Title:

The reliability of a heat acclimation state test prescribed from metabolic heat production intensities

Running Title:

Reliability of a heat acclimation state test

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Abstract

Acclimation state indicates an individual's phenotypic response to a thermally stressful environment, where changes in heat dissipation capacity are determined during a heat acclimation state test (HAST). Variations in thermoregulatory and sudomotor function are reported while exercising at intensities relative to maximal oxygen uptake. This inter-individual variation is not true when intensity is prescribed to elicit a fixed rate of metabolic heat production ($\dot{H}_{prod}$). This study investigated the reliability of peak T_{re} and two composite measures (sweat gain and sweat setpoint) derived from indices of thermosensitivity during a HAST prescribed from $\dot{H}_{prod}$ intensities.

Fourteen participants (mean ± SD; age 23 ± 3 years, stature 174 ± 7 cm, body mass 75.0 ± 9.4 kg, body surface area 1.9 ± 0.1 m^2, peak oxygen consumption [$\dot{V}O_{2peak}$] 3.49 ± 0.53 $L.min^{-1}$) completed a lactate threshold-$\dot{V}O_{2peak}$ test and two duplicate $\dot{H}_{prod}$ HASTs on a cycle ergometer. The HAST consisted of three, 30-minute periods of exercise at fixed $\dot{H}_{prod}$ intensities relative to body mass (3, 4.5 and 6 $W.kg^{-1}$), within hot dry conditions (44.7 ± 1.8°C and 18.1 ± 4.7 % relative humidity).

Peak T_{re} (38.20 ± 0.36 *vs* 38.16 ± 0.42°C, p = 0.54), sweat setpoint (36.76 ± 0.34 and 36.79 ± 0.38°C, p = 0.68) and sweat gain (0.37 ± 0.14 and 0.40 ± 0.18 $g.sec^{-1}.°C^{-1}$, p = 0.40) did not differ between HASTs. Typical error of measurement (TEM), coefficient variation (CV) and intra-class coefficient of correlation (ICC) were 0.19°C, 0.5% and 0.80 for peak T_{re}, 0.21°C, 0.6% and 0.65 for sweat setpoint and 0.09 $g.sec^{-1}.°C^{-1}$, 28% and 0.68 for sweat gain, respectively.

The use of fixed $\dot{H}_{prod}$ intensities relative to body mass is a reliable method for measuring T_{re} and ascertaining sweat setpoint during a HAST, whereas, sweat gain displays greater variability. A $\dot{H}_{prod}$ HAST appears sufficiently reliable for quantifying heat acclimation state, where TEM in peak T_{re} and sweat setpoint are small enough to identify physiologically meaningful improvements post intervention.

Key words:

Metabolic heat production, Thermosensitivity, Reliability, Heat acclimation state, Heat, Heat Acclimation

Abbreviations:

Body surface area (BSA)
Change in rectal temperature (ΔT_{re})
Coefficient variation (CV)
Heart rate (HR)
Heat acclimation state tests (HAST)
Intra-class correlation coefficient (ICC)
Limits of agreement (LOA)
Maximal oxygen uptake ($\dot{V}O_{2max}$)
Metabolic heat production ($\dot{H}_{prod}$)
Nude body mass (NBM)
Peak oxygen consumption ($\dot{V}O_{2peak}$)
Peak ratings of perceived exertion (RPE_{peak})
Peak thermal sensation (TSS_{peak})
Ratings of perceived exertion (RPE)
Rectal temperature (T_{re})
Relative humidity (RH)
Respiratory exchange ratios (RER)
Standard deviation (SD)
Standard error (SE)
Sweat rate ($\dot{m}_{sw}$)
Thermal sensation (TSS)
Typical error of measurement (TEM)
Urine osmolality (U_{osm})
Urine specific gravity (U_{sg})

1. Introduction

An individual's primary phenotypic response to a thermally stressful environment is indicated by acclimation state (Havenith and Middendorp, 1990). Acclimation state changes are determined during an incremental, sub-maximal heat acclimation state test (HAST). HASTs are predominantly used to pre-screen individuals to determine changes in heat dissipation capacity under fixed heat stress and evaluate the effectiveness of heat alleviating strategies, such as heat acclimation protocols. Previously, HASTs (Havenith and Middendorp, 1986, 1990), have identified two composite measures of sweat setpoint and sweat gain, derived from indices of thermosensitivity including, sudomotor (sweat rate [$\dot{m}_{sw}$]) function and thermoregulatory (rectal temperature [T_{re}]) responses to exercise. Figure 1 demonstrates the linear $\dot{m}_{sw}$-T_{re} relationship for slope to provide a measure of sweat gain (an assessment of sudomotor sensitivity), and the x-intercept represents the point of sweating above baseline, known as the sweat setpoint (Havenith and Middendorp, 1990). When comparing between-individuals, a greater magnitude in sweat gain and a lower sweat setpoint may permit effective regulation in body temperature within thermally challenging environments. Superior sweat gains and reductions in sweat setpoint after an intervention may indicate a greater change in heat acclimation state, demonstrating improved adaptive responses in thermoregulation and sudomotor function at rest and during exercise under thermal stress. Therefore, the improved body temperature regulation would reduce physiological strain and improve aerobic performance (Sawka et al., 2011).

Individuals with larger aerobic capacities are thought to be partially heat acclimated by exhibiting lower resting and exercising heart rate and core temperatures, and superior sudomotor capacities within hot-dry conditions (Havenith and Middendorp, 1986; Pandolf, 1998). However, these trained individuals exercise at greater intensity compared to untrained individuals, when exercising at similar percentages of maximal oxygen uptake (% $\dot{V}O_{2max}$), thus generating greater metabolic heat due to larger absolute oxygen uptake (Gagnon et al., 2008; Mora-Rodriguez et al., 2010). Consequently, Jay et al. (2011) demonstrated how large variations in $\dot{V}O_{2peak}$ between-groups matched for body mass and body surface area (BSA) may induce greater changes in T_{re} (ΔT_{re}) during relative intensity exercise. Conversely, improving exercise intensity prescriptions between-independent groups by using fixed rates of metabolic heat production ($\dot{H}_{prod}$) provided similar thermoregulatory responses between trained and untrained individuals (Jay et al., 2011). Previous HASTs have prescribed exercise intensities relative to $\dot{V}O_{2max}$, therefore, researchers might have observed greater T_{re} in individuals with a larger aerobic capacity (Jay et al., 2011), indicating a lower acclimation state, yet superior sweat gains, indicative of high acclimation state. Consequently, prescribing intensity of exercise using $\dot{H}_{prod}$ per unit mass ($W.kg^{-1}$) may reduce systematic bias in ΔT_{re}

between-independent groups of varying biophysical characteristics or fitness levels (Jay et al., 2011; Cramer and Jay, 2014). Thus, previous studies may be confounded by methodological limitations, including exercising at different $\dot{H}_{prod}$ as well as failure to control for body mass and BSA, which in turn generated type 1 errors. If previous HASTs were performed between-independent groups, pre to post intervention (i.e. heat acclimation), where alterations in body mass or training status may occur, the changes within T_{re} and local sweat rates may have been misinterpreted and at risk of being considered practically meaningful, instead of being attributed to the intervention itself and not a difference in exercise intensity.

A new HAST must prescribe $\dot{H}_{prod}$ intensities, which elicit reliable changes in core temperature and thermosensitivity, while minimising measurement error within biological variations and instrument noise (Atkinson and Nevill, 1998). A reliable test would also promote greater confidence in thermosensitive adaptations within- and between-groups, after acute and chronic heat acclimation protocols. Previous studies report coefficient variation (CV%) for sudomotor (11% local [Hayden et al., 2004] and 4.7% whole-body sweat rates [Brokenshire et al., 2009]), T_{re} (0.3% [Hayden, et al 2004], 0.6% [Brokenshire et al., 2009] and 0.34% [Mee et al., 2015]), and heart rate (3.9% [Hayden, et al 2004], 3% [Brokenshire et al., 2009] and 1% [Mee et al., 2015]) variables during running and cycling heat stress tests, respectively. However, it is difficult to make comparisons between studies of different magnitudes of heat stress, duration, mode and intensity of exercise.

While acknowledging the pioneering work of Havenith and Middendorp (1986, 1990), recent methodologies by Jay et al. (2011) and Cramer and Jay (2014), have included the prescription of $\dot{H}_{prod}$ ($W.kg^{-1}$) exercise intensities. This may enable accurate and reliable measures of core temperature and thermosensitivity between individuals to determine heat acclimation state, evaluate pre to post intervention efficacy between-independent groups and further support the proposal that $\dot{H}_{prod}$ may be an optimal method to prescribe heat acclimation (Gibson et al., 2015). However, the reliability of T_{re}, sweat gain and sweat setpoint is unknown while exercising at variable $\dot{H}_{prod}$ exercise intensities within a HAST, but is required for confident interpretations to be made regarding heat acclimation state. The aim of this study was to examine the reliability of a new HAST which prescribes $\dot{H}_{prod}$ intensities relative to body mass. It was hypothesised there would be agreement and no significant difference in (1) the T_{re} or composite measures of sweat gain and sweat setpoint, and (2) physiological and perceptual measures between both $\dot{H}_{prod}$ HASTs.

2. Methods

2.1 Participants

Fourteen active, moderately trained ($\dot{V}O_{2peak}$ >45 ml.kg^{-1}.min^{-1}) male participants (mean ± standard deviation [SD]; age 23 ± 3 years, stature 174 ± 7 cm, nude body mass [NBM] 75.0 ± 9.4 kg, BSA 1.9 ± 0.1 m^{2} and peak oxygen consumption [$\dot{V}O_{2peak}$] 3.49 ± 0.53 L.min^{-1}) volunteered and provided written informed consent for the study. The study was approved by the Institution Research Ethics and Governance Committee and conducted in accordance with the Declaration of Helsinki of 1975, as revised in 2008. Participants had not been exposed to hot conditions (>25°C) in the 3 months prior to the investigation. Participants abstained from caffeine, alcohol and prolonged strenuous activity for 24 hours prior to testing. They also refrained from food 2 hours before exercise and arrived in a euhydrated state indicated by a urine osmolality <700 mOsm.kg^{-1} and specific gravity <1.020 (Sawka et al., 2007).

2.2 Experimental design

After completing an incremental cycling lactate threshold (LT) to $\dot{V}O_{2peak}$ test, participants completed two $\dot{H}_{prod}$ HASTs, separated by 48 hours.

2.3 Measurements and equipment

All tests were completed on a cycle ergometer (Monark 620 Ergomedic, Varberg, Sweden). During each visit, participants produced fresh, mid-flow urine samples to determine hydration indices of urine osmolality (U_{osm}) and specific gravity (U_{sg}), assessed using a Pocket Pal-Osmo (Vitech Scientific, Ltd) and hand-held refractometer (Atago Co., Tokyo, Japan), respectively. Stature and NBM were measured using physician (Detecto Scale Company, USA) and weighing scales (Adam Equipment Co LTD., Milton Keynes, UK). T_{re} was assessed continuously and displayed on logging monitors (YSI, 4600 series, YSI, Hampshire, UK), using a single use rectal probe (449H, Henleys Medical, Hertfordshire, UK), placed ~10cm past the anal sphincter. Once heart rate (HR) monitors (Accurex+, Polar Electro, Oy, Kempele, Finland) were attached a 15-min rest period occurred to obtain baseline measures. During cycling exercise, HR, T_{re}, perceptual ratings of perceived exertion (RPE) (Borg, 1982) and thermal sensation (TSS) (Toner et al., 1986) were recorded at 5 minute increments.

Peak perceptual scores (RPE_{peak} and TSS_{peak}) were recorded during each HAST. Non-urine fluid loss was estimated to the nearest gram for each period of exercise using scales placed in the corner of the environmental chamber. Difference between pre and post exercise, towel-dried NBM determined non-urine fluid loss, which was corrected for urine output (zero incidences), but not for insensible respiratory water and metabolic losses, which was assumed to be similar between tests.

2.4 Peak oxygen uptake test

$\dot{V}O_{2peak}$ was determined during an incremental LT-$\dot{V}O_{2peak}$ test within temperate conditions (21.6 ± 0.9°C and 30.8 ± 3.2% relative humidity [RH]). The test started with a 5 min warm-up at 95 W and increased by 24 W every 3 min, with blood samples taken within the final 30 s of each 3 min stage. Blood samples were collected into lithium-heparin coated microvette tubes and analysed using an automated analyser (YSI 2300 Plus, Yellow Springs Instruments, Ohio, USA). This was calibrated immediately before each test using the manufacturers 5 $mmol.L^{-1}$ standard, set to self-calibrate every 45 min and verified after each test using the same manufacturer standard (YSI 2427; CV = 5.5%). When LT turnpoint was reached, determined by a sudden and sustained increase in blood lactate concentration around 2-5 $mmol.L^{-1}$ (Bourdon, 2000), no further samples were taken and intensity was increased 24 $W.min^{-1}$ until volitional exhaustion (Hayes, et al., 2014). Expired air was collected to measure oxygen uptake using open-circuit spirometry for approximately 45 s during the final minute of each stage. Pulmonary gases (oxygen [O_2] and carbon dioxide [CO_2]), temperature and expired air volume were sampled using a Servomex 4100 xentra gas analyser (Servomex International Ltd, Crowborough, UK; CV; O_2 = 1.5%; CO_2 = 1.9%). A two-point calibration occurred using nitrogen and a mixture of gases of known O_2 and CO_2 quantities (BOC, UK) prior to each test. Both HR and RPE were continuously monitored throughout and recorded within the final 15 s of each stage.

2.5 Heat acclimation state test (HAST) protocol

HASTs were completed within an environmental chamber (TISS, Hampshire, UK) under hot-dry conditions (44.7 ± 1.8°C and 18.1 ± 4.7% RH, 29.9 ± 1.1°C wet bulb globe temperature). Participants were aware they could stop exercising at any time and were removed from the chamber if T_{re} reached 39.7°C (zero incidences). The $\dot{H}_{prod}$ HAST simulated the protocol of Havenith and Middendorp (1986), consisting of three 30 min exercise blocks. The intensity across blocks was increased by a fixed rate of $\dot{H}_{prod}$ relative to body mass (3, 4.5 and 6 $W.kg^{-1}$) and set as an external mechanical power output (W). Each 30 min block was designed to enable T_{re} to reach steady state and was separated by a short (<3 min) break period, where NBM and non-urine fluid loss were recorded. The relationship between $\dot{m}_{sw}$-T_{re} was modelled using a linear regression (method of least squares). The x-intercept and slope represent a T_{re} setpoint for the onset of sweating and a sweat gain respectively (Figure 1). The ΔT_{re} was calculated for each block of exercise.

2.6 Metabolic heat production intensities

In accordance with the recommendations of Jay et al. (2011) and Cramer and Jay (2014), $\dot{H}_{prod}$ was prescribed as an external mechanical power output (W). Metabolic energy expenditure (*M*) was estimated from known values of O_2 uptake and respiratory exchange ratios (RER) during sub-maximal cycling within the LT test, using the equation of Nishi (1981):

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

where: the caloric equivalents per litre of oxygen 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 (BM) to obtain a relative measure ($W.kg^{-1}$):

$$\dot{H}_{prod} = (M - \mathrm{W}) / \mathrm{BM}$$

2.7 Statistical analyses

All data are reported as mean ± standard deviation (SD). Data were assessed for normality and sphericity prior to statistical analysis. Between-test comparisons of sweat gain, sweat setpoint and physiological responses during both HASTs were analysed using a paired samples *t-test*. Non-parametric datasets, including RPE_{peak} and TSS_{peak} were analysed using a Wilcoxon signed-rank test with Bonferroni correction applied. A composite battery of reliability statistics including relative (Pearson's correlation coefficients and ICC) and absolute (CV and limits of agreement) measures were implemented within this study to improve the scientific robustness when evaluating thermosensitive measures (Atkinson and Nevill, 1998; James et al., 2014). Standard (SEM) and typical error of measurement (TEM) were calculated from the SD of the mean difference between the two $\dot{H}_{prod}$ HAST, $\sqrt{1}$ then subtracted by the ICC (SEM, Atkinson and Nevill, 1998) or divided by $\sqrt{2}$ (TEM, Hopkins, 2000), and expressed as a mean CV (%). Meaningful differences between related samples during both HASTs were evaluated using Cohen's *d* and confidence intervals (CI) (Lakens, 2013). Effect size was categorised as small (0.2), medium (0.5) and large (0.8) (Cohen, 1988). Pearson product moment correlation coefficient and ICC were calculated and categorised as small (<0.3), moderate (0.3-0.6) and large (>0.6). Bias and 95% limits of agreement (LOA) were determined from Bland-Altman plots, which investigated systematic and random error trends. Adjusted r^2 and standard error (SE) of the slope were identified within the $\dot{m}_{sw}$-T_{re} linear regression relationship. Statistical significance was accepted as $P \leq 0.05$. Data were analysed using SPSS (version 20.0).

3. Results

3.1 Physical characteristics

All participants arrived in a similar physiological state for both HASTs, with no difference in resting HR (67 ± 12 and 66 ± 12 b.min^{-1}, t = 0.47, p = 0.64), T_{re} (37.28 ± 0.32 and 37.27 ± 0.31ºC, t = 0.07, p = 0.95), U_{osm} (300 ± 247 and 387 ± 212 mOsm.kg^{-1}, t = 0.38, p = 0.71), U_{sg} (1.008 ± 0.007 and 1.010 ± 0.007, t = 0.04, p = 0.97) or pre exercise NBM (75.0 ± 9.8 and 75.0 ± 9.5 kg, t = 0.10, p = 0.92).

3.2 Criteria of heat acclimation state

No difference was found in the goodness of fit of the linear model through the sets of $\dot{m}_{sw}$ and T_{re} data with adjusted r^2 and SE of 0.89 and 9.1% for HAST 1, and 0.85 and 13.1% for HAST 2. No differences were found in T_{repeak} (38.20 ± 0.36 *vs* 38.16 ± 0.42°C, t = 0.63, p = 0.54) or pre to post ΔT_{re} (0.93 ± 0.35 *vs* 0.88 ± 0.32°C, t = 0.77, p = 0.45) within both HASTs (Table 1). Bland-Altman plots presented in Figure 2 display bias (± 95% LoA) for T_{repeak} (0.04 ± 0.53°C) and pre to post ΔT_{re} (0.05 ± 0.47°C). Sweat setpoint (36.76 ± 0.34 *vs* 36.79 ± 0.38°C, t = 0.43, p = 0.68) and sweat gain (0.37 ± 0.14 *vs* 0.40 ± 0.18 g.sec^{-1}.°C^{-1}, t = 0.87, p = 0.40) did not differ between the HASTs. Cohen's d (95% CI) was 0.08 (-0.15, 0.21) and 0.19 (-0.05, 0.11) for sweat setpoint and sweat gain, respectively. TEM (CV) was 0.21°C (0.6%) and 0.09 g.sec^{-1}.°C^{-1} (28%) for sweat setpoint and sweat gain, respectively. Large correlations were observed for sweat gain (r = 0.69 and ICC = 0.72) and sweat setpoint (r = 0.62 and ICC = 0.65). Bland-Altman plots presented in Figure 3 display bias (± 95% LoA) for sweat setpoint (0.03 ± 0.60°C) and sweat gain (0.03 ± 0.26 g.sec^{-1}.°C^{-1}), respectively. Plotted regression lines through data points within Figure 3 display a slope close to zero (-0.03) and no correlation coefficient (r = 0.03) in sweat setpoint, whereas, a statistically significant ($p < 0.01$), small correlation coefficient (r = 0.29) and a larger slope (-0.27), may therefore display the presence of heteroscedasticity in sweat gain.

3.3 Sudomotor function, thermoregulatory and cardiovascular measures

No differences were found between the HASTs for total non-urine fluid losses (t = 0.17, p = 0.87) or HR_{peak} (t = 1.76, p = 0.10) (Table 1). Nor were differences found between the HASTs for mean average T_{re}, non-urine fluid loss or HR between block 1, 2 and 3. HAST data during individual blocks are displayed within Table 2.

3.4 Perceptual measures

RPE_{peak} (14 ± 2 *vs* 14 ± 3, Z = -0.318, p = 0.75) and TSS_{peak} (7 ± 1 *vs* 7 ± 1, Z = -1.342, p = 0.18) did not differ between HASTs, and presented low variability (TEM [CV], 1 [7.3%] and 0 [4.9%]) and large correlations (r = 0.85 and 0.76), respectively.

4. Discussion

4.1 Overview

The aim of this study was to determine the reliability of a HAST, prescribed from fixed rates of $\dot{H}_{prod}$ relative to body mass. The main findings from the current study present small bias, acceptable and strong correlations between the repeated HASTs. This was apparent within the mean average T_{re}, ΔT_{re} and composite measures of sweat gain and sweat setpoint, which determine heat acclimation state. Traditional markers of heat acclimation adaptation, such as rectal temperature, heart rate and non-urine fluid loss presented similar findings. Finally, T_{re} and sweat setpoint provides more accurate and reliable measures, compared to sweat gain, displayed by low within-participant variability and typical error.

4.2 Core temperature

As thermosensitive measures are a control property of T_{re} and not the prescription of fixed $\dot{H}_{prod}$ during exercise, the reliability of peak, pre to post changes and mean average T_{re} were assessed. T_{repeak} and pre to post ΔT_{re} presented no statistically significant test re-test difference ($p = 0.54$ and $p = 0.45$), large correlation (ICC = 0.80 and 0.74), low TEM (0.19°C and 0.17°C) and a CV of 0.5% and 24%, respectively. T_{repeak} was found to be greater than those reported in other heat stress tests (0.13°C [0.34%] and 0.93, Mee et al., 2015), while pre to post ΔT_{re} presented moderate variation when expressed as a percentage of the mean score. Mean average T_{re} during each 30-min block of the HAST which contributes to the derivative calculation of sweat setpoint, presented small levels of within-participant variation for block 1 (TEM [CV] = 0.16°C [0.4%]), block 2 (0.14°C [0.4%]) and block 3 (0.14°C [0.4%]), and large correlations (ICC = 0.75, 0.81 and 0.86, respectively) between HASTs. These data are in agreement with Hayden et al. (2004), who reported TEM (CV) in T_{re} of 0.20°C (0.3%) during cycling at relative intensities for 60 minutes and Brokenshire et al. (2009), who reported aural temperatures of 0.10°C (0.6%) during cycling at relative intensities for three 20 minute blocks separated by a rest period, both within hot and humid conditions (36°C and 60% RH, and 35°C and 46% RH, respectively). However, all the aforementioned studies set relative or generic intensities which present various biophysical complications (Cramer and Jay, 2014) and validity issues if tested between-independent groups or post interventions, where training adaptations and body mass changes are expected. Therefore, prescribing fixed $\dot{H}_{prod}$ intensities may ensure fair comparisons in thermosensitive criteria across independent groups, irrespective of intervention, training status or anthropometric characteristics. Furthermore, this thermoregulatory data may aid the interpretations of meaningful changes and evaluate the efficacy of an intervention, if repeated after a heat acclimation protocol.

4.3 Sweat setpoint

Sweat setpoint demonstrated no statistically significant test re-test difference ($p = 0.68$), alongside a low TEM (0.21°C) and a CV of 0.6%, which fall within predefined acceptable and reliable limits (Atkinson and Nevill, 1998). Brengelmann et al. (1994) suggested the reproducibility and therefore the smallest worthwhile change in sweat setpoint to be less than 0.10°C. However, Brengelmann et al. (1994) determined reproducibility by externally cooling then heating skin temperature, over extended periods on multiple occasions to identify difference in two sweat thresholds, whilst using live sweat rate data recordings. More recently, Cheuvront et al. (2009) identified mean range in sweat setpoint of +0.50 to -0.25°C, and sweat gain of +0.15 to -0.30 $mg.cm^{-2}.min^{-1}.°C^{-1}$, due to various physiological effects such as dehydration, heat acclimation, sleep deprivation and exercise intensity. Although the TEM in the present study is higher than that reported (>0.10°C) within Brengelmann et al. (1994), there are vast differences within study methods. Nonetheless, the TEM obtained in the present study does fall well within the LOA found by Cheuvront et al. (2009). Consequently, it is within reason to suggest that the sweat setpoint in the present study demonstrates an acceptable standard of reliability for a HAST that prescribes $\dot{H}_{prod}$ intensities set relative to body mass. Previous studies investigating sweat setpoint reductions after intervention strategies have reported improvements ranging from -0.1 to -0.5°C (Gonzalez et al., 1974; Nadel et al., 1974; Roberts et al., 1977; Havenith and Middendorp, 1986). Although these tests were used to evaluate the changes in sweat setpoint post intervention, they were undertaken using relative intensities and therefore may present inherent validity issues if tested between-independent groups of varying fitness levels and biophysical characteristics pre to post interventions. The large between-study differences are attributed to the nature and design of the intervention, i.e. training in temperate conditions (Nadel et al., 1974; Roberts et al., 1977), passive heat exposure (Nadel et al., 1974; Roberts et al., 1977) or a combination of training and heat acclimation (Nadel et al., 1974; Gonzalez et al., 1974; Roberts et al., 1977; Havenith and Middendorp, 1986). Furthermore, it may be suggested that for improvements in sweat setpoint post intervention to be meaningful they must be greater than the TEM (0.21°C) in this study when prescribing $\dot{H}_{prod}$ intensities within- or between-groups. The intra-individual variability within chronic adaptations in thermosensitive variables, which determine heat acclimation state (signal) are largely dependent on measurement variability (signal to noise ratio) (Cheuvront et al., 2009). It has been observed that sweat onset is a more favourable, sensitive variable when measuring changes in thermosensitivity, as displayed within this study and by a lower within-individual CV (9.6%) compared to sweat rate (22.3%) within Kenefick et al. (2012). However, the sensitivity of the HAST with $\dot{H}_{prod}$ intensities after an intervention such as heat acclimation is still required.

4.4 Sweat gain

To the authors' knowledge no previous studies report reliability data on sudomotor sensitivity, defined as the slope of the linear $\dot{m}_{sw}$-T_{re} relationship (Cheuvront et al., 2009). As opposed to sweat setpoint results, sweat gain presented poorer reliability ($p = 0.40$, TEM = 0.09 $g.sec^{-1}.°C^{-1}$, CV = 28% and ICC = 0.72). These results are contributed by the sensitivity of sweat gland activity and sweat output (Kondo et al., 2001), and are dependent upon the responses towards the ΔT_{re} while exercising at low and moderate $\dot{H}_{prod}$ intensities. The variability in sudomotor function is displayed within the non-urine fluid loss comparisons in mean bias and CV during low intensity exercise within block 1 (22 mL and 22%) and moderate intensity exercise within block 3 (4 mL and 10%). The appearance of heteroscedasticity and larger variations (CV = 28%) observed in the second criterion of heat acclimation state can also be attributed to the inter-individual variances in the ΔT_{re} from pre to post exercise. The two indices of thermosensitivity that contribute to the calculation of sweat gain, presented smaller variability of 8.5% for total non-urine fluid loss, yet the ΔT_{re} presented far higher variations of 24% between HASTs. Furthermore, it is evident that the reliability of T_{re} and non-urine fluid loss measures during each 30-min block improves as exercise intensity increases within the HAST, as opposed to the overall ΔT_{re} from pre to post (Table 2). Therefore, the greater variability within sweat gain, appears to be a consequence of the sources of error associated with measuring T_{re} pre to post exercise and the variability in non-urine fluid loss during earlier stages of the HAST, as the associated slow responses to thermal transients whilst using rectal temperatures (Sawka and Wenger, 1988) are presumed similar within-individuals between-trials.

4.5 Mean average responses to incremental metabolic heat production intensities

It is recognised that large differences within study methodologies prevent direct comparisons, however, TEM (CV) for mean average HR during blocks 1, 2 and 3 were; 5 (4.9%), 6 (5.5%) and 8 $b.min^{-1}$ (6.5%), are greater than those previously reported during submaximal exercise in temperate (6.1%, Wilmore et al., 1998), and hot (3.9% and 3%, Hayden et al., 2004 and Brokenshire et al., 2009, respectively) conditions. Moreover, HR_{peak} (10 $b.min^{-1}$ [7.1%]) was also greater than those reported (2 $b.min^{-1}$ [1%]) within a running heat tolerance test by Mee et al. (2015). This study presents low within-participant variability in physiological and perceptual responses towards incremental exercise intensities, set at a fixed rate of $\dot{H}_{prod}$ relative to body mass, under hot-dry conditions. The peak measures of T_{re}, HR, RPE and TSS, and overall non-urine fluid loss, known to change with interventions such as heat acclimation, presented high correlation coefficients (>0.7) and low CV (<10%). Moreover, this was also observed for mean average T_{re}, HR and non-urine fluid loss during the individual blocks of increasing exercise intensities of 3, 4.5 and 6 $W.kg^{-1}$ within the HAST. However, it must be acknowledged that some measures were more variable at lower intensities such as fluid loss, whereas mean average T_{re} remained consistent throughout. When testing within- or between-individuals, these responses would not be confounded by differences in protocol and therefore may be useful for practitioners investigating pre to post intervention changes in physiological and perceptual adaptations when changes in body mass or training status are expected.

4.6 Inter-individual variances

Results from this study highlight a large range of inter-individual heat acclimation states, contributed by varied sudomotor function and thermoregulatory measures within the sample tested. Sweat setpoint ranged from 36.27 to 37.48°C, and sweat gain from 0.16 to 0.80 $g.sec^{-1}.°C^{-1}$, which reflects inter-individual variability in thermosensitivity within a homogenous sample of similar fitness, age and anthropometric characteristics. The authors suggest these ranges in sweat setpoint and sweat gain may define (± 2.5%) low (37.5°C and 0.20 $g.sec^{-1}.°C^{-1}$) and high (36.3°C and 0.80 $g.sec^{-1}.°C^{-1}$) acclimation states within similar populations. Therefore, such variances in HAST criteria and physiological responses, suggest exercise intensity when prescribed at a fixed rate of $\dot{H}_{prod}$ and expressed relative to body mass, underpins and provides an equal physiological response to thermal stress without systematic differences, as similarly found by Cramer and Jay (2014).

4.7 Limitations

The linear regression model to determine thermosensitivity and heat acclimation state criteria only includes three data points. Therefore, future studies should consider increasing the number of exercise bouts and associated data points to improve the robustness of heat acclimation state determination. In addition to adopting new methods of technical absorbent material or live ventilated capsule monitoring to determine local sweat rate. Furthermore, SE within both of the linear regression models presented larger variability in HAST 2 compared to HAST 1, which may have contributed to the variability observed in ΔT_{re} and sweat gain measures. Variable sensitivity responses of sweat gland activity and output may inhibit the determination of sweat gain and sweat setpoint (Cheuvront et al., 2009), whilst using the linear regression model within this study. It has been observed that a late phase of sweating onset causes a biphasic and flatter slope, which may display lower values for sweat setpoint, therefore warranting continuous monitoring of sudomotor function to better quantify thermosensitivity. Although the environmental conditions within this study appear uncompensable, where those with a larger aerobic capacity have distinct physiological advantages during exercise (Pandolf, 1979; Cheung and McLellan, 1998; Selkirk and McLellan, 2001; Selkirk et al., 2008), the population were of similar aerobic fitness and comparisons were made within-individuals, where results presented highly reliable measures. Furthermore, no correlations appear between fitness level and sweat setpoint ($r = -0.1$) or sweat gain ($r = 0.2$).

5. Conclusion

This study is the first to assess the reliability of a HAST which prescribes exercise intensity at a fixed rate of $\dot{H}_{prod}$ relative to body mass. The $\dot{H}_{prod}$ HAST appears reliable, presenting low typical error and good agreement for core temperature and sweat setpoint. Sweat gain however, shows far greater between-test variability which may further warrant the sole use of sweat setpoint when heat acclimation state is to be determined. This study also demonstrates the reliability and variability displayed within physiological and perceptual responses towards exercise of varying $\dot{H}_{prod}$ intensities, although future studies are required to test the $\dot{H}_{prod}$ HAST post intervention. While investigating sweat setpoint and thermoregulatory improvements after heat acclimation protocols, experimenters can be confident that an observed change above 0.21°C and 0.19°C, respectively, are a result of the intervention and not error. This is applicable when healthy, moderately trained populations exercise within hot-dry conditions during a HAST, that is prescribed at a fixed rate of $\dot{H}_{prod}$ relative to body mass.

6. References

Atkinson, G. and Nevill, A.M. (1998). Statistical methods for assessing measurement error (reliability) in variables relevant to sports medicine. *Sports Medicine* 26, 217-238.

Borg, G. (1982). Psychophysical bases of perceived exertion. *Medicine and Science in Sports and Exercise,* 14 (5), pp. 377-381.

Bourdon, P. (2000). Blood lactate transition thresholds: concepts and controversies. In: Gore CJ, ed. *Physiological Tests for Elite Athletes*. Champaign, IL: Human Kinetics: 50–65.

Brengelmann, G. L., Savage, M. V., & Avery, D. H. (1994). Reproducibility of core temperature threshold for sweating onset in humans. *Journal of Applied Physiology*, *77*(4), 1671-1677.

Brokenshire, C. S., Armstrong, N., & Williams, C. A. (2009). The reliability of adolescent thermoregulatory responses during a heat acclimation protocol. *Journal of sports science & medicine*, *8* (4), 689.

Cheung, S. S. and McLellan, T. M. (1998). Heat acclimation, aerobic fitness, and hydration effects on tolerance during uncompensable heat stress. *Journal of Applied Physiology*, *84* (5), 1731-1739.

Cheuvront, S. N., Bearden, S. E., Kenefick, R. W., Ely, B. R., DeGroot, D. W., Sawka, M. N., & Montain, S. J. (2009). A simple and valid method to determine thermoregulatory sweating threshold and sensitivity. *Journal of Applied Physiology*, *107*(1), 69-75.

Cramer, M. N., and Jay, O. (2014). Selecting the correct exercise intensity for unbiased comparisons of thermoregulatory responses between groups of different mass and surface area. *Journal of Applied Physiology*, *116*(9), 1123-1132.

Cohen, J. (1988). Statistical Power Analysis for the Behavioral Sciences. Hillsdale, NJ: Lawrence Erlbaum Associates.

Gagnon, D., Jay, O., Lemire, B., & Kenny, G. P. (2008). Sex-related differences in evaporative heat loss: the importance of metabolic heat production. *European journal of applied physiology*, *104*(5), 821-829.

Gibson, O. R., Mee, J. A., Tuttle, J. A., Taylor, L., Watt, P. W., and Maxwell, N. S. (2015a). Isothermic and fixed intensity heat acclimation methods induce similar heat adaptation following short and long-term timescales. Journal of Thermal Biology, 49, 55–65.

Gonzalez, R. R., Pandolf, K. B., and Gagge, A. P. (1974). Heat acclimation and decline in sweating during humidity transients. *Journal of applied physiology*,*36* (4), 419.

Hayden, G., Milne, H. C., Patterson, M. J., and Nimmo, M. A. (2004). The reproducibility of closed-pouch sweat collection and thermoregulatory responses to exercise–heat stress. *European journal of applied physiology*, *91*(5-6), 748-751.

Hayes, M., Castle, P. C., Ross, E. Z., and Maxwell, N. S. (2014). The influence of hot humid and hot dry environments on intermittent-sprint exercise performance. *International journal of sports physiology and performance*, *9*(3), 387-396.

Havenith, G., and van Middendorp, H. (1986). Determination of the individual state of acclimatization. Report Institute for Perception IZF 1986-27.

Havenith, G., and van Middendorp, H. (1990). The relative influence of physical fitness, acclimatization state, anthropometric measures and gender on individual reactions to heat stress. *European journal of applied physiology and occupational physiology*, *61* (5-6), 419-427.

Hopkins, W.G. (2000) Measures of Reliability in Sports Medicine and Science. *Sports Medicine* 30, 1-15.

James, C. A., Richardson, A. J., Watt, P. W., and Maxwell, N. S. (2014). Reliability and validity of skin temperature measurement by telemetry thermistors and a thermal camera during exercise in the heat. *Journal of thermal biology*, *45*, 141-149.

Jay, O., Bain, A. R., Deren, T. M., Sacheli, M., and Cramer, M. N. (2011). Large differences in peak oxygen uptake do not independently alter changes in core temperature and sweating during exercise. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *301* (3), R832-R841.

Kenefick, R. W., Cheuvront, S. N., Elliott, L. D., Ely, B. R., and Sawka, M. N. (2012). Biological and analytical variation of the human sweating response: implications for study design and analysis. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *302*(2), R252-R258.

Kondo, N., Shibasaki, M., Aoki, K., Koga, S., Inoue, Y., and Crandall, C. G. (2001). Function of human eccrine sweat glands during dynamic exercise and passive heat stress. *Journal of Applied Physiology*, *90* (5), 1877-1881.

Lakens, D., (2013). Calculating and reporting effect sizes to facilitate cumulative science: a practical primer for t-tests and ANOVAs. *Frontiers in psychology*, 4, 863-874.

Mee, J. A., Doust, J., and Maxwell, N. S. (2015). Repeatability of a running heat tolerance test. *Journal of thermal biology*, *49*, 91-97.

Mora-Rodriguez, R., Del Coso, J., Hamouti, N., Estevez, E., and Ortega, J. F. (2010). Aerobically trained individuals have greater increases in rectal temperature than untrained ones during exercise in the heat at similar relative intensities. *European journal of applied physiology*, *109*(5), 973-981.

Nadel, E. R., Pandolf, K. B., Roberts, M. F., and Stolwijk, J. A. (1974). Mechanisms of thermal acclimation to exercise and heat. *Journal of Applied Physiology*, *37* (4), 515-520.

Nishi, Y. (1981). Measurement of thermal balance in man. In: *Bioengineering, Thermal Physiology and Comfort*, edited by Cena K and Clark J. New York, NY: Elsevier, p. 29–39.

Pandolf, K. B. (1979). Effects of physical training and cardiorespiratory physical fitness on exercise-heat tolerance: recent observations. *Medicine and Science in Sports* 11: 60–65.

Pandolf, K. B. (1998). Time course of heat acclimation and its decay. *International journal of sports medicine*, *19*, S157-60.

Roberts, M. F., Wenger, C. B., Stolwijk, J. A. J., and Nadel, E. R. (1977). Skin blood flow and sweating changes following exercise training and heat acclimation. *J Appl Physiol* 43: 133–137.

Sawka, M. N., Burke, L. M., Eichner, E. R., Maughan, R. J., Montain, S. J., and Stachenfeld, N. S. (2007). American College of Sports Medicine position stand. Exercise and fluid replacement. *Medicine and science in sports and exercise*, *39* (2), 377-390.

Sawka, M. N., and Wenger, C. B. (1988). *Physiological responses to acute exercise-heat stress* (No. USARIEM-M-14/88). ARMY RESEARCH INST OF ENVIRONMENTAL MEDICINE NATICK MA.

Sawka, M. N., Leon, L. R., Montain, S. J., and Sonna, L. A. (2011). Integrated physiological mechanisms of exercise performance, adaptation, and maladaptation to heat stress. *Comprehensive Physiology*. 1:1883-1928.

Selkirk, G. A., and McLellan, T. M. (2001). Influence of aerobic fitness and body fatness on tolerance to uncompensable heat stress. *Journal of Applied Physiology*, *91*(5), 2055-2063.

Selkirk, G. A., McLellan, T. M., Wright, H. E. and Rhind, S. G. (2008). Mild endotoxemia, NF-κB translocation, and cytokine increase during exertional heat stress in trained and untrained individuals. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *295* (2), R611-R623.

Toner, M. M., Drolet, L. L., and Pandolf, K. B. (1986). Perceptual and physiological responses during exercise in cool and cold water. *Perceptual and motor skills*, *62* (1), 211-220.

Wilmore, J.H., Stanforth, P.R., Turley, K.R., Gagnon, J., Daw, E.W., Leon, A.S., Rao, D.C., Skinner, J.S. and Bouchard, C. (1998). Reproducibility of cardiovascular, respiratory, and metabolic responses to submaximal exercise: The HERITAGE Family Study. *Medicine and science in sports and exercise*, *30*(2), 259-265.

Vitae

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Ash completed his BSc (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 MPhil 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).

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Jeanne’s research interest is exercise tolerance with a particular focus on the power - endurance relationship, exercise intensity domains, training adaptations and neuromuscular fatigue. She has a publication record of over 30 peer reviewed papers in the area whether investigating exercise tolerance in cycle ergometry or in swimming. Jeanne's applied work in swimming physiology is internationally recognized with regular invitations to speak at several overseas conferences.

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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 MPhil/PhD 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 also leads the Environmental Extremes Laboratory which sits within the Centre for Sport and Exercise Science and Medicine.