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# Repeatability of a running heat tolerance test

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# ABSTRACT

At present there is no standardised heat tolerance test (HTT) procedure adopting a running mode of exercise. Current HTTs may misdiagnose a runner's susceptibility to a hyperthermic state due to differences in exercise intensity. The current study aimed to establish the repeatability of a practical running test to evaluate individual's ability to tolerate exercise heat stress. Sixteen (8M, 8F) participants performed the running HTT (RHTT) (30 min, 9 km  $h^{-1}$ , 2% elevation) on two separate occasions in a hot environment (40 °C and 40% relative humidity). There were no differences in peak rectal temperature (RHTT1:  $38.82 \pm 0.47$  °C, RHTT2:  $38.86 \pm 0.49$  °C, Intra-class correlation coefficient (ICC)=0.93, typical error of measure (TEM)=0.13 °C), peak skin temperature (RHTT1: 38.12 ± 0.45, RHTT2: 38.11 ± 0.45 °C, ICC=0.79, TEM=0.30 °C), peak heart rate (RHTT1:  $182 \pm 15$  beats min<sup>-1</sup>, RHTT2:  $183 \pm 15$  beats min<sup>-1</sup>, ICC=0.99, TEM=2 beats min<sup>-1</sup>), nor sweat rate  $(1721 \pm 675 \text{ g h}^{-1}, 1716 \pm 745 \text{ g h}^{-1}, \text{ ICC}=0.95, \text{ ICC}=0.95,$ TEM = 162 g h<sup>-1</sup>) between RHTT1 and RHTT2 (p > 0.05). Results demonstrate good agreement, strong correlations and small differences between repeated trials, and the TEM values suggest low withinparticipant variability. The RHTT was effective in differentiating between individuals physiological responses; supporting a heat tolerance continuum. The findings suggest the RHTT is a repeatable measure of physiological strain in the heat and may be used to assess the effectiveness of acute and chronic heat alleviating procedures.

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## 1. Introduction

During exercise in a hot environment, active muscles perform work causing an increase in body heat content. These changes are modulated by the rate of relative heat production (Cramer and Jay, 2014), and represent the rate of change in body heat storage, which in turn reflects the balance between metabolic heat production, heat absorbed from the environment and total body heat loss (Jay and Kenny, 2007). Individuals vary in their ability to withstand heat stress, with some demonstrating a decreased capability to dissipate heat and greater body heat content under the same exercise heat stress (Epstein, 1990). These individuals have been described as heat intolerant and are often characterized by an earlier and greater rise in body temperature, a greater storage of metabolic heat, a higher physiological strain to moderate intensity exercise in the heat and reduced sweating sensitivity (Epstein et al., 1983; Moran et al., 2004).

An individual's heat intolerant state may be temporary or permanent (Epstein, 1990; Moran et al., 2007; Ruell et al., 2014),

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stemming from transient predisposing factors, such as an acute injury to the thermoregulatory centre, insufficient heat acclimation, dehydration or infectious disease (Epstein, 1990). In addition, a lasting thermoregulatory dysfunction may stem from conditions such as cardiac disease, impairment to sweat glands (Epstein, 1990), or differences in gene expression (Moran et al., 2006). Congenital factors such as ectodermal dysplasia may also compromise heat tolerance in some individuals (Epstein, 1990). Aside from these predisposing factors, the high exercise intensity that endurance runners experience during competitions combined with extreme ambient conditions, may elicit unavoidable uncompensable heat production. The evaporative heat loss requirement to maintain a thermal steady state exceeds the maximal evaporative capacity of the individual in the given environment causing a continual rise in body temperature. The work by Nielsen (1996) provides data to suggest a marathon runner may experience up to a 1 °C rise every  $\sim$ 9 min when racing in high ambient conditions (35  $^{\circ}$ C, > 60% relative humidity (RH)), when radiant and convective heat loss is negligible. This rate of rise in core temperature would result in the runner reaching a core body temperature of 40 °C within 25-30 min, with the immediate dangers of heat exhaustion. High incidences of exertional heat illness (EHI) have been reported in long distance runners, with 31% and 53% of the total cases of EHI during the 1992 New Orleans U.S. Olympic Trials and the 1996 Atlanta Olympics respectively,

*Abbreviations:* CV, Coefficient of Variation; EHI, Exertional Heat Illness; ICC, Intraclass Correlation Coefficient; IDF, Israeli Defence Force; LOA, Limits of Agreement; RHTT, Running Heat Tolerance Test; TEM, Typical Error of Measure

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occurring in long distance runners (Martin, 1997). Whether heat intolerance is permanent or acquired the consequences of EHI among endurance athletes emphasise the importance of a running specific test to evaluate individual's ability to withstand exercise heat stress.

Experimental procedures have been applied to cause a rise in core temperature under resting and exercise conditions to challenge the thermoregulatory responses (Inoue et al., 2005; Johnson et al., 2013; Kenney and Hodgson, 1987; Montain et al., 1994). These procedures are used as a method of assessing the ability of an individual to withstand heat stress and evaluate heat dissipating mechanisms. The Israeli Defence Force (IDF) developed a heat tolerance test (HTT) to evaluate whether military personnel's experience of EHI, was temporary or permanent, supporting a safe return to duty (Moran et al., 2004). The protocol involves 120 min walking on a treadmill at a pace of 5 km  $h^{-1}$  and a 2% gradient in ambient conditions of 40 °C and 40% relative humidity (RH). Heat tolerance is determined at the end of the exposure, whereby peak rectal temperature (Tr<sub>peak</sub>)  $\leq$  38.0 °C, peak heart rate (HR<sub>peak</sub>)  $\leq$  120 beats min<sup>-1</sup>, and sweat rate (SR)  $\geq$  780 g h<sup>-1</sup>. Moran and colleagues (2007) acknowledge larger deviations from the specified criteria indicate a greater state of heat intolerance, whereas a pronounced plateau in both Tr and HR is a definitive sign of heat tolerance.

There is an instant elevation in the rate of thermogenesis at the onset of physical activity. As exercise intensity increases, especially in an uncompensable environment, a thermal imbalance persists. This results in a continually positive rate of change in body heat storage, increasing body heat content and a sustained rise in core temperature, giving a graded increase of heat strain (Jay and Kenny, 2007). The IDF HTT may be appropriate for specific occupational situations due to the low to moderate intensity coupled with the long exposure time that is likely to be experienced in military scenarios. Acknowledging the work carried out by the IDF, limitations associated with the HTT remain when examining endurance runners. EHI is compounded by uncompensable heat stress which is in turn influenced by the duration and intensity of exercise. The relative work intensity of an endurance runner training and competing in the heat is markedly higher compared with occupational activities. Therefore, the IDF HTT may not be applicable to an endurance population due to the duration and intensity of protocol. Nielsen, (1966) reported a 23% increase in heat production when running compared with walking on a 10% gradient at a matched energy production. Furthermore, a greater absolute exercise intensity has been shown to result in an increased heat production, irrespective of aerobic fitness (Jay et al., 2011; Mora-rodriguez et al., 2010). Consequently, the low intensity nature of current HTT may misrepresent the metabolic heat production of endurance runners and potentially misdiagnose their susceptibility to a hyperthermic state, pointing to the benefit of a running HTT (RHTT). At present there is no standardised HTT procedure adopting a running mode of exercise which may offer greater ecological validity to endurance runners.

Moran and colleagues (2004) assessed the heat tolerance of nineteen male participants and concluded that the duration of a HTT cannot be shorter than 120 min, since tolerance at 60 min was unable to predict tolerance at 120 min. The work by Epstein and colleagues (1983) and more recently by Moran and colleagues (2007) contradicts these findings, as the rate of increase in rectal temperature (Tr) and heart rate (HR) during the first 20–30 min was considerably different between those individuals deemed heat intolerant and those heat tolerant. This evidence suggests that it may be plausible to assess an individual's ability to withstand exercise heat stress in 30 min. A shorter RHTT requiring no prior testing would provide a more time efficient screening procedure for runners.

To assess and monitor changes in heat tolerance a protocol needs to reliable to minimise measurement error due to biological variation and equipment noise (Atkinson and Nevill, 1998). Typically, the assessment of reliability occurs on performance markers more often than physiological markers, and especially thermoregulatory markers. When the reliability of physiological markers has been assessed, it is often been between two pieces of equipment measuring the same physiological variable. Consequently, there is limited evidence comparing physiological markers during repeated trials using a set intensity exercise protocol. The reproducibility of mean aural temperature and mean heart rate during a fixed intensity cycling heat stress test, which involved three 20 min cycle bouts separated by 8 min rest was assessed in adolescents (Brokenshire et al., 2009). ICC of 0.58 and 0.95 for mean aural temperature and mean HR, respectively and a CV 0.1% and 3% for mean aural temperature and mean HR, respectively were reported and assumed indicative of strong measurement reproducibility. Determining the repeatability of physiological measures during a heat tolerance test would provide greater confidence in observed adjustment in heat tolerance following acute and chronic heat-alleviating interventions.

This study aimed to establish the repeatability of a practical running test to evaluate individual's heat tolerance. It was hypothesised that the RHTT would be repeatable, evidenced by small variations in physiological measures between repeated trials. The findings may enable specific guidance on preparation required prior to training or competing in high ambient conditions. These findings may also enable researchers and practitioners to use the RHTT to track accurately and interpret the changes in physiological variables resulting from acute and chronic interventions to alleviate heat strain in relation to the measurement error.

# 2. Materials and methods

## 2.1. Participants

Sixteen (8 males; 8 females) healthy individuals who typically perform a minimum of 9 miles per week within their weekly training, volunteered and provided written informed consent to participate in the current study (Mean  $\pm$  SD, age 23  $\pm$  5 years, body mass  $67.07 \pm 10.96$  kg, height  $1.76 \pm 0.10$  m, body surface area  $1.82 \pm 0.19$  m<sup>2</sup>, sum of four skin folds  $43 \pm 15$  mm, speed at lactate threshold  $11.7 \pm 1.8$  km h<sup>-1</sup> and  $\dot{VO}_2$  max  $48.8 \pm 6.5$  ml kg<sup>-1</sup> min<sup>-1</sup>). The study was approved by the institution's ethics committee and conducted in accordance with the guidelines of the revised Declaration of Helsinki 2013.

## 2.2. Preliminary testing

During the first visit to the laboratory, anthropometric variables were measured, followed by a graded exercise test. Height and body mass were recorded using a stadiometer (Detecto, USA). Body surface area was calculated from the measurements of body mass and height (DuBois and DuBois, 1916). Sum of skin folds was determined from four sites (Durnin and Womersley, 1974); the bicep, triceps, subscapular and supra-iliac area using Harpenden skin fold callipers (Harpenden, UK). A graded exercise test was performed to determine participants lactate threshold and maximal aerobic capacity ( $\dot{VO}_2$  max) using a motorised treadmill (PPS55 sport-1, Woodway, Germany), according to the British Association of Sport and Exercise Science Guidelines (Jones, 2007).

To determine the lactate threshold, participants performed five to nine, 3 min, incremental ( $0.8 \text{ km h}^{-1}$ ) stages on a treadmill. The initial running speed was set between 8 km h<sup>-1</sup> and 10 km h<sup>-1</sup>. On completion of the stage a capillary blood sample was taken

from a fingertip and analysed for lactate concentration (YSI 2300 Plus, Yellow Springs Instruments, Ohio, USA). Lactate threshold was determined using the point at which blood lactate increased 1 mM above resting value (Jones and Doust, 1998).

Following a ~15 min recovery participants performed 1 min incremental (1% gradient) stages to volitional exhaustion. Expired air was collected for approximately 45 s during each stage using open-circuit spirometry.  $\dot{VO}_2$  max was determined when three out of the following four criteria where achieved; a plateau in  $\dot{VO}_2$ , RER > 1.15, HR reached 10 beats min<sup>-1</sup> from age predicted max and blood lactate > 8 mM.

#### 2.3. Running heat tolerance test

All participants performed the trials at the same time of day (between 0700 h and 1000 h) to control for time of day effects (Winget et al., 1985). Experimentation occurred during the UK winter (mean ambient temperature of 5 °C); therefore participants had been absent from repeated external heat exposure for the previous 3 months. To control for hormonal effects, female participants (n=3) were tested during the early-follicular phase (1-7)days after the onset of menstruation) of their menstrual cycle. Female participants (n=5) taking oral contraceptive performed the experimental sessions during the no pill phase of oral contraceptive use. These timings were selected as they have been shown to be effective in controlling for hormonal fluctuations (Gagnon and Kenny, 2011). Twenty four hours prior to conducting the trials participants were instructed to maintain normal hydration and refrain from the consumption of alcohol, and exhaustive exercise. Two hours prior to arrival, participants were instructed to consume  $3-5 \text{ ml kg}^{-1}$  of water to ensure adequate hydration (Sawka et al., 2007). On arrival to the laboratory, participants voided their bladder to provide a urine sample. Euhydration, permitting continuation of the protocol was confirmed when two out of the following three criteria were achieved, an osmolality value of  $\leq$  700 mOsm kg<sup>-1</sup> H<sub>2</sub>O, a urine specific gravity value of  $\leq$  1.020 and body mass within 1% of daily average (Sawka et al., 2007). These experimental controls were not violated for any participant for any of the preliminary or experimental procedures.

The RHTT consisted of 30 min running at  $9 \text{ km h}^{-1}$  and 2% gradient in ambient conditions of  $40.0 \pm 0.5$  °C and  $39.9 \pm 1.3\%$  RH. Towel-dried nude body mass was measured and recorded to the nearest gram before and after all trials as a measure of SR (Adam GFK 150, Adam Equipment Inc., USA). Values were uncorrected for respiratory and metabolic weight losses, since these were assumed as similar between trials due to the matched exercise intensity and environmental conditions. Participants inserted a rectal thermometer (Henley, Reading, UK) 10 cm past the anal sphincter to measure Tr. Exercise was terminated if Tr  $\ge$  39.7 °C, or the participant withdrew due to volitional exhaustion (zero violations). Skin temperature  $(T_{Skin})$  was recorded using skin thermistors (Eltek Ltd, Cambridge, UK) attached to four sites; the midpoint of the right pectoralis major ( $T_{chest}$ ), midpoint of the triceps brachii lateral head  $(T_{arm})$ , right rectus femoris  $(T_{upper leg})$  and right gastrocnemius lateral head ( $T_{\text{lower leg}}$ ) and connected to a Squirrel temperature logger (Squirrel 1000 series, Eltek Ltd., UK). After a 20 min stabilisation period, resting measures were recorded and participants entered the environmental chamber (TISS, Hampshire, UK). At 5 min intervals, HR (Polar Electro, Kempele, Finland) and Tr were recorded. At 10 min intervals ratings of perceived exertion (RPE) (Borg, 1962) and thermal sensation (TS) (Toner et al., 1986) were recorded. Participants repeated the process between 5 and 7 days later to assess the repeatability of the RHTT.

#### 2.4. Equations

2.4.1. Mean skin temperature

$$= (0.3 \cdot [T_{chest} + T_{upperarm}]) + (0.2 \cdot [T_{upperleg} + T_{lowerleg}])$$
  
(Ramanathan, 1964).

#### 2.4.2. Physiological Strain Index

The Physiological Strain Index (PSI) was calculated from exercising *HR* and *Tr* using the following equation:

$$PSI = 5(Tr_t - Tr_0) \cdot (39.5 - Tr_0)^{-1} + 5(HR_t - HR_0) \cdot (180 - HR_0)^{-1}$$

where  $Tr_0$  and  $HR_0$  are the initial Tr and HR, and  $Tr_t$  and  $HR_t$  are simultaneous measurements taken at any time (Moran et al., 1998).

## 2.5. Statistical analysis

All data were first checked for normality using the Shapiro-Wilk method. Physiological measures during RHTT1 and RHTT2 were examined using a battery of reliability statistics. As a measure of retest correlation intra-class correlation (ICC) with 95% confidence intervals (95% CI) were calculated for each variable. Typical error of the measure (TEM) was calculated from the standard deviation of the mean difference for each pair of trials using the formula  $\text{TEM}=\text{SD}_{(\text{diff})}/\sqrt{2}$  and expressed as a percentage of its respective mean to form the coefficient of variation (CV). Bland-Altman limits of agreement plots showing the mean bias and 95% confidence intervals were produced. The individual participant differences, between the two trials for each variable were plotted against the respective individual means. Paired sampled t test were calculated to identify any differences in physiological measures between RHTT1 and RHTT2. Effect sizes (cohens d) were calculated to analyse the magnitude of the interaction, (Lakens, 2013). All data was analysed using a standard statistical package (SPSS version 20.0), and reported as mean  $\pm$  standard deviation. Statistical significance was accepted at the level of  $p \le 0.05$ .

#### 3. Results

Measures of Intra-class correlation coefficient (ICC), typical error of the measure (TEM), the coefficient of variation (CV) and mean bias with limits of agreement (LOA) for key markers of heat tolerance are presented in Table 1. Strong correlations were observed in Tr<sub>peak</sub> (ICC=0.93),  $T_{skinpeak}$  (ICC=0.95), HR<sub>peak</sub> (ICC=0.99), PSI<sub>peak</sub> (ICC=0.98), and SR (ICC=0.95) between RHTT1 and RHTT2 (Fig. 1). These observed similarities are further supported by a low TEM and CV for the key markers of heat tolerance between RHTT1 and RHTT2 (Table 1). Fig. 2 demonstrates small mean bias and LOA between trials for physiological measures between the two trials. In addition, there were no differences observed in Tr<sub>peak</sub> ( $t_{(15)}$ = -0.785, p=0.445, d=0.20),  $T_{skinpeak}$  ( $t_{(15)}$ =0.223, p=0.827, d=0.), HR<sub>peak</sub> ( $t_{(15)}$ = -1.005, p=0.331, d=0.25), PSI<sub>peak</sub> ( $t_{(15)}$ = -0.969, p=0.348, d=0.24), and SR ( $t_{(15)}$ =0.087, p=0.931, d=0.02) between RHTT1 and RHTT2.

Strong correlations were observed in resting Tr (Tr<sub>rest</sub>) (ICC=0.88 (0.38, 0.90), *p* < 0.001), mean Tr (Tr<sub>mean</sub>) (ICC=0.92 (0.77, 0.97), *p* < 0.001), resting HR (HR<sub>rest</sub>) (ICC=0.91 (0.76, 0.97), *p* < 0.001) and mean HR (HR<sub>mean</sub>) (ICC=0.97 (0.98, 0.99), *p* < 0.001) between RHTT1 and RHTT2. These observed similarities are further supported by a low TEM and CV for Tr<sub>rest</sub> (0.17 °C, 0.45%), Tr<sub>mean</sub> (0.15 °C, 0.39%), HR<sub>rest</sub> (3 beats min<sup>-1</sup>, 5%) and

Mean $\pm$ SD reliability statistics for physiological variables during repeated trials of the RHTT.								
Tr <sub>peak</sub> (°C)	T <sub>skinpeak</sub> (°C)	$\mathrm{HR}_{\mathrm{peak}}$ (beats $\mathrm{min}^{-1}$ )	PSI <sub>peak</sub>					

_		Tr <sub>peak</sub> (°C)	T <sub>skinpeak</sub> (°C)	$HR_{peak}$ (beats min <sup>-1</sup> )	PSI <sub>peak</sub>	$SR (g h^{-1})$
	RHTT1 RHTT2 ICC (95% CI) TEM (CV %) Mean bias (LOA)	$\begin{array}{l} 38.82 \pm 0.47 \\ 38.86 \pm 0.49 \\ 0.93 \; (0.89, \; 0.99), \; p < 0.001 \\ 0.13 \; (0.34) \\ -0.04 \; (-0.41, \; 0.33) \end{array}$	$\begin{array}{l} 38.12 \pm 0.45 \\ 38.11 \pm 0.45 \\ 0.95 \ (0.85, \ 0.98), \ p < 0.001 \\ 0.14 \ (0.37) \\ 0.01 \ (-0.38, \ 0.40) \end{array}$	$\begin{array}{l} 182\pm15\\ 183\pm15\\ 0.99\ (0.96,\ 0.99),\ p<0.001\\ 2\ (1)\\ -1\ (-8,\ 6) \end{array}$	$\begin{array}{l} 8.7 \pm 1.5 \\ 8.8 \pm 1.5 \\ 0.98 \ (0.94, \ 0.99), \ p < 0.001 \\ 0.3 \ (3) \\ - 0.10 \ (-0.93, \ 0.72) \end{array}$	$\begin{array}{l} 1,721\pm 675\\ 1,716\pm 745\\ 0.95\ (0.86,\ 0.98),\ p<0.001\\ 162\ (9)\\ -\ 133\ (-\ 492,\ 227) \end{array}$

Notes:  $Tr_{peak}$  = peak rectal temperature,  $T_{skinpeak}$  = peak skin temperature,  $HR_{peak}$  = peak heart rate,  $PSI_{peak}$  = peak physiological strain index, SR = sweat rate, RHTT = running heat tolerance test.

HR<sub>mean</sub> (3 beats min<sup>-1</sup>, 2%). There were small mean bias and LOA for Tr<sub>rest</sub> [-0.04, (-0.50, 0.42)], Tr<sub>mean</sub> [-0.04, (-0.45, 0.37)], HR<sub>rest</sub> [0, (-8, 8)] and HR<sub>mean</sub> [1, (-7.55, 9.87)]. Furthermore, there were no differences in Tr<sub>rest</sub> ( $t_{(15)} = -0.722$ , p = 0.481, d = 0.18) Tr<sub>mean</sub> ( $t_{(15)} = -0.770$ , p = 0.453, d = 0.19), HR<sub>rest</sub> ( $t_{(15)} = -0.301$ , p = 0.768, d = 0.08) and HR<sub>mean</sub> ( $t_{(15)} = -1.003$ , p = 0.332, d = 0.25) between RHTT1 and RHHT2.

Strong correlations were observed for peak RPE (ICC=0.96, (0.88, 0.99), p < 0.001) and peak TS (ICC=0.92, (0.76, 0.97), p < 0.001) between RHTT1 and RHTT2. These observed similarities are further supported by a low TEM and CV for peak RPE (0.6%,

4%), and peak TS (0.2%, 3.2%). There were small mean bias and LOA for peak RPE [-0.1, (-1.7, 1.6)], peak TS [0.1, (-0.6, 0.7)] between RHTT1 and RHTT2. Furthermore, there were no differences in peak RPE ( $t_{(15)} = -0.293$ , p = 0.774, d = 0.08) and peak TS ( $t_{(15)} = 0.145$ , p = 0.0.27, d = 0.28) between RHTT1 and RHHT2.

## 4. Discussion

The principle aim of the current study was to examine the repeatability of a RHTT from two trials separated by 5–7 days. Classic



**Fig. 1.** Peak rectal temperature ( $Tr_{peak}$ ) (A), peak heart rate ( $HR_{peak}$ ) (B) sweat rate (SR) (C) and peak skin temperature ( $T_{skinpeak}$ ) during RHTT1 (*x*-axis) and RHTT2 (*y*-axis). Dotted line represents line of equality. N = 16.



**Fig. 2.** Bland–Altman plots with mean bias (solid line) and 95% limits of agreement (dotted line) for peak rectal temperature  $(Tr_{peak})$  (A), peak heart rate (HR<sub>peak</sub>) (B) sweat rate (SR) (C) and peak skin temperature ( $T_{skinpeak}$ ) (D). N=16.

markers of heat tolerance and heat acclimation, namely  $Tr_{peak}$ ,  $T_{skinpeak}$  HR<sub>peak</sub> and SR were used for analysis (Moran et al., 2007; Sawka et al., 2011). The main finding from the current study is that the RHTT had good agreement, strong correlations and small differences between repeated trials and the TEM values for these classic markers suggested low within-participant variability. Interestingly, values of  $Tr_{peak}$ ,  $T_{skinpeak}$  HR<sub>peak</sub> and SR appear to be spread along a continuum, suggesting greater face validity in contrast to the dichotomous classification previously suggested (Kresfelder et al., 2006; Moran et al., 2007). The data presented in the current study demonstrates that the RHTT has strong repeatability and is able to differentiate between individuals responses to the RHTT, supporting the use of the RHTT in future investigations to gauge individual's running heat tolerance, and to monitor the extent of acute and chronic heat alleviating protocols.

To the authors knowledge there are no studies accessing the repeatability of physiological measures during a fixed intensity running protocol making acceptable levels of reliability *a priori* difficult to determine. Furthermore, there is no literature assessing the reliability of the classical IDF HTT which would offer comparison. As a general rule, a correlation coefficient over 0.90 is considered to be high, between 0.70–0.80 moderate, and below 0.70 to be low for physiological tests (Vincent, 1995). In the current study, ICC values for  $Tr_{peak}$ ,  $T_{skinpeak}$  HR<sub>peak</sub> PSI<sub>peak</sub> and SR were all equal or greater than 0.93; based on these criteria, it is reasonable to suggest that the physiological measures during the RHTT have an acceptable level of reliability between repeated trials based on ICC.

The data in the current study demonstrates less variability than available data on fixed intensity cycling protocols in the heat using similar physiological variables. The CV of 0.3% and 1% for Tr<sub>peak</sub> and HR<sub>peak</sub> respectively, compare favourably to the CV of 0.3% and 3.9% for mean Tr and mean HR reported during a 60 min, fixed intensity cycling protocol in hot humid conditions (Hayden et al., 2004). Furthermore, Brokenshire and collegues (2009) investigated the repeatability of HR during a fixed intensity cycling heat stress test, which involved three 20 min cycle bouts separated by 8 min rest. The ICC of 0.95 and CV of 3% for mean HR were reported and assumed indicative of strong measurement reproducibility. The ICC of 0.99 and CV of 1% in the current study, again compare favourably to these findings highlighting the strength of the RHTT in terms of low measurement error.

To aid interpretation and presentation of results when assessing individual's heat tolerance, researchers have dichotomized the population and categorized individuals as either tolerant or intolerant (Kresfelder et al., 2006; Moran et al., 2007). Altman and Royston (2006) reported several problems with dichotomizing data. Statistical power to detect a relationship may be reduced, there is an increased risk of reporting a false positive, a possibility of underestimating the extent of the variation in the outcome between groups, and using two groups conceals any non-linearity in the relationship. Altman and Royston (2006) state using multiple categories is generally favoured to dichotomizing data; with four or five groups the loss of information can be quite small but there are complexities to analysis. Indeed, Moran and colleagues (2007) acknowledge that the larger the deviations from normal values the more pronounced the state of heat intolerance, thus implying a continuum of heat tolerance. Similarly work from, Taylor and Cotter (2006) propose that heat adaptation is a continuum, with the position of an individual along the continuum representing progressive increases in heat tolerance. The findings in the present study demonstrate clearly that an individual's heat tolerance, represented by the  $Tr_{peak}$ ,  $T_{skin_{peak}}$ .  $HR_{peak}$  PSI<sub>peak</sub> and SR, is a continuous variable; demonstrating that individual's heat tolerance may be more accurately categorised on a continuum. Consequently, these findings support the use of the RHTT to track changes in individual's ability to withstand exercise heat stress from acute and chronic heat-alleviating interventions.

Cases of EHI can occur among endurance athletes, in extreme circumstances leading to death; however, the epidemiology is not well documented in the literature. Martin (1997) reported the highest incidence of EHI occurred in long distance runners during the 1992 New Orleans U.S. Olympic Trials and the 1996 Atlanta Olympics; with long distance runners accounting for 31% and 53% respectively, of the total cases of EHI. Furthermore, Nielsen (1996) provides data to suggest the incidence of heat illness is unavoidable for endurance runners when competing in a high ambient temperature combined with high relative humidity without a severe reduction in endurance performance; highlighting the importance of thorough preparation to prevent the incidence of a heat illness. The findings in the current study could be applied in a manner that would serve to minimise the number of athletes that suffer from hyperthermia, by supporting a more complete evaluation and subsequent preparation prior to training and competing in the heat (Johnson et al., 2013).

The typical error of measurement expressed as a coefficient of variation can be used to estimate sample size for future studies using the RHTT when a smallest worthwhile change is known; using the formula  $N=8 \text{ s}^2/\text{d}^2$  (where 's' = typical error expressed as a coefficient of variation and 'd' = the smallest worthwhile change (Hopkins, 2000). A change of 0.4 °C typically represents a significant reduction in Tr following acute and chronic heat alleviating procedures (Buono et al., 1998; Patterson et al., 2004; Castle et al., 2006; Garrett et al., 2009). Accordingly, in the current study to detect a 0.4 °C change, a sample size of six participants would be sufficient when Tr<sub>peak</sub> is the key variable of concern, assuming similar variability among the participants recruited.

## 5. Limitations

The RHTT adopts a fixed absolute workload, which is an appropriate procedure to assess heat tolerance when the experimental design is a repeated measure. However, the fixed absolute workload may limit applicability of the test when comparing between groups, especially when they are not matched. The recent work by Cramer & Jay (2014) reports when comparing between individuals or groups unmatched from a biophysical perspective, exercise intensity should be administered using fixed heat production relative to body mass to prevent the introduction of systematic bias. Future research is warranted to quantify the potential differences in metabolic heat production between participants of differing body mass, body surface area and fitness during the RHTT to ensure unbiased comparison of thermoregulatory responses between individuals (Cramer and Jay, 2014). Furthermore, the role of the RHTT in identifying individual's susceptibility to exertional heat illness has not been identified within the current study. Collecting RHTT data on individuals who have previously incurred exertional heat illness would reinforce the value of the RHTT in identifying individuals susceptibility to experiencing an exertional heat illness.

#### 6. Conclusion

The main finding from the current study was that the RHTT had good agreement, strong correlations and small differences between repeated trials and the TEM values for these classic markers suggested low within-participant variability. In addition, data is presented to demonstrate that the RHTT is capable of differentiating between individual's responses to the RHTT when adopting a running mode of exercise; providing evidence that a 30 min HTT protocol is sufficient in duration. Furthermore, the findings from the present study challenge the previous reports that an individual's heat tolerance is dichotomous, with individual responses spread over a continuum. These findings can be applied in a manner to observe individuals thermoregulatory responses over time, providing information to physiologist, coaches and medical staff to make decisions regarding athlete's safety prior to training and competing in the heat. In addition, the RHTT could be used as a tool for investigating the effect of acute and chronic heatalleviating procedures in relation to measurement error.

## **Conflict of Interest**

The authors declare that they have no competing interests such as funding or personal financial interest.

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