significant differences were detected between HST1 and HST2 in end osm_u ($t[13] = 3.094$; $p = 0.009$) and resting col_u ($t[15] = 2.416$; $p = 0.029$) (Table 2).

3.3. Perceptual

Perceptual data is presented in Table 3, representative of $n = 16$. Intraclass correlation coefficients indicated good correlations in resting TS (ICC = 0.782), thermal sensation at 90-min (TS_{90}) (ICC = 0.889), and mean thermal sensation (TS_{mean}) (ICC = 0.889), while moderate correlations were present for rating of perceived exertion (RPE_{90}) (ICC = 0.630), mean rating of perceived exertion (RPE_{mean}) (ICC = 0.630), and thermal comfort at 90-min (TC_{90}) (ICC = 0.513). Remaining variables produced poor correlations (ICC <0.5). Mean biases for all variables were below their respective calculated TEM, except for resting TS (Fig. 5: upper) which was higher (mean bias = 0.813; TEM (CoV) = 0.59 (7.7%) (Table 3). Magnitude of change (Fig. 3) for perceptual variables showed trivial or small changes except for resting TS (−0.97: large) and mean TS (−0.63: medium). Thus, significant differences were detected between HST1 and HST2 in resting TS ($t[15] = 3.896$; $p = 0.001$) and TS_{mean} ($t[15] = 2.535$; $p = 0.023$) (Table 3).

3.4. Performance

Performance data is presented in Table 4, representative of $n = 7$. In the first trial, seven participants reached the incremental protocol to exhaustion. Whereas in the second trial, eleven reached the incremental protocol to exhaustion. Remaining participants failed due to reaching the ethical cut off Tre of 39.5 °C prior to attaining the incremental test to exhaustion in at least one of the trials. Intraclass correlation coefficients indicated excellent correlations in PPO (ICC = 0.923) and TTE (ICC = 0.910), as well as a moderate correlation in end $\overline{T}_{re}$ (ICC = 0.579) (Table 4). End HR ($n = 7$) produced an ICC of 0.000. Following a sensitivity analysis, an extreme outlier was found and upon removal from the dataset for this variable, improved to 0.875 ($n = 6$). The mean biases of each variable were below their calculated TEM (Table 4). Performance variables produced trivial-small magnitudes of change, except for PPO (0.52: medium; Fig. 3), however no statistical differences were detected between HST1 and HST2 ($P > 0.05$) (Table 4).

Table 2
Mean hydration status measures pre-to-post repeated cycling HSTs in hot-humid conditions (35 °C, 60%RH) (n = 16).

Measure	HST 1		HST2		Statistics					
					p (t (df))	ICC		TEM (CoV %)	Bland-Altman	
	Mean	95%CI	Mean	95%CI		PE	95%CI		Mean Bias	LoA
osm$_u$ R	532	327–736	415	250–581	0.182 (1.410 (13))	0.541<	0.080–0.812	218 (46.05)	116.143	−488.144–720.429
osm$_u$ 90	619	454–783	439	284–593	**0.009** (3.094 (13))	0.689<	0.309–0.879	154 (29.16)	180.321	−247.059–607.702
SG$_u$ R	1.0136	1.0089–1.0182	1.0104	1.0063–1.0145	0.135 (1.581 (15))	0.530<	0.065–0.806	0.0056 (0.56)	0.003	−0.012–0.019
SG$_u$ 90	1.0168	1.0132–1.0205	1.0130	1.0085–1.0174	0.096 (1.774 (15))	0.343	−0.167–0.708	0.0062 (0.61)	0.004	−0.013–0.021
col$_u$ R	3	2–3	2	2–3	**0.029** (2.416 (15))	0.124	−0.381–0.572	0.8 (31.80)	0.688	−1.544–2.919
col$_u$ 90	4	3–5	4	3–4	0.203 (1.331 (15))	0.423	−0.075–0.752	0.9 (25.00)	0.438	−2.14–3.015
BM R (kg)	80.2	74.1–86.3	80.5	74.6–86.5	0.109 (−1.705 (15))	0.998*	0.993–0.999	0.55 (0.68)	−0.331	−1.854–1.192
BM E (kg)	79.2	73.2–85.2	79.2	73.2–85.2	0.979 (−0.026 (15))	0.997*	0.990–0.999	0.68 (0.85)	−0.006	−1.88–1.868
ΔBM (kg)	−0.8	−1.2–0.4	−1.2	−1.8–0.6	0.121 (1.643 (15))	0.500<	0.024–0.792	0.65 (64.17)	0.375	−1.415–2.165
Hb R	14.9	14.4–15.4	14.8	14.2–15.4	0.649 (0.465 (15))	0.671<	0.280–0.871	0.61 (4.10)	0.100	−1.588–1.788
Hb E	15.3	14.8–15.9	15.1	14.7–15.5	0.321 (1.027 (15))	0.584<	0.142–0.832	0.58 (3.83)	0.213	−1.41–1.835
Hct R (%)	42.9	39.7–46.1	42.9	40.7–45.2	0.913 (−0.111 (15))	0.639<	0.226–0.857	3.17 (7.38)	−0.125	−8.945–8.695
Hct E (%)	44.1	40.3–47.8	43.9	41–46.9	1.000 (0.000 (15))	0.915*	0.775–0.969	1.80 (4.08)	0.000	−5.21–5.21
ΔPV (%)	−3.3	−10.8–4.2	−3.5	−9.5–2.6	0.958 (0.054 (15))	0.525<	0.058–0.804	9.03 (−265.66)	−0.172	−24.849–25.193

Notes: HST = heat stress test; ICC = intraclass correlation; n = number of participants; 95%CI = 95% confidence intervals; TEM = technical error of measurement; LoA = limits of agreement; PE = point estimate; osm$_u$ = urine osmolality; SG$_u$ = urine specific gravity; col$_u$ = urine colour; BM = body mass; Hb = haemoglobin; Hct = haematocrit; % PVChange = percentage plasma volume change;* ***significance level P < 0.05.*

Table 3
Mean perceptual measures pre-to-post repeated cycling HSTs in hot-humid conditions (35 °C, 60%RH) (n = 16).

Measure	HST 1		HST2		Statistics					
					p (t (df))	ICC		TEM (CoV %)	Bland-Altman	
	Mean	95%CI	Mean	95%CI		PE	95%CI		Bias	LoA
RPE 90	17	16–19	16	15–18	0.323 (1.022 (15))	0.630	0.213–0.853	1.74 (10.37)	0.625	−4.169–5.419
RPE$_{mean}$	12	11–12	11	10–12	0.432 (0.808 (15))	0.630	0.212–0.853	0.87 (7.63)	0.250	−2.177–2.677
TS R	8	7–9	7	7–8	**0.001** (3.896 (15))	0.782	0.482–0.918	0.59 (7.70)	0.813	−0.822–2.447
TS 90	11	10–12	11	9–12	0.150 (1.518 (15))	0.877	0.684–0.955	0.81 (7.50)	0.438	−1.822–2.697
TS$_{mean}$	10	9–11	9	8–10	**0.023** (2.535 (15))	0.889	0.712–0.960	0.61 (6.32)	0.544	−1.138–2.225
TC R	2	1–2	2	1–2	0.423 (0.824 (15))	0.049	−0.444–0.519	0.64 (38.88)	0.188	−1.597–1.972
TC 90	4	4–5	4	3–5	0.289 (1.098 (15))	0.513	0.041–0.798	0.80 (19.66)	0.313	−1.919–2.544
TC$_{mean}$	3	3–3	3	2–3	0.300 (1.074 (15))	0.252	−0.262–0.654	0.66 (22.90)	0.250	−1.575–2.075

Notes: HST = heat stress test; ICC = intraclass correlation; n = number of participants; 95%CI = 95% confidence intervals; TEM = technical error of measurement; LoA = limits of agreement; PE = point estimate; RPE = rate of perceived exertion; RPE$_{mean}$ = mean RPE; TS = thermal sensation; TS$_{mean}$ = mean TS; TC = thermal comfort; TC$_{mean}$ = mean TC;* ***significance level P < 0.05.*

Table 4
Mean performance measures pre-to-post repeated cycling HSTs in hot-humid conditions (35 °C, 60%RH) (n = 7).

Measure	HST 1		HST2		Statistics					
					p (t (df))			TEM (CoV %)	Bland-Altman	
	Mean	95%CI	Mean	95%CI		PE	95%CI		Bias	LoA
TTE (s)	557	319–794	546	292–801	0.832 (0.222 (6))	0.910*	0.763–0.968	85.50 (15.50)	10.143	−226.84–247.126
PPO (w)	231	191–271	240	199–281	0.220 (−1.370 (6))	0.923*	0.795–0.972	12.09 (5.13)	−8.857	−42.377–24.663
HR End (b.min-1)	172	157–188	177	171–184	0.525 (−0.675 (6))	0.000	−0.482–0.482	13.86 (7.92)	−5.000	−43.425–33.425
Tr End (°C)	38.57	38.13–39.01	38.50	38.08–38.92	0.689 (0.420 (6))	0.579<	0.134–0.829	0.32 (0.83)	0.071	−0.811–0.954

Notes: HST = heat stress test; ICC = intraclass correlation; n = number of participants; 95%CI = 95% confidence intervals; TEM = technical error of measurement; LoA = limits of agreement; PE = point estimate; TTE(s) = time to exhaustion in seconds; PPO = peak power output; HR End = end exercise heart rate; Tr End = end exercise rectal temperature;* ***significance level P < 0.05.*

4. Discussion

The primary aim of this study was to assess the test re-test of responses during a cycling HST consisting of 90-min steady-state followed by an incremental test to exhaustion in hot-humid conditions (35 °C; 60%RH) 7 days apart. Physiological variables (Table 1) demonstrated favourable repeatability. However, HR$_{90}$ (d = 0.54), $\overline{T}_{sk}90$ (d = −0.71) and $\overline{T}_{sk}$ (d = −0.71) showed medium differences between trials. Similarly, many hydration status variables indicated strong repeatability, except for end osm$_u$ (d = −0.83: large) and resting col$_u$ (d = −0.6: medium). Resting (d = −0.97) and mean TS (d = -0.63) showed a large and medium difference, respectively, between trials, with the remaining variables indicating trivial-small changes (Table 3). Performance measures all showed good repeatability between trials. There appears to be some variability between HST1 and HST2 suggesting a familiarisation effect, evidenced by the number of participants capable of reaching the incremental protocol to exhaustion in HST2 compared to HST1.

Resting $\overline{T}_{re}$ and resting HR are commonly desribed to be indicative of heat adaptation (Garrett et al., 2014, 2019; Moss et al., 2020; Shaw et al., 2022), and showed good repeatability between prolonged repeated trials in hot conditions. Resting $\overline{T}_{re}$ showed a trivial difference between trials (−0.05, −1.11 to 1.11 °C) comporable to previous, shorter duration trials where differences of −0.1 °C (37.1 ± 0.4 to 37.1 ± 0.3 °C) (Che Jusoh et al., 2015), and −0.04 °C (−0.45 to 0.37 °C) (Mee et al., 2015) were reported in cyclists and runners, respectively. This is important as the range between the core temperature starting point and the ethical cut off point (Akerman et al., 2016; Shaw et al., 2022) was extremely similar between trials. Similarly, resting HR in the present study indicated a trivial difference in the second trial compared to the first (−3, −26 to 21 b min^{-1}), although Mee et al. (2015) reported a difference of 0 (−8 ±8 b min^{-1}) during a 30-min running heat tolerance test in 40 ± 0.5 °C and 39.9 ± 1.3%RH.

Reducing RPE can can potentially increase exercise capacity, enabling participants to tolerate prolonged steady-state exercise bouts (Tyler et al., 2016), and has been observed in heat acclimation studies previously (Moss et al., 2020; Shaw et al., 2022; Tyler et al., 2016). The current study did not identify any meaningful differences in mean RPE (0.3; −1.9, 2.1) nor specifically at 90-min (−1; 4, 5). Mean RPE data was comparable to previous findings of 0 (12.1 ± 1 to 12.1 ± 1) (Che Jusoh et al., 2015) and 0 (15.0 ± 1.7 to 15.0 ± 1.7) (Marino et al., 2002) during a 45-min cycling trial working at 55% $\dot{V}O_{2peak}$ in a wet bulb globe temperature (WBGT) of 26.7 ± 0.8 °C, and a 60-min cycling trial using participants' own bicycle mounted to an electromagnetic cycle trainer in 33 ± 0.7 °C and 63 ± 2%RH, respectively.

Given that these – and the majority of the remaining – variables show good repeatability and are in agreement with previous research suggests that confidence can be gained – in post-HA trials using this specific

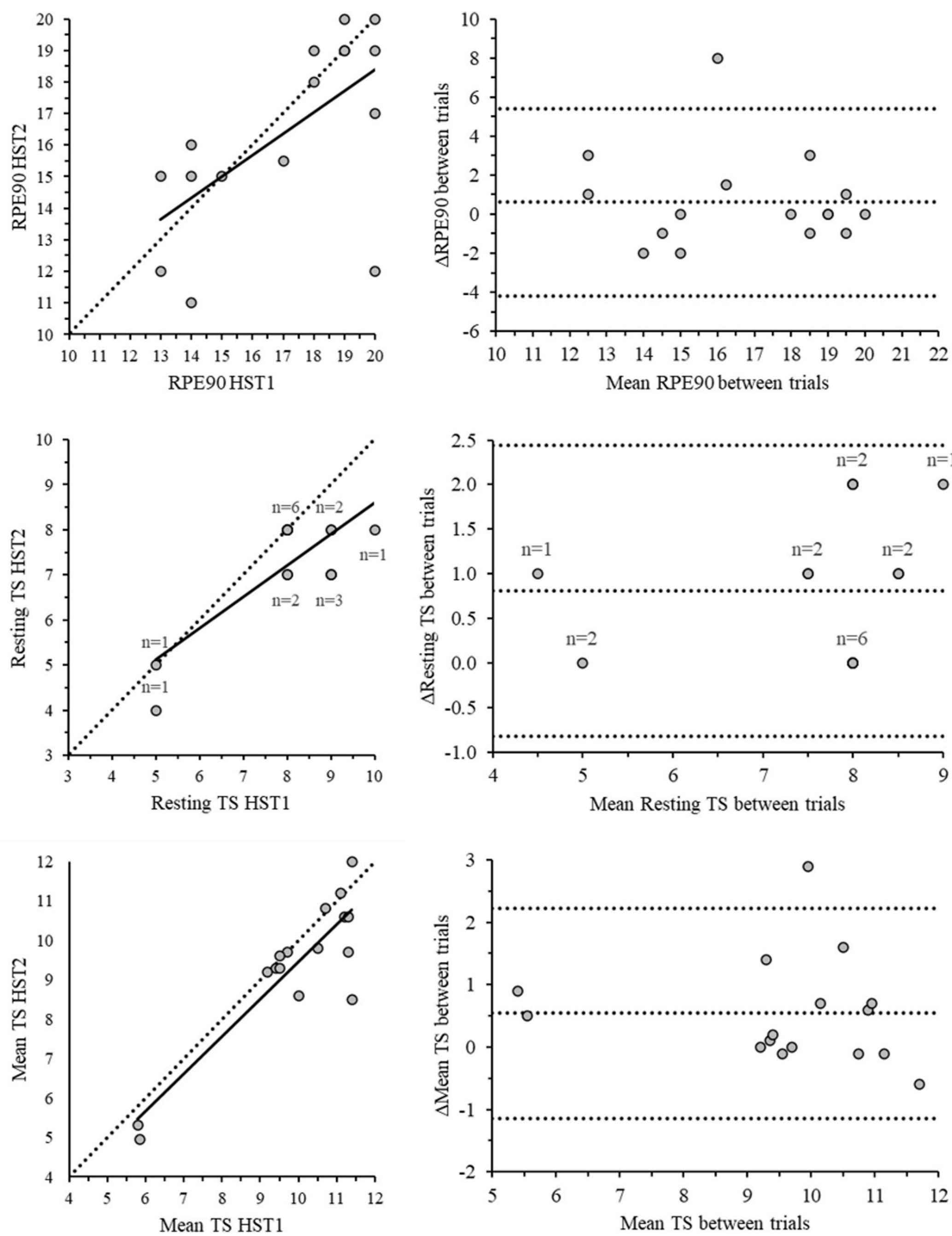

Fig. 4. Scatter plots (left) post HST 1 (x-axis) and HST 2 (y-axis), and Bland-Altman plots (right) with mean bias (middle dotted line) and 95% limits of agreement (outer dotted lines) for RPE90 (upper), resting TS (middle), and mean TS (lower). $N = 16$.

protocol and population – in the argument that a meaningful change has occurred in results where a HA intervention has been implemented and is not a result of equipment noise. However, some of the variables in present study did not indicate good repeatability and so this protocol cannot be regarded as wholly repeatable.

Heart rate at 90-min indicated a significant increase (+8, −36 to 20 b $\min^{-1}$; P = 0.047) in the second trial comapared to the first (Table 1). This could be explained as an example of the potential for greater

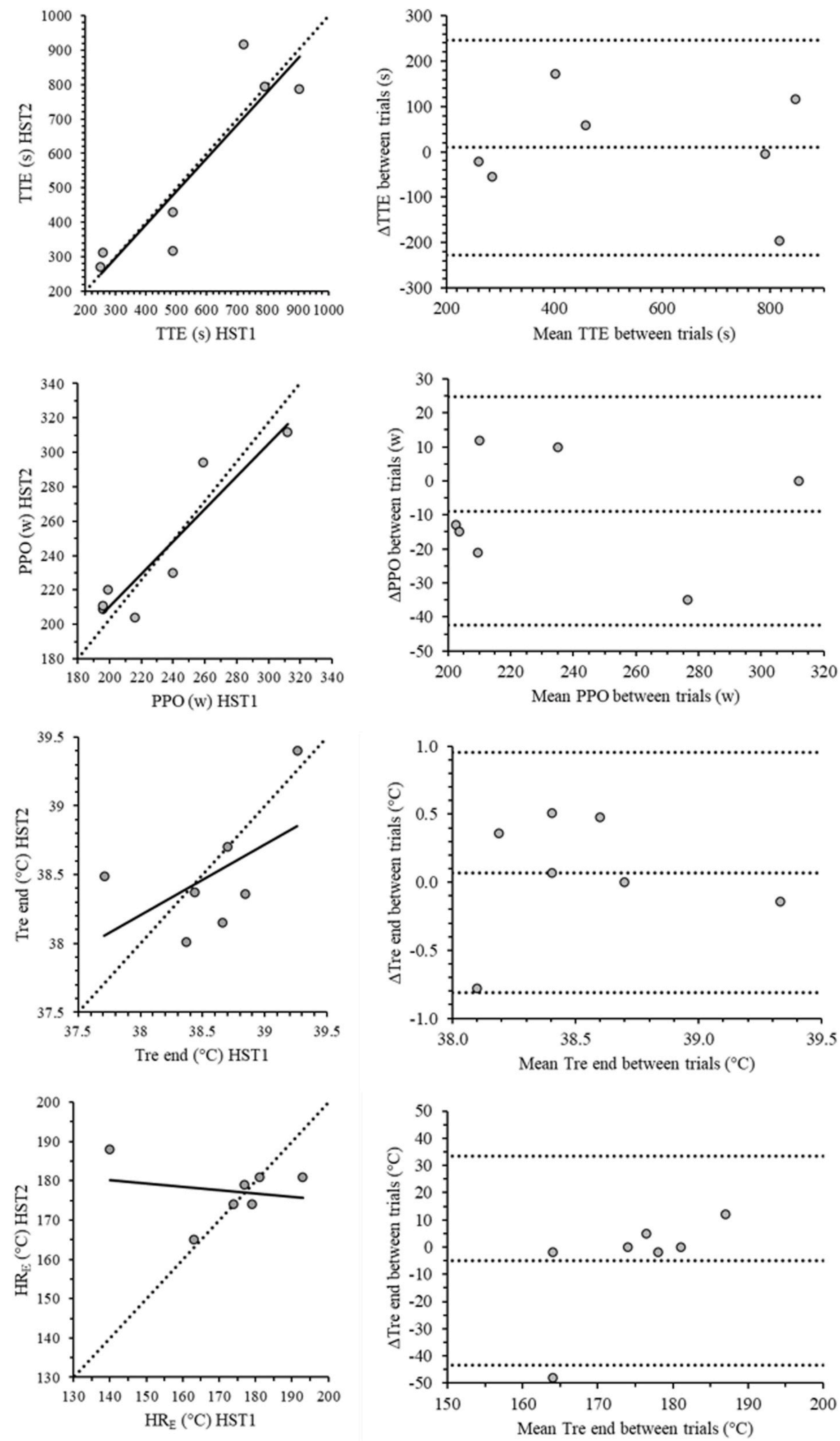

Fig. 5. Scatter plots (left) post HST 1 (x-axis) and HST 2 (y-axis), and Bland-Altman plots (right) with mean bias (middle dotted line) and 95% limits of agreement (outer dotted lines) for TTE (upper), PP (upper middle), end Tre (lower middle), and end HR (lower). $n = 7$.

variance when utilizing prolonged duration trials as in shorter trials HR measures have been shown to be consistent between trials (Che Jusoh et al., 2015; Marino et al., 2002; Mee et al., 2015; Tyler and Sunderland, 2008), with the exception of the third trial in the study by Marino et al. (2002) who reported a significant decrease in mean HR (−3, 170 ± 11 to 167 ± 11 b min^{-1}; P < 0.05) compared to the second. This finding of the current study is unlikely to be an acclimation effect as PV was consistent between trials (−0.2; −24.9, 25.2%), which has been described to stabilise HR during exercise-heat bouts reducing cardiovascular strain (Moss et al., 2020; Shaw et al., 2022; Tyler et al., 2016) which was not the case here.

Mean (−0.5; −1.1, 2.2; P = 0.023) and resting (−0.8; −0.8, 2; P = 0.001) TS (Fig. 4) were significantly decreased in the second trial compared to the first. Mee et al. (2015) reported a slight change in TS_{peak} (−0.1; −0.6, 0.7) however their protocol was much shorter (30-min) than the current studies' and their mode of exercise was running. The change indicated in mean TS in the current study could potentially be as a result of the concurrent significant redcution in $\overline{T}_{sk}$ (−0.29; −0.51, 1.10 °C), which contradicts prior research which has not previously reported this in repeated exercise bouts in hot conditions (Barnett and Maughan, 1993; Che Jusoh et al., 2015; Marino et al., 2002; McLellan et al., 1995; Mee et al., 2015; Schabort et al., 1998; Tyler and Sunderland, 2008). Therefore, this is unlikely to be resultant of an acclimation effect, but rather another possible example of potential greater variance consequent of prolonged duration protocols.

One problem associated with conducting research is adherence to controls by participants, particularly those inexperienced with experimental protocols and the necessity of strict adherence of preparation for performance. Hence, hydration measures – specifically resting $colour_u$ – may have contributed in addition to the above to the changes observed by increasing the amount of body water available at the beginning of the trial. By increasing hydration via water intake, and therefore increasing plasma volume, it is possible that by doing so increased physiological functionality (Garrett et al., 2014; Lorenzo et al., 2010; Racinais et al., 2012; Scoon et al., 2007) leading to the decrease in TS observed further leading to increased confidence to navigate the second trial better than the first.

Statistical analyses with the intended purpose of identifying repeatable methods for particular variables has been questioned in the past – namely Bland and Altman (2010). It is described that measures of correlation coefficients are "… totally inappropriate …" (Bland and Altman, 2010) when determining repeatability between repeated measures using the same method. However, in exercise-heat stress studies assessing the repeatability of measures during exercise-heat trials, ICCs are present (Mee et al., 2015; Shaw et al., 2022). Despite ICC having appropriate and valuable uses in particular research scenarios (Atkinson and Nevill, 1998; Mundel et al., 2023; Portney, 2020; Shrout and Fleiss, 1979; Vaz et al., 2013)Mundel et al. (2023) state that ICC – as well as *r* – should not be used to extrapolate to new individuals or between different measurements. For the current scenario, they provide an important indication of the distribution of data points within this study in a straight line, regardless of the direction of said line, therefore we have utilised these statistical tools in tandem with more informative illustrations of test re-test repeatability in the form of scatter plots and Bland-Altman plots, TEM and CoV (Mee et al., 2015).

The repeatability observed was good considering that a familiarisation trial was not included. A familiarisation trial is understood to be an important component to minimize any potential learning effect (Che Jusoh et al., 2015; Jeukendrup et al., 1996; Marino et al., 2002; Tyler and Sunderland, 2008) but is not always included in repeatability studies (e.g., Mee et al. (2015)). Despite the lack of familiarisation, Mee et al. (2015) determined good agreement between their variables. In the current study where the cohort was of mixed ability, it is possible that a learning effect was present despite no meaningful significant correlation between change in variables and fitness which could be explained by: i) changes in hydration prior to the second trial where mean resting col_u was significantly improved (P < 0.05); ii) participants applied themselves differently in the second trial compared to the first where the number of participants reaching the incremental protocol to exhaustion (7–11); or iii) participants percieved the environmental conditions to be less stressful in the second trial compared to the first where resting (P < 0.01) and mean TS (P < 0.05) were significantly reduced. Therefore, to reduce the impact of learning, if not remove completely, at least one familiarisation trial may be beneficial to improve confidence.

5. Limitations

This study was part of a larger HA study where the first trial was used as a familiarisation and the second trial was used as a pre-intervention baseline. Therefore, this study did not include a familiarisation trial per se prior to experimental testing due to time constraints with certain participants preparing for endurance competition in hot conditions. Future repeatability studies should include a familiarisation prior to the first experimental trial to further improve confidence. Urine and blood samples were analysed in duplicate and analysing in triplicate would increase accuracy of measurements. Power analysis was not performed on measures to determine the necessary sample size to accurately justify the size of the current cohort. However, accuracy could be improved with greater sample size.

6. Conclusion

Thirty-one of the 37 variables measured in this study showed favourable repeatability between repeated cycling HSTs in hot-humid conditions evidenced by moderate to excellent ICC ratings, trivial to small magnitudes of change (*d*), lower mean biases compared to their respective calculated TEMs, and statistical non-significance *despite* the lack of a familiarisation trial. The observed statistically significant changes in the present study could potentially be explained by this. Data suggests the presence of a familiarisation effect by way of more participants reaching the incremental protocol to exhaustion, and increased HR suggestive of greater work at the end of the endurance exercise phase.

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Author contributions

Jake Shaw is the primary researcher. Edward Cole was an investigator and internal reviewer. Andrew J. Simpson was an internal reviewer and contributor. Chris Tyler was involved in data collection and external review. Andrew T. Garrett was the project supervisor.

Declaration of competing interest

None of the authors had any competing interests in the conceptualization, conduction, analysis, or reporting of this manuscript.

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