

Title:

Isothermic and fixed intensity heat acclimation methods induce similar heat adaptation following short and long-term timescales.

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## Abstract

Heat acclimation requires the interaction between hot environments and exercise to elicit thermoregulatory adaptations. Optimal synergism between these parameters is unknown. Common practise involves utilising a fixed workload model where exercise prescription is controlled and core temperature is uncontrolled, or an isothermic model where core temperature is controlled and work rate is manipulated to control core temperature.

Following a baseline heat stress test; twenty four males performed a between groups experimental design performing short term heat acclimation (STHA; five 90 min sessions) and long term heat acclimation (LTHA; STHA plus further five 90 min sessions) utilising either fixed intensity ( $50\% \dot{V}O_{2peak}$ ), continuous isothermic (target rectal temperature  $38.5^{\circ}\text{C}$  for STHA and LTHA), or progressive isothermic heat acclimation (target rectal temperature  $38.5^{\circ}\text{C}$  for STHA, and  $39.0^{\circ}\text{C}$  for LTHA). Identical heat stress tests followed STHA and LTHA to determine the magnitude of adaptation.

All methods induced equal adaptation from baseline however isothermic methods induced adaptation and reduced exercise durations (STHA =  $-66\%$  and LTHA =  $-72\%$ ) and mean session intensity (STHA =  $-13\% \dot{V}O_{2peak}$  and LTHA =  $-9\% \dot{V}O_{2peak}$ ) in comparison to fixed ( $p < 0.05$ ). STHA decreased exercising heart rate ( $-10 \text{ b}\cdot\text{min}^{-1}$ ), core ( $-0.2^{\circ}\text{C}$ ) and skin temperature ( $-0.51^{\circ}\text{C}$ ), with sweat losses increasing ( $+0.36 \text{ L}\cdot\text{hr}^{-1}$ ) ( $p < 0.05$ ). No difference between heat acclimation methods, and no further benefit of LTHA was observed ( $p > 0.05$ ). Only thermal sensation improved from baseline to STHA ( $-0.2$ ), and then between STHA and LTHA ( $-0.5$ ) ( $p < 0.05$ ). Both the continuous and progressive isothermic methods elicited exercise duration, mean session intensity, and mean  $T_{rec}$  analogous to more efficient administration for maximising adaptation.

Short term isothermic methods are therefore optimal for individuals aiming to achieve heat adaptation most economically, i.e. when integrating heat acclimation into a pre-competition taper. Fixed methods may be optimal for military and occupational applications due to lower exercise intensity and simplified administration.

## Highlights

- Isothermic and fixed intensity heat acclimation methods elicit equal adaptation.
- Isothermic heat acclimation is more appropriate for athletes due to more efficient procurement.
- Progressive increases in target core temperature do not increase the extent of adaptation.

## 1. Introduction

Repeated exposure to stressful hot environments initiates the heat-adapted phenotype. The heat-adapted phenotype is acquired most effectively when hot and humid environmental conditions and physical work (intensity, duration and frequency) interact to stress thermoregulatory and cardiovascular systems (Sawka et al., 2011); this process is known as heat acclimation (Garrett et al., 2011). Primary adaptations induced by heat acclimation include decreased core temperature (Armstrong and Maresh, 1991; Buono et al., 1998; Garrett et al., 2011) and reduced heat storage (Aoyagi et al., 1997) facilitated by increased sudomotor function (Chinevere et al., 2008; Lorenzo and Minson, 2010; Machado-Moreira et al., 2006; Martinez et al., 2012), increased skin blood flow (Lorenzo and Minson, 2010), and cardiovascular adjustments eliciting greater maintenance of stroke volume and reduced heart rate at a given workload (Frank et al., 2001). These adaptations contribute to a decreased thermal and perceptual strain (Castle et al., 2011), ultimately facilitating increased exercise performance in hot and cool environments (Lorenzo et al., 2010). Heat acclimation is often categorised into short term (STHA) and long term (LTHA) induction periods. LTHA, the traditional time scale, generally comprises  $\geq 10$  daily heat exposures (Garrett et al., 2011), potentiating the most complete phenotypic adaptation. STHA utilises  $\leq 5$  daily exposures, facilitating rapid, but, incomplete adaptation ( $\sim 75\%$  compared to LTHA, (Pandolf, 1979)). Notwithstanding, STHA still remains an effective tool used by practitioners for augmenting adaptation before exposure to hot environments, improving tolerance to exercise or work (Garrett et al., 2012, 2009).

Increased core temperature is a fundamental requirement for inducing heat acclimation (Regan et al., 1996; Taylor and Cotter, 2006). Isothermic heat acclimation (also known as controlled hyperthermia) is imposed based upon endogenous (internal) criteria (Castle et al., 2012; Garrett et al., 2014, 2012, 2009; Hom et al., 2012; Machado-Moreira et al., 2006; Magalhães et al., 2010a, 2010b; Patterson et al., 2014, 2004), and might provide sustained targeting and attainment of specific and individualised internal temperatures through a combination of active and passive heat acclimation (Fox et al., 1963). The balance between work and rest to target and maintain specific core temperatures ensures a consistency, or a progression of endogenous heat strain to induce adaptation, albeit requiring alterations in administration throughout each session. Implementation of fixed intensity heat acclimation methods is in comparison relatively simple, with participants maintaining a fixed workload throughout each active acclimation session (Amorim et al., 2011; Castle et al., 2011; Cheung and McLellan, 1998; Houmard et al., 1990; Kresfelder et al., 2006; Lorenzo and Minson, 2010; Lorenzo et al., 2010; Marshall et al., 2007; Nielsen et al., 1997,

1993; Sandström et al., 2008; Watkins et al., 2008; Yamada et al., 2007). Fixed methods derive exercise workloads from a pre acclimation baseline, and the exogenous (external) environment are consistent day-on-day. Though this method may provide sufficient heat strain during the initial sessions of heat acclimation regimens, fixed methods may not achieve the desired, nor optimally potentiating stimuli – increased core temperature, as the thermal strain relative to the start of acclimation diminishes with ensuing adaptation (Taylor and Cotter, 2006; Taylor, 2014). During both STHA and LTHA, relative workload and the thermal strain of heat acclimation are likely to reduce during fixed intensity as on-going adaptation is seen. Isothermic heat acclimation, where endogenous thermal stimulus is consistently targeted throughout, may positively sustain the rate of adaptation, or advance adaptation should a progressive increase in core temperature be implemented (Taylor and Cotter, 2006; Taylor, 2014). Progressive isothermic methods have only previously been implemented using models where the environmental conditions or workload for acclimation are increased (Burk et al., 2012; Chen et al., 2013; Daanen et al., 2011), this presumably to offset the aforementioned ongoing adaptation. These progressive methods are not certain to increase core temperature in the manner that a progressive increase in the isothermic target temperature would. Varied administration of heat acclimation methods has likely produced different phenotypic adaptive responses. The mode of exercise, relative exercise intensity and climatic conditions may modulate different degrees of adaptation (Taylor and Cotter, 2006). Should the anticipated core temperature changes be observed between methods it is likely that fixed heat acclimation methods are analogous to a reduction in the potentiating stimuli for adaptation and consequently the rate of adaptation would decrease from STHA to LTHA. The isothermic continuous method should theoretically sustain potentiating stimuli and consequently sustain the rate of adaptation from STHA to LTHA. Finally a progressive isothermic method could theoretically be used to increase potentiating stimuli and may increase the rate of adaptation from STHA to LTHA.

The aim of the present study was to determine whether any differences in heat adaptation occurred between an established exogenous controlled, fixed intensity heat acclimation method, an endogenous controlled, isothermic heat acclimation method, and a stepwise progressive endogenous isothermic heat acclimation method, after STHA and LTHA periods. No direct comparison has been made of the observed adaptation and administration differences between isothermic and fixed heat acclimation methods across STHA and LTHA timescales; additionally evidence is limited in support of a stepwise progression in thermal strain to increase the rate of adaptation from STHA to LTHA. We hypothesised that the rate of phenotypic adaptation would be greater in isothermic heat acclimation

methods in comparison to fixed methods due to sustained strain. It was additionally hypothesised that a greater rate of adaptation would be induced by utilising a progressive model. It was also hypothesised that implementation of isothermic heat acclimation would require reduced exercise durations and lower average sessional exercise intensities, in spite of initially higher exercise intensities, which would favour athletes in the pre-competition taper.

## **2. Methods**

### **2.1 Participants**

Twenty-four healthy males were assigned into fixed intensity (FIXED), or isothermic heat acclimation (ISO) groups, ISO was then subdivided into continuous isothermic heat acclimation (ISO<sub>CONT</sub>), or progressive isothermic heat acclimation (ISO<sub>PROG</sub>) groups; participants were matched for peak oxygen uptake ( $\dot{V}O_{2peak}$ ) and anthropometric characteristics. Data are presented in Table 1. Confounding variables of smoking, caffeine, glutamine, alcohol, generic supplementation, prior thermal, hypoxic and hyperbaric exposures were all controlled in line with previous work in the field (Gibson et al., 2014; Taylor et al., 2011). Following institutional ethics approval and full description of experimental procedures, all participants completed medical questionnaires and provided written informed consent following the principles outlined by the Declaration of Helsinki of 1975, as revised in 2013. The experimental design for the study is presented in Figure 1 with full explanation of the heat acclimation methods contained within the “Heat Acclimation Methods” section 2.4 which follows.

### **2.2 Preliminary Testing**

Participants consumed 500 mL of water 2 h before all preliminary and experimental exercise sessions (Sawka et al., 2007). A urine osmometer (Alago Vitech Scientific, Pocket PAL-OSMO, UK) was used to ensure consistent hydration prior to each experimental session (Garrett et al., 2014). Participants were deemed euhydrated and subsequently able to commence further preliminary, and experimental procedures if urine osmolality was  $<700$  mOsm $\cdot$ kg $^{-1}$  H $_2$ O (Sawka et al., 2007). Prior to the initial  $\dot{V}O_{2peak}$  experimental trial, height (cm) using a fixed stadiometer (Detecto Physicians Scales; Cranlea & Co., Birmingham, UK), and body density, using calipers (Harpenden, Burgess Hill, UK) and a four site skin fold calculation (Durnin and Womersley, 1974) were determined, later body fat (%) was calculated from body density (Siri, 1956) and body surface area (Du Bois and Du Bois, 1916). Nude body mass (NBM) was recorded to 0.01 kg from digital scales (ADAM GFK 150, USA), relative metabolic heat production (MHP;

(W.kg<sup>-1</sup>) was calculated to describe the initial requirements of each heat acclimation method in accordance with the guidelines of Cramer and Jay (2014).

$\dot{V}O_{2peak}$  (L.min<sup>-1</sup>) was determined from an incremental test on a cycle ergometer (Monark e724, Vansbro, Sweden) in temperate laboratory conditions (20°C, 40% relative humidity (RH)). Starting intensity was set at 80W, with resistance applied to the flywheel eliciting a 24 W.min<sup>-1</sup> increase at the constant cadence of 80 rpm. Expired metabolic gas was measured using online gas analysis (Metamax 3X, Cortex, Germany);  $\dot{V}O_{2peak}$  was considered as the highest  $\dot{V}O_2$  obtained in any 10 s period. Heart rate (HR; b.min<sup>-1</sup>) was recorded continually during all exercise tests by telemetry (Polar Electro Oyo, Temple, Finland). Saddle position was adjusted by the participant to their preferred cycling position and remained unchanged for all experimental trials. Heat acclimation workloads were subsequently calculated using linear regression utilising power: $\dot{V}O_2$  data collected following the incremental test.

### **2.3 Heat Stress Testing**

A running Heat Stress Test (HST) was performed as a preliminary test (HST1), then 48 h after STHA (HST2), and 48 h after LTHA (HST3) inside a purpose built environmental chamber with temperature and humidity (40.0 ± 0.1°C and 28.4 ± 6.6% RH) controlled using automated computer feedback (WatFlow control system; TISS, Hampshire, UK) and no additional convective cooling provided e.g. motorised fan. All HST were performed between 08:00 and 10:00 h (Drust et al., 2005). Following confirmation of adequate hydration, participants inserted a single-use disposable rectal thermistor (Henleys Medical, UK, Meter logger Model 401, Yellow Springs Instruments, Yellow Springs, Missouri, USA; accuracy ± 0.20°C) 10 cm past the anal sphincter to measure rectal temperature ( $T_{rec}$ ). Skin temperature ( $T_{sk}$ ) was measured using a data logger (Squirrel Meter Logger, Grant Instruments, Cambridge, UK) and skin thermistors attached to the right-hand side of the body using zinc oxide tape (Cramer Products Inc., Kansas, USA) at the pectoralis major muscle belly, lateral head of triceps brachii, rectus femoris muscle belly and lateral head of the gastrocnemius (Ramanathan, 1964). Mean skin temperature was calculated according to the formula of Ramanathan (1964). Absolute sweat loss (L.h<sup>-1</sup>) was estimated using the change in towel-dried NBM from the pre-to-post exercise periods and adjusted based upon the HST duration. Participants were not permitted to consume any fluid between pre and post-test measurement of NBM. No correction was made for insensible water loss and loss of mass associated with the respiratory exchange of O<sub>2</sub> and CO<sub>2</sub> (Dion et al., 2013); all were assumed to be similar between HSTs due to the equal length of each trial at each time point (table 2).

After a 20 min seated stabilisation period in temperate laboratory conditions, resting measures were taken after which participants entered the environmental chamber to perform 30 min running at 9 km.h<sup>-1</sup> and 2% elevation. HR, T<sub>rec</sub> and T<sub>sk</sub> were recorded every 5 min. Ratings of perceived exertion (Borg et al., 1985) and thermal sensation (Toner et al., 1986) were recorded every 10 min. HSTs was terminated if T<sub>rec</sub> ≥ 39.7°C (zero incidences), or the participant withdrew due to volitional exhaustion, or inability to maintain the running speed despite strong verbal encouragement.

## 2.4 Heat Acclimation Methods

Each heat acclimation testing session was conducted at the same time of day (07:00 - 11:00 h) to control for effects of daily variation in performance (Shido et al., 1999). Following provision of a urine sample and NBM, each participant inserted the rectal thermistor described in the HST and affixed a HR monitor upon which time resting measures were taken after 5 min seated in temperate laboratory conditions. Participants subsequently mounted a cycle ergometer (Monark, e724, Vansbro, Sweden) located inside the environmental chamber where conditions were consistent for all groups (40.2 ± 0.4°C, 39.0 ± 7.8% RH). The FIXED participants performed ten sessions of 90 min of continuous cycling exercise at a workload corresponding to 50%  $\dot{V}O_{2peak}$ . ISO<sub>CONT</sub> participants exercised initially at a workload corresponding to 65% of  $\dot{V}O_{2peak}$  until a target T<sub>rec</sub> of 38.5°C was achieved for all ten heat acclimation sessions. ISO<sub>PROG</sub> participants exercised initially at a workload corresponding to 65% of  $\dot{V}O_{2peak}$  targeting a T<sub>rec</sub> of 38.5°C for the first five sessions, then progressing to a T<sub>rec</sub> of 39.0°C for the final five sessions. Once target T<sub>rec</sub> had been reached, power was adjusted every 5 min, first by a 25%  $\dot{V}O_{2peak}$  reduction and then adjusted (± 5%  $\dot{V}O_{2peak}$ , or seated rest) to maintain the desired experimental T<sub>rec</sub> for a total session duration of 90 min within the environmental chamber; workloads at the onset of exercise are presented in Table 1. During each testing session HR, T<sub>rec</sub> and power output, were recorded every 5 min. Mean T<sub>rec</sub> reflects the average T<sub>rec</sub> recorded throughout each acclimation method. T<sub>recfinal60min</sub> quantifies the mean T<sub>rec</sub> between minutes 30 and 90 of the acclimation session to reflect the temperature following the initial rate of increase. Exercising duration was defined as the total time exercising (power output >1 W) during acclimation sessions reflecting the physical work demands throughout each 90 min session. Mean Session Intensity (% $\dot{V}O_{2peak}$  and W.kg<sup>-1</sup>) was calculated from the relative exercise intensity during each 5 min period throughout all of the 90 min acclimation sessions. This contrasted the Mean Exercise Intensity (% $\dot{V}O_{2peak}$  and W.kg<sup>-1</sup>), which reflected the mean relative exercise intensity only (power

output >1 W), thus excluding periods of rest within isothermic methods. Data for these variables are provided in Table 2.

## 2.5 Statistical Analyses

All outcome variables were first checked for normality using Kolmogorov-Smirnov and sphericity using the Greenhouse Geisser method prior to further analysis. Two way mixed design ANOVA were performed to determine differences in dependent variables between heat acclimation methods for STHA and LTHA timescales, and between heat acclimation methods and HST1, HST2 and HST3. Adjusted Bonferroni comparisons were used as post hoc analyses, determining where differences existed within ANOVA where a time or interaction was found. Data are reported as mean  $\pm$  SD, with two-tailed significance was accepted at  $p < 0.05$ .

## 3. Results

### 3.1 Method Administration and Thermoregulatory and Physiological Responses to Short and Long Term Heat Acclimation

Differences ( $p < 0.05$ ) were observed with increased exercise duration, total work done and duration  $T_{rec} \geq 38.5^\circ\text{C}$  in all methods from STHA to LTHA, data are presented in Table 2. Mean session intensity, mean session power and the time to target  $T_{rec}$  increased from STHA to LTHA in ISO<sub>CONT</sub> and ISO<sub>PROG</sub>, with mean  $T_{rec}$  and mean  $T_{rec\text{final}60}$ , reducing in FIXED. The duration  $T_{rec} \geq 39.0^\circ\text{C}$  increased from STHA to LTHA in ISO<sub>PROG</sub> only. No difference was observed for mean exercise intensity ( $f = 1.935, p = 0.179$ ), mean exercise power ( $f = 1.061, p = 0.315$ ), change in  $T_{rec}$  ( $f = 0.866, p = 0.363$ ), rate of  $T_{rec}$  increase ( $f = 2.158, p = 0.157$ ), or mean HR ( $f = 3.026, p = 0.097$ ) between STHA and LTHA.

A between heat acclimation methods interaction effect was observed for exercise duration ( $f = 13.090, p < 0.001$ ), and the time to target  $T_{rec}$  ( $f = 6.500, p = 0.006$ ), mean session intensity ( $f = 6.727, p = 0.006$ ), mean  $T_{rec}$  ( $f = 7.063, p = 0.005$ ), mean  $T_{rec\text{final}60}$  ( $f = 11.073, p = 0.001$ ), duration  $T_{rec} \geq 38.5^\circ\text{C}$  ( $f = 14.608, p < 0.001$ ), duration  $T_{rec} \geq 39.0^\circ\text{C}$  ( $f = 28.262, p < 0.001$ ), mean exercise power ( $f = 3.765, p = 0.040$ ), change in  $T_{rec}$  ( $f = 5.277, p = 0.014$ ) and mean HR ( $f = 11.073, p = 0.001$ ). Post hoc analysis is presented in Table 2 for clarity. No between group interaction was observed for total work done ( $f = 0.011, p = 0.989$ ), mean exercise intensity ( $f = 3.186, p = 0.062$ ), rate of  $T_{rec}$  increase ( $f = 0.884, p = 0.428$ ), or for the post hoc analysis of mean session power ( $f = 4.822, p = 0.019$ ).

### 3.2 Daily responses to heat acclimation



Resting  $T_{rec}$  ( $f = 3.048, p = 0.002$ ), resting HR ( $f = 3.085, p = 0.002$ ), and sessional sweat loss (%NBM) ( $f = 3.798, p < 0.000$ ) all demonstrated improvements overall as the number of heat acclimation sessions increased. Post hoc analysis revealed resting  $T_{rec}$  was reduced ( $p < 0.05$ ) from session one before session eight, nine and ten, resting HR was reduced ( $p < 0.05$ ) from session one before session nine and ten and sweat loss was increased ( $p < 0.05$ ) from session one following session eight, nine and ten. No between group effect was observed for resting  $T_{rec}$  ( $f = 1.146, p = 0.311$ ), resting HR ( $f = 1.553, p = 0.076$ ) and sessional sweat loss ( $f = 1.007, p = 0.453$ ). Data are presented in Figure 3.

### 3.3 Heat Stress Testing - Resting Adaptations

Resting HR ( $f = 7.730, p = 0.001$ ) and resting  $T_{rec}$  ( $f = 7.372, p = 0.004$ ) reduced with heat acclimation; post hoc analysis revealed a reduction ( $p < 0.05$ ) in both measures from HST1 to HST2 and HST1 to HST3, but no difference between HST2 and HST3 ( $p > 0.05$ ), data are presented in Table 3. No between heat acclimation method interaction was observed for either resting HR ( $f = 0.819, p = 0.521$ ) or resting  $T_{rec}$  ( $f = 0.750, p = 0.537$ ).

### 3.4 Heat Stress Testing - Exercising Adaptations

Mean exercising HR ( $f = 23.887, p < 0.001$ ), mean  $T_{rec}$  ( $f = 11.067, p < 0.001$ ), sweat loss ( $f = 10.516, p < 0.001$ ), mean  $T_{sk}$  ( $f = 10.516, p < 0.001$ ) and peak  $T_{sk}$  ( $f = 13.185, p < 0.001$ ) reduced with heat acclimation; post hoc analysis revealed a reduction ( $p < 0.05$ ) in mean exercising HR, mean  $T_{rec}$ , mean  $T_{sk}$ , and peak  $T_{sk}$ , and increase in sweat loss from HST1 to HST2 and HST1 to HST3, but no difference between HST2 and HST3 ( $p > 0.05$ ), (Table 3). No between heat acclimation method interaction was observed for mean exercising HR ( $f = 0.431, p = 0.786$ ), mean  $T_{rec}$  ( $f = 0.213, p = 0.930$ ), sweat loss ( $f = 2.183, p = 0.870$ ) or peak  $T_{sk}$  ( $f = 2.008, p = 0.111$ ). No changes were observed between HSTs, or between heat acclimation methods for exercise duration ( $f = 2.333, p = 0.125$ ) and ( $f = 0.333, p = 0.854$ ), change in exercising HR ( $f = 0.529, p = 0.593$ ) and ( $f = 2.318, p = 0.073$ ), the change  $T_{rec}$  ( $f = 0.126, p = 0.295$ ) and ( $f = 0.975, p = 0.432$ ), or the rate of  $T_{rec}$  increase ( $f = 1.257, p = 0.295$ ) and ( $f = 0.975, p = 0.432$ ) respectively.

### 3.5 Heat Stress Testing - Perceptual Changes

Peak thermal sensation ( $f = 8.316, p = 0.001$ ) and mean thermal sensation ( $f = 5.573, p = 0.007$ ) reduced with heat acclimation, post hoc analysis revealed a reduction ( $p < 0.05$ ) in peak thermal sensation from HST1 to HST2, HST1 to HST3, and HST2 to HST3 and mean thermal sensation from HST2 to HST3 (Table 3). No between heat acclimation

method interaction was observed for either peak thermal sensation ( $f = 1.137, p = 0.352$ ) or mean thermal sensation ( $f = 1.150, p = 0.346$ ). No changes were observed between HSTs, or between heat acclimation methods for peak rating of perceived exertion ( $f = 2.891, p = 0.067$ ) and ( $f = 2.194, p = 0.086$ ) respectively, or mean rating of perceived exertion ( $f = 1.787, p = 0.180$ ) and ( $f = 0.705, p = 0.593$ ), respectively.

#### **4. Discussion**

The aim of this experiment was to determine whether there was a difference in measures of heat adaptation to STHA and LTHA between a fixed intensity heat acclimation method, a controlled isothermic heat acclimation method and a progressive isothermic heat acclimation method. It was observed that equal heat adaptation was induced between all methods over STHA with no significant additional benefit from our LTHA timescale. Relative to Fixed intensity methods, Isothermic methods are the favourable form of administration with equal adaptation induced following reduced exercise durations and mean session intensity.

##### **4.1 Differences in Heat Acclimation methods.**

No difference in the magnitude of adaptation existed between fixed intensity, continuous isothermic, and progressive isothermic heat acclimation methods, however during STHA and LTHA, the FIXED mode of heat acclimation was inferior to isothermic heat acclimation methods (ISO<sub>CONT</sub> and ISO<sub>PROG</sub>) when considering applied practical perspectives in accordance with established recommendations for interacting physical training and heat acclimation to maintain performance (Aoyagi et al., 1997). Isothermic methods achieved adaptation with reduced exercise durations (STHA and LTHA) and mean session intensity (STHA and LTHA), which is desirable for athletic applications as an effective means for reducing the volume of physiological strain of exercise in the heat. This application perhaps contrasts military and occupational applications for which the FIXED method may be optimal due to lower intensity of work and simplified administration facilitating implementation for large cohorts, or a research perspective when matching of training stimulus is required. Exercising durations were lower in isothermic methods (66-79% of session duration) compared to fixed intensity heat acclimation (>99% of session duration). It is noteworthy that the time taken to achieve the target  $T_{rec}$  in ISO<sub>CONT</sub> and ISO<sub>PROG</sub> increased from STHA to LTHA by 6.6% and 10.0% respectively, thus demonstrating the effects of ongoing adaptation, and using the ISO<sub>PROG</sub> method the greater work required to attain a higher  $T_{rec}$ . Higher initial work intensity balanced by increased rest periods are congruous with typical training regimes, therefore isothermic methods may be more appropriate when

integrating heat acclimation into a training taper (Mujika et al., 2004) prior to competition due to sport specificity (Houmard et al., 1990), particularly when acknowledging that the duration of a typical fixed heat acclimation session is at the upper end of that desirable for typical pre-competition training session, with the repeated sessions exceeding the typical volume of a typical endurance training taper (Spilsbury et al., 2014).

#### **4.2 Adaptations made during short and long term heat acclimation**

Isothermic heat acclimation methods were more favourable than FIXED at targeting and sustaining specific  $T_{rec}$  (i.e.  $\geq 38.5^{\circ}\text{C}$ ) thus delivering greater elevations in thermal strain, notably the important potentiating stimuli of increased core temperature over both STHA and LTHA (Regan et al., 1996; Taylor and Cotter, 2006). This statement can be evidenced by ISO<sub>CONT</sub> and ISO<sub>PROG</sub> evoking greater mean  $T_{rec}$ , mean  $T_{rec\text{final}60}$  and ISO<sub>PROG</sub> eliciting favourable duration  $T_{rec}\geq 38.5^{\circ}\text{C}$ , duration  $T_{rec}\geq 39.0^{\circ}\text{C}$ , change in  $T_{rec}$  and mean heart rate (HR) when compared to FIXED (Table 2). Isothermic heat acclimation increased the duration spent above the minimum proposed  $T_{rec}$  of  $38.5^{\circ}\text{C}$  (Fox et al., 1963) in comparison to FIXED during STHA (Duration  $T_{rec}\geq 38.5^{\circ}\text{C}$ ; ISO<sub>CONT</sub> =  $\sim 32$  min.session, ISO<sub>PROG</sub> =  $\sim 49$  min.session; FIXED  $\sim 24$  min.session) and LTHA (Duration  $T_{rec}\geq 38.5^{\circ}\text{C}$ ; ISO<sub>CONT</sub> =  $\sim 32$  min.session, ISO<sub>PROG</sub> =  $\sim 46$  min.session; FIXED  $\sim 18$  min.session), with potential for more complete phenotypic adaptation as a result of consistently longer durations at higher core temperatures (Patterson et al., 2004; Regan et al., 1996; Taylor and Cotter, 2006). Contrary to our hypothesis however, the rate or magnitude of adaptation was not different utilising our between methods, low statistical power was observed for the interaction effect in our data (change HR  $\eta^2 = 0.18$ , sweat loss  $\eta^2 = 0.17$ ,  $T_{sk}$   $\eta^2 = 0.16$ , all other variables  $\eta^2 \leq 0.10$ ), this may suggest the present study is under powered, or more likely that no difference will be observed when using sample sizes representative of other research in the field, and based upon *a priori* calculations. The between method statistical analysis implemented may additionally have yielded different observations than that of a repeated measures within method design, with the latter potentially influencing the ability to determine differences in adaptation between STHA and LTHA. FIXED heat acclimation remains a simple method for eliciting adaptation through consistent workloads, however core temperature increases could only be sustained throughout acclimation should a higher exercise intensity, longer exercise duration or elevated exogenous environmental temperature be progressively implemented to counteract the reduced endogenous strain (Galloway and Maughan, 1997; Gibson et al., 2014; Nielsen et al., 1993; Périard et al., 2012). This observation is further evidenced by decreased mean  $T_{rec}$  and mean  $T_{rec\text{final}60}$  as adaptation occurred from STHA to LTHA sessions in FIXED.

Within the isothermic methods, failure for ISO<sub>PROG</sub> to confer greater adaptation than ISO<sub>CONT</sub>, suggests a minimum thermoregulatory strain sufficient to elicit physiological adaptations are surpassed by both isothermic, and also fixed methods. Comparison of method administration data (Table 2) suggests individual variability still occurs within methods, particularly isothermic methods as during STHA, when both ISO<sub>CONT</sub> and ISO<sub>PROG</sub> are performing the same intended protocol, differences are observed in the exercising duration and duration  $T_{rec} = 38.5^{\circ}\text{C}$ . This is likely to be due to subtle differences evoked by prescribing workloads based upon a  $\% \dot{V}O_{2peak}$ . Irrespective of the variation between ISO<sub>CONT</sub> and ISO<sub>PROG</sub>, both elicit greater potentiating stimuli for adaptation than FIXED. Isothermic methods attain the optimal internal temperature for adaptation ( $38.5^{\circ}\text{C}$ ) for greater durations throughout STHA and LTHA, in line with seminal work in the field (Fox et al., 1963). Isothermic data are similar to that observed during short duration, high exercise intensity heat acclimation ( $75\% \dot{V}O_{2peak}$  for  $30 - 35 \text{ min} \cdot \text{day}^{-1}$ ), which was found to elicit identical adaptation to a longer duration, low exercise intensity heat acclimation method ( $50\% \dot{V}O_{2peak}$  for  $60 \text{ min} \cdot \text{day}^{-1}$ ) similar to FIXED (Houmard et al., 1990). Short duration, moderate intensity exercise-heat stress followed by passive rest (isothermic methods) more closely representing competition or training, is equally as effective as longer, lower intensity exposures at inducing adaptations. The benefit of isothermic methods being higher initial workloads reduce exercising durations in comparison to lower intensity continuous fixed methods. The differences in core temperature, and subsequent duration and intensity of work performed between continuous and progressive isothermic methods occur as a result of recent observations that absolute  $\dot{V}O_2$  is most closely related to metabolic heat production (Smoljanic et al., 2014) (Table 1), and that to ensure equal comparison between groups, workload could be more closely controlled using a workload prescription method other than  $\% \dot{V}O_{2peak}$  (Cramer and Jay, 2014), the authors propose that prescribing heat acclimation utilising workloads known to elicit desired rates of metabolic heat production may reduce variations in heat gain particularly in an unacclimated individual with relatively lower sweat and evaporative losses. Additionally intermittent exercise, which the latter stages of isothermic protocols can mimic, is known to elicit greater thermal and cardiovascular strain than continuous exercise of the same average intensity (Taylor and Cotter, 2006).

#### **4.3 Physiological mechanisms of heat acclimation**

Resting  $T_{rec}$  and HR reductions were observed towards the latter end of the sessional heat acclimation data (Figure 2) and during HSTs following STHA and LTHA (Figure 3). The same magnitude and rate of adaptation indicated that

these primary physiological adaptations to heat acclimation regimes occurred using all methods (Garrett et al., 2011; Sawka et al., 2011; Taylor, 2014). Resting and mean exercising  $T_{rec}$  reduced after STHA, but were not further enhanced after LTHA for all methods in accordance with previous work (Buono et al., 1998; Kampmann et al., 2008). Though comparable with some previous data to determine temperature responses to heat stress (Druyan et al., 2013; Moran et al., 2006), the mean/change  $T_{rec}$  observed during the HSTs were not as high as observed using alternative protocols to determine physiological responses to heat stress (Magalhães et al., 2010a; Périard et al., 2012). This may have affected the ability of the test to determine core temperature differences augmented by the different heat acclimation methods, or between STHA and LTHA. Additionally, a cycling rather than running heat stress test may have yielded different  $T_{rec}$  responses specific to the exercise modality of the heat acclimation methods. This may also be true of a test implementing workloads specific to one particular exercise domain or prescribing an intensity more closely reflecting athletic competition or occupational activity. Modified temperature thresholds and plasticity of the hypothalamic neurons within the thermoregulatory centre (Boulant, 2006) and afferent peripheral-central drive (Horowitz, 2014) are proposed mechanisms for this phenomenon, although the molecular role of prostaglandin E2 (PGE2), cyclooxygenase (COX)-2 and orexin cannot be excluded (Shin et al., 2013). Change in  $T_{rec}$  did not attenuate from HST1 following STHA or LTHA, therefore adaptations did not offset rate of heat gain (Schlader et al., 2011a). Reduced HR following STHA, combined with lower  $T_{rec}$ , indicated lower overall physiological strain during HST2, but a further five days of any heat acclimation method did not elicit further adaptations at HST3 (Kampmann et al., 2008).

Sweat losses increased (Figure 3), likely contributing, alongside reduced skin blood flow (Kenefick et al., 2007) to the reduced mean and peak  $T_{sk}$  following STHA, but was not enhanced by a LTHA period of 10 days. Though commonly reported as an adaptation following LTHA (Buono et al., 2009; Chinevere et al., 2008), improved sweat loss after STHA is not unique to our data (Machado-Moreira et al., 2006). Our data show enhanced sudomotor function from STHA in all methods of heat acclimation; as such different work duration or intensity does not induce different sweat rate adaptation. It is conceivable that other thermoregulatory adaptations inhibited the requirement for elevated sweat loss during HST3 rather than a plateau in adaptation being apparent. This is somewhat supported by our daily heat acclimation session data (Figure 2), whereby increased sweat losses were observed beyond the STHA timescale. It is likely that a lowered internal temperature threshold for sweating was induced by each heat acclimation method (Armstrong and Kenney, 1993; Cotter et al., 1997; Gonzalez et al., 1974; Hessemer et al., 1986;

Nadel et al., 1974; Patterson et al., 2004; Roberts et al., 1977; Shido et al., 1999). In conjunction with reduced  $T_{rec}$ , this adaptation afforded participants an improved centrally-mediated tolerance to exercise-heat stress with the increased sweat loss a consequence of a greater duration spent sweating within the session. This adaptation is facilitated by an earlier onset of sweating (Shido et al., 1999). Heat acclimation is also known to induce peripheral changes at the sweat gland to sweat response during exercise-heat stress (Buono et al., 2009; Fox et al., 1964; Lorenzo and Minson, 2010). Increased cholinergic sensitivity of the eccrine sweat gland or increased glandular hypertrophy is induced by heat acclimation (Sato and Sato, 1983). Sweat adaptations through central (threshold for sweat onset) and peripheral (sweat gland function) mechanisms combine with reductions in the core temperature threshold for cutaneous vasodilation (Buono et al., 1998; Fujii et al., 2012; Hessemer et al., 1986; Nielsen et al., 1997; Yamazaki and Hamasaki, 2003) to confer adaptation decreasing mean and peak  $T_{sk}$  by sweat evaporation (Figure 3). These adaptations combined to reduce  $T_{sk}$ , permitting greater direction of cardiac output to active muscles, as opposed to cutaneous anatomy (González-Alonso et al., 1999) reducing cardiovascular strain in the heat as evidenced by our heart rate data.

The observation that thermal sensation further reduced from STHA to LTHA is potentially beneficial for performance in the heat (Figure. 3), it is believed that thermal discomfort drives true behavioural thermoregulation (Flouris, 2011). Initiation of each of these response pathways during exercise elicit behavioural responses are known to lower work rate (Tucker et al., 2006, 2004). This is an undesirable with regards to optimal performance in the heat however the role of thermal comfort/sensation and pacing are yet to be fully elucidated with contrasting data at present likely due to difference experimental design (Barwood et al., 2014, 2012; Schlader et al., 2011b).

#### **4.4 Applications for practitioners**

Environmental conditions in this study were at the upper range of that typically prescribed for heat acclimation. Current practice dictates practitioners aiming to induce heat acclimation would typically administer exercise-heat stress environments at lower ambient temperature and humidity. We propose that in environments of lower heat stress, that isothermal heat acclimation would provide optimal conditions for adaptation compared to fixed intensity methods, where adaptations would not be augmented to the same extent on a sessional basis due to reductions in strain during the acclimation process. This is most relevant during LTHA. With reference to isothermic modes of heat acclimation, as exogenous heat stress reduces, the exercise or training load would increase,

potentially reducing the efficacy of this method. Hotter or more humid conditions would offset this, eliciting more rapid increases in  $T_{rec}$  than cooler conditions (Gibson et al., 2014). Integration of heat acclimation into an athletic taper (Garrett et al., 2011; Mujika et al., 2004) is potentially problematic, requiring acknowledgement of increased work demands of exercise in increasing thermal environments (Galloway and Maughan, 1997), which subsequently decreases with attainment of heat acclimation (Sawka et al., 1983). We suggest practitioners wishing to induce heat acclimation at a time of athletic taper should prescribe isothermic heat acclimation under conditions of greater exogenous heat stress than forthcoming competition, to facilitate maximal thermal adaptation for reduced exercise training requirement. The reduction in training volume being an essential component of the taper (Spilsbury et al., 2014), establishing cardiorespiratory, vascular, haematological and neuromuscular changes which ultimately contribute towards optimal performance (Mujika et al., 2004). Additionally due to the greater absolute  $\dot{V}O_2$ , and consequently metabolic heat production, for the same relative workload athletes typically exhibit, the rate of rectal temperature increase is likely to be greater in a trained vs untrained population, reducing the duration taken to reach the 38.5°C core temperature target facilitating greater rest periods.

#### **4.5 Future research directions**

Future work could involve the implementation of an isothermic method where workload is implemented using a fixed relative metabolic heat production or relative power, as opposed to a relative workload such as  $\% \dot{V}O_{2max}$ , which may further optimise adaptation by reducing our observed individual variability associated with metabolic heat production and retention (Cramer and Jay, 2014). Additionally due to the linear relationship between HR and  $\dot{V}O_2$ , this physiological measurement may be viable for prescribing work rate during heat acclimation. A comparison of the inducibility of changes in sweat composition, skin blood flow and plasma volume expansion in response to the different heat acclimation modes are yet to be elucidated. It also remains uncertain whether isothermic heat acclimation is a more efficient method than fixed intensity heat acclimation for preparing highly trained individuals for exercise heat stress (Garrett et al., 2012). Highly trained individuals are likely to be able to sustain the greater absolute workloads required of the isothermic methods, with higher metabolic heat production elevating the rate of core temperature more rapidly (Cramer et al., 2012; Garrett et al., 2012, 2011) and thus, giving greater competition specificity to their acclimation, further enhancing the efficacy of isothermic methods for this population.

## **5. Conclusions**

All heat acclimation methods tested in this study were able to induce the heat-adapted phenotype following five days of heat acclimation and therefore have merit towards attenuating increased physiological strain when exercising in the heat. Based upon our data, the implementation of ten days of heat acclimation did not elicit greater adaptation than five days with the exception of thermal sensation. We have identified that no difference in the extent of adaptation exists between fixed intensity, continuous isothermic, and progressive isothermic heat acclimation methods. Isothermic methods may be more favourable for athletes aiming to integrate heat acclimation into a pre competition taper due to reduced exercise durations and mean session intensities.

## **Abbreviations**

FIXED: Fixed intensity heat acclimation experimental group; HR: Heart rate; HST: Heat Stress Test; ISO<sub>CONT</sub>: Continuous isothermic heat acclimation experimental group; ISO<sub>PROG</sub>: Progressive isothermic heat acclimation experimental group; LTHA: Long term heat acclimation; STHA: Short term heat acclimation; T<sub>rec</sub>: Rectal temperature; T<sub>sk</sub>: Skin temperature; V O<sub>2peak</sub>: Peak Oxygen Uptake.

## **Competing interests**

The authors declare that they have no competing interests.

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Table 1. Mean  $\pm$  SD Participant characteristics and descriptive data for the initial workload in fixed intensity heat acclimation (FIXED), continuous isothermic heat acclimation (ISO<sub>CONT</sub>), and progressive isothermic heat acclimation (ISO<sub>PROG</sub>) experimental groups.

|  | FIXED<br>(n = 8) | ISO <sub>CONT</sub><br>(n = 8) | ISO <sub>PROG</sub><br>(n = 8) |
|--|------------------|--------------------------------|--------------------------------|
| Age<br>(years)   | 19.9 $\pm$ 1.0   | 22.6 $\pm$ 5.5                 | 26.1 $\pm$ 4.9*                |
| Height<br>(cm)   | 179.3 $\pm$ 5.8  | 177.9 $\pm$ 5.8                | 179.5 $\pm$ 6.6                |
| Body Mass<br>(kg)  | 79.2 $\pm$ 18.3  | 74.2 $\pm$ 6.9                 | 75.1 $\pm$ 8.8                 |
| Body Mass Index<br>(kg.m <sup>-2</sup> )                   | 24.6 $\pm$ 5.7   | 23.4 $\pm$ 1.7                 | 23.4 $\pm$ 3.2                 |
| Body Surface Area<br>(m <sup>2</sup> )                     | 1.97 $\pm$ 0.21  | 1.92 $\pm$ 0.11                | 1.94 $\pm$ 0.11                |
| Body fat<br>(%)  | 14.9 $\pm$ 7.7   | 14.8 $\pm$ 2.2                 | 14.1 $\pm$ 3.5                 |
| $\dot{V}O_{2peak}$<br>(L.min <sup>-1</sup> )               | 3.61 $\pm$ 0.90  | 3.63 $\pm$ 0.69                | 3.80 $\pm$ 0.55                |
| Initial Workload<br>(W.kg <sup>-1</sup> )                  | 1.6 $\pm$ 0.5    | 2.2 $\pm$ 0.3*                 | 2.4 $\pm$ 0.4*                 |
| Initial metabolic heat production<br>(W.kg <sup>-1</sup> ) | 9.5 $\pm$ 3.3    | 11.1 $\pm$ 1.4                 | 11.9 $\pm$ 2.0                 |

\*denotes significantly difference from FIXED ( $p < 0.05$ )

Table 2. Mean  $\pm$  SD Protocol, thermoregulatory and physiological response data for STHA, then LTHA of fixed intensity heat acclimation (FIXED), continuous isothermic heat acclimation (ISO<sub>CONT</sub>), and progressive isothermic heat acclimation (ISO<sub>PROG</sub>) experimental groups.

|   | STHA             |                     |                     | LTHA               |                     |                     |
|---|------------------|---------------------|---------------------|--------------------|---------------------|---------------------|
|   | FIXED            | ISO <sub>CONT</sub> | ISO <sub>PROG</sub> | FIXED              | ISO <sub>CONT</sub> | ISO <sub>PROG</sub> |
| Exercising Duration (min)                             | 450 $\pm$ 0      | 337 $\pm$ 47 #      | 263 $\pm$ 47 †      | 900 $\pm$ 0 *      | 707 $\pm$ 102 * #   | 598 $\pm$ 87 * †    |
| Time to target T <sub>rec</sub> (min)                 | 89 $\pm$ 2       | 49 $\pm$ 12 #       | 43 $\pm$ 10 #       | 89 $\pm$ 2         | 54 $\pm$ 14 * #     | 52 $\pm$ 10 * #     |
| Mean Session Intensity (% $\dot{V}O_{2peak}$ )        | 50.0 $\pm$ 0.0   | 40.6 $\pm$ 6.2 #    | 33.5 $\pm$ 7.2 #    | 50.0 $\pm$ 0.0     | 43.9 $\pm$ 6.0 *    | 38.1 $\pm$ 7.1 * #  |
| Mean Exercise Intensity (% $\dot{V}O_{2peak}$ )       | 50.0 $\pm$ 0.0   | 55.2 $\pm$ 6.4      | 57.7 $\pm$ 5.3      | 50.0 $\pm$ 0.0     | 56.7 $\pm$ 5.8      | 57.5 $\pm$ 4.4      |
| Mean Session Power (W.kg <sup>-1</sup> )              | 1.6 $\pm$ 0.4    | 1.4 $\pm$ 0.1       | 1.3 $\pm$ 0.3       | 1.6 $\pm$ 0.4      | 1.5 $\pm$ 0.2 *     | 1.4 $\pm$ 0.3 *     |
| Mean Exercise Power (W.kg <sup>-1</sup> )             | 1.6 $\pm$ 0.4    | 1.9 $\pm$ 0.3       | 2.2 $\pm$ 0.5 #     | 1.6 $\pm$ 0.4      | 2.0 $\pm$ 0.2       | 2.2 $\pm$ 0.5 #     |
| Total Work Done (kJ)                                  | 3352 $\pm$ 815   | 2789 $\pm$ 358      | 2590 $\pm$ 560      | 6701 $\pm$ 1603 *  | 6113 $\pm$ 834 *    | 5880 $\pm$ 1484 *   |
| Mean T <sub>rec</sub> (°C)                            | 38.03 $\pm$ 0.16 | 38.18 $\pm$ 0.12    | 38.26 $\pm$ 0.18 #  | 37.92 $\pm$ 0.15 * | 38.16 $\pm$ 0.12 #  | 38.26 $\pm$ 0.20 #  |
| Mean T <sub>recfinal60min</sub> (°C)                  | 38.28 $\pm$ 0.18 | 38.44 $\pm$ 0.13    | 38.60 $\pm$ 0.18 #  | 38.16 $\pm$ 0.18 * | 38.43 $\pm$ 0.14 #  | 38.63 $\pm$ 0.21 #  |
| $\Delta$ T <sub>rec</sub> (°C)                        | 1.72 $\pm$ 0.58  | 1.74 $\pm$ 0.19     | 2.15 $\pm$ 0.38     | 1.63 $\pm$ 0.60    | 1.78 $\pm$ 0.22     | 2.27 $\pm$ 0.29 #   |
| Rate T <sub>rec</sub> increase (°C.hr <sup>-1</sup> ) | 1.43 $\pm$ 0.43  | 1.98 $\pm$ 0.31     | 2.42 $\pm$ 0.54     | 1.34 $\pm$ 0.46    | 1.99 $\pm$ 0.36     | 2.36 $\pm$ 0.50     |
| Duration T <sub>rec</sub> $\geq$ 38.5°C (min)         | 118 $\pm$ 53     | 161 $\pm$ 62        | 244 $\pm$ 62 †      | 176 $\pm$ 86 *     | 318 $\pm$ 118 *#    | 462 $\pm$ 120 * †   |



|   |          |          |          |          |         |              |
|---|----------|----------|----------|----------|---------|--------------|
| Duration $T_{rec} \geq 39.0^\circ\text{C}$<br>(min) | 17 ± 21  | 5 ± 14 + | 38 ± 32  | 21 ± 24  | 13 ± 24 | 146 ± 70 * † |
| Mean HR<br>(b.min <sup>-1</sup> )                   | 155 ± 13 | 150 ± 9  | 142 ± 11 | 150 ± 13 | 150 ± 9 | 143 ± 11     |

\* denotes significantly different ( $p < 0.05$ ) from STHA (within group), # denotes significantly different ( $p < 0.05$ ) from FIXED (within timescale), † denotes significantly different ( $p < 0.05$ ) from FIXED and ISO<sub>CONT</sub> (within timescale), + denotes significantly different ( $p < 0.05$ ) from ISO<sub>PROG</sub> within timescale.

Table 3. Mean  $\pm$  SD Heat Stress Test data at baseline (HST1), post five HA sessions (HST2) and post ten HA sessions (HST3) of the fixed intensity heat acclimation (FIXED), continuous isothermic heat acclimation (ISO<sub>CONT</sub>), and progressive isothermic heat acclimation (ISO<sub>PROG</sub>) experimental groups.

|  | HST1             |                     |                     | HST2              |                     |                     | HST3              |                     |                     |
|--|------------------|---------------------|---------------------|-------------------|---------------------|---------------------|-------------------|---------------------|---------------------|
|  | FIXED            | ISO <sub>CONT</sub> | ISO <sub>PROG</sub> | FIXED             | ISO <sub>CONT</sub> | ISO <sub>PROG</sub> | FIXED             | ISO <sub>CONT</sub> | ISO <sub>PROG</sub> |
| Duration (min)                               | 25.0 $\pm$ 8.0   | 29.4 $\pm$ 1.8      | 29.4 $\pm$ 1.8      | 25.0 $\pm$ 8.0    | 30.0 $\pm$ 0.0      | 30.0 $\pm$ 0.0      | 25.6 $\pm$ 8.2    | 30.0 $\pm$ 0.0      | 30.0 $\pm$ 0.0      |
| Rest HR (b.min <sup>-1</sup> )               | 74 $\pm$ 8       | 71 $\pm$ 9          | 63 $\pm$ 10         | 65 $\pm$ 11*      | 66 $\pm$ 8*         | 59 $\pm$ 9*         | 69 $\pm$ 9*       | 63 $\pm$ 4*         | 56 $\pm$ 12*        |
| Change HR (b.min <sup>-1</sup> )             | 107 $\pm$ 9      | 113 $\pm$ 13        | 118 $\pm$ 11        | 115 $\pm$ 16      | 105 $\pm$ 12        | 111 $\pm$ 9         | 111 $\pm$ 11      | 110 $\pm$ 10        | 115 $\pm$ 10        |
| Mean exercising HR (b.min <sup>-1</sup> )    | 161 $\pm$ 10     | 159 $\pm$ 9         | 154 $\pm$ 17        | 152 $\pm$ 12*     | 147 $\pm$ 10*       | 145 $\pm$ 13*       | 153 $\pm$ 9*      | 148 $\pm$ 8*        | 144 $\pm$ 18        |
| Rest T <sub>rec</sub> (°C)                   | 37.23 $\pm$ 0.35 | 37.05 $\pm$ 0.21    | 36.94 $\pm$ 0.40    | 36.94 $\pm$ 0.36* | 36.95 $\pm$ 0.21*   | 36.73 $\pm$ 0.41*   | 36.90 $\pm$ 0.40* | 36.96 $\pm$ 0.19*   | 36.75 $\pm$ 0.2     |
| Change T <sub>rec</sub> (°C)                 | 1.18 $\pm$ 0.44  | 1.61 $\pm$ 0.31     | 1.48 $\pm$ 0.19     | 1.25 $\pm$ 0.57   | 1.39 $\pm$ 0.35     | 1.44 $\pm$ 0.25     | 1.20 $\pm$ 0.47   | 1.28 $\pm$ 0.38     | 1.41 $\pm$ 0.3      |
| Rate T <sub>rec</sub> (°C.hr <sup>-1</sup> ) | 2.35 $\pm$ 0.87  | 3.21 $\pm$ 0.62     | 2.97 $\pm$ 0.39     | 2.49 $\pm$ 1.13   | 2.77 $\pm$ 0.71     | 2.87 $\pm$ 0.49     | 2.39 $\pm$ 0.94   | 2.56 $\pm$ 0.75     | 2.82 $\pm$ 0.7      |
| Mean T <sub>rec</sub> (°C)                   | 37.77 $\pm$ 0.30 | 37.76 $\pm$ 0.17    | 37.56 $\pm$ 0.39    | 37.58 $\pm$ 0.34* | 37.57 $\pm$ 0.19*   | 37.41 $\pm$ 0.41*   | 37.45 $\pm$ 0.30* | 37.52 $\pm$ 0.27*   | 37.35 $\pm$ 0.4     |
| Sweat Loss (L.hr <sup>-1</sup> )             | 1.45 $\pm$ 0.50  | 1.61 $\pm$ 0.43     | 1.28 $\pm$ 0.42     | 1.88 $\pm$ 0.75*  | 1.95 $\pm$ 0.36*    | 1.96 $\pm$ 0.80*    | 2.16 $\pm$ 0.61*  | 2.17 $\pm$ 0.61*    | 1.73 $\pm$ 0.5      |
| Peak T <sub>sk</sub> (°C)                    | 37.72 $\pm$ 0.98 | 37.40 $\pm$ 0.78    | 37.52 $\pm$ 0.48    | 36.95 $\pm$ 1.11* | 36.74 $\pm$ 0.32*   | 36.81 $\pm$ 0.84*   | 37.16 $\pm$ 0.83* | 36.73 $\pm$ 0.62*   | 36.73 $\pm$ 0.8     |
| Mean T <sub>sk</sub> (°C)                    | 35.70 $\pm$ 1.07 | 36.07 $\pm$ 0.67    | 36.05 $\pm$ 0.52    | 35.47 $\pm$ 1.12* | 35.65 $\pm$ 0.38*   | 35.38 $\pm$ 0.86*   | 35.74 $\pm$ 0.99* | 34.93 $\pm$ 0.71*   | 35.13 $\pm$ 0.7     |
| Peak RPE                                     | 13 $\pm$ 3       | 16 $\pm$ 3          | 15 $\pm$ 2          | 14 $\pm$ 4        | 15 $\pm$ 3          | 14 $\pm$ 3          | 14 $\pm$ 3        | 14 $\pm$ 3          | 14 $\pm$ 4          |

|          |           |           |           |           |           |           |             |             |            |
|----------|-----------|-----------|-----------|-----------|-----------|-----------|-------------|-------------|------------|
| Mean RPE | 13 ± 3    | 14 ± 3    | 13 ± 1    | 13 ± 3    | 14 ± 3    | 13 ± 2    | 13 ± 3      | 13 ± 3      | 13 ± 2     |
| Peak TSS | 7.1 ± 1.0 | 6.9 ± 0.8 | 6.7 ± 0.5 | 6.8 ± 0.8 | 6.7 ± 1.0 | 6.7 ± 0.4 | 6.5 ± 1.0*# | 6.3 ± 1.0*# | 6.3 ± 0.5* |
| Mean TSS | 6.8 ± 1.0 | 6.3 ± 0.6 | 6.3 ± 0.4 | 6.5 ± 0.9 | 6.3 ± 0.8 | 6.3 ± 0.5 | 6.0 ± 0.8#  | 6.1 ± 0.5#  | 6.2 ± 1.0# |

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\* denotes HST significantly different (p <0.05) from HST1 overall. # denotes HST significantly different (p <0.05) from HST2 overall.

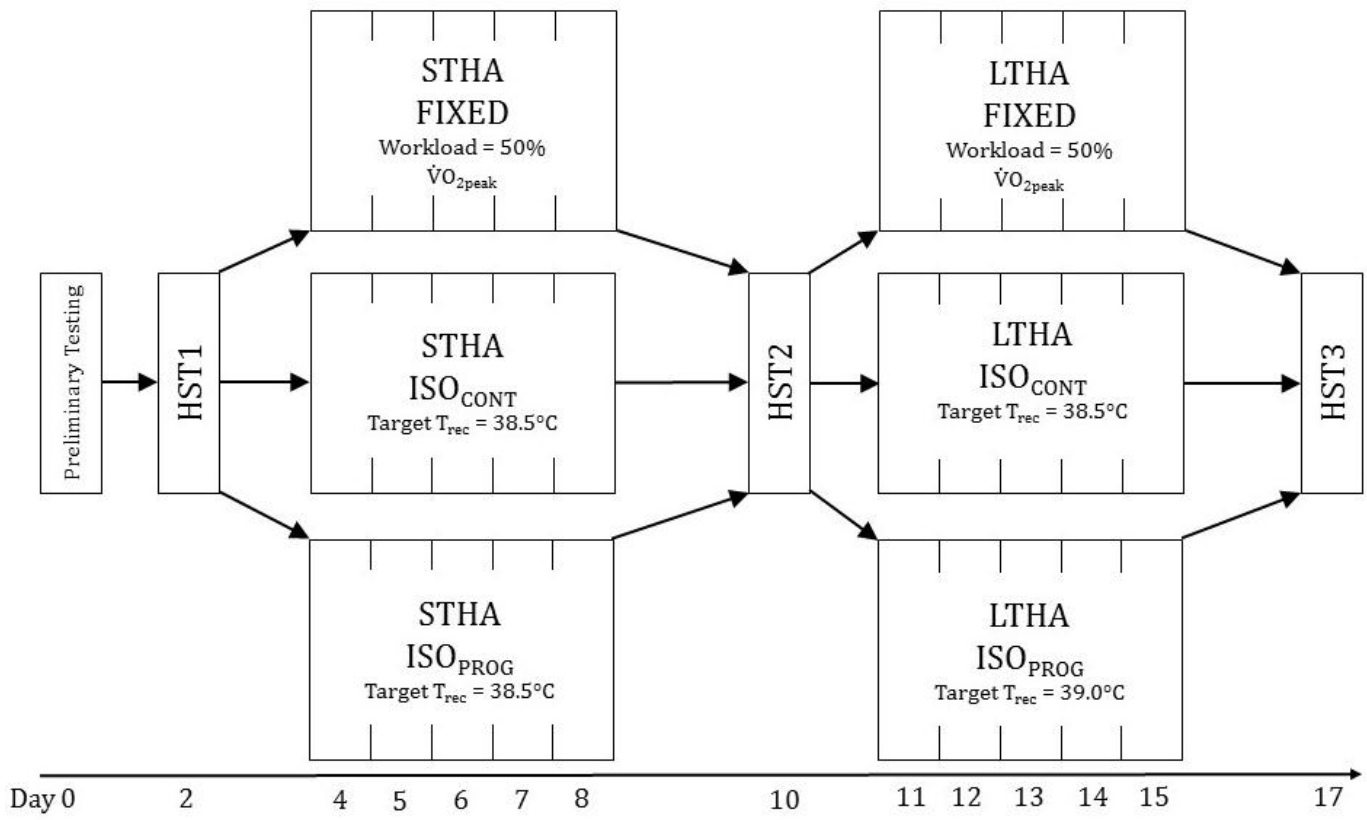
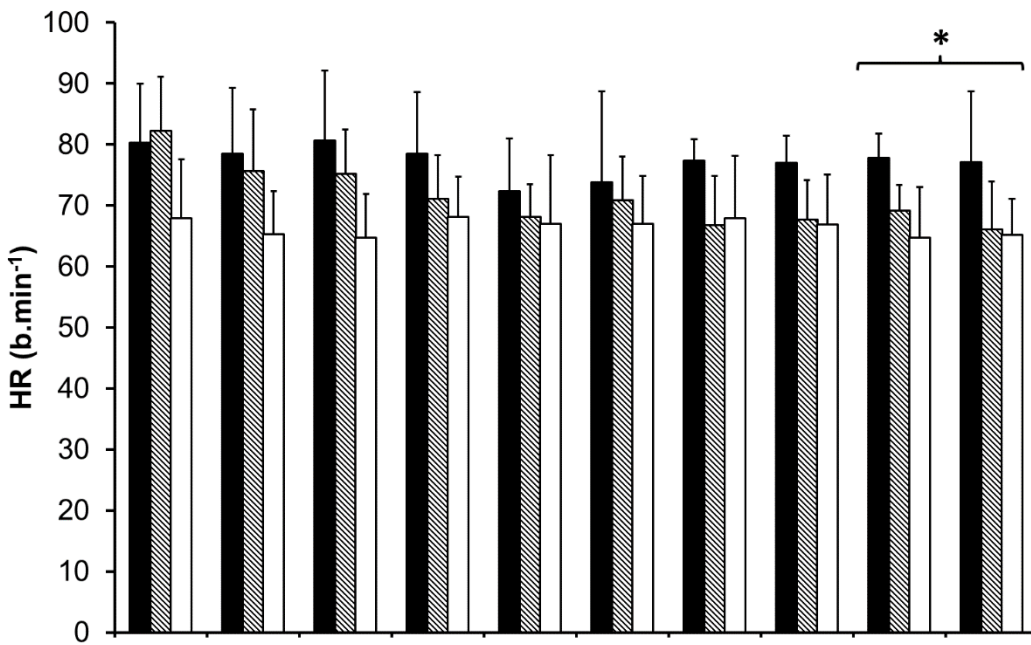
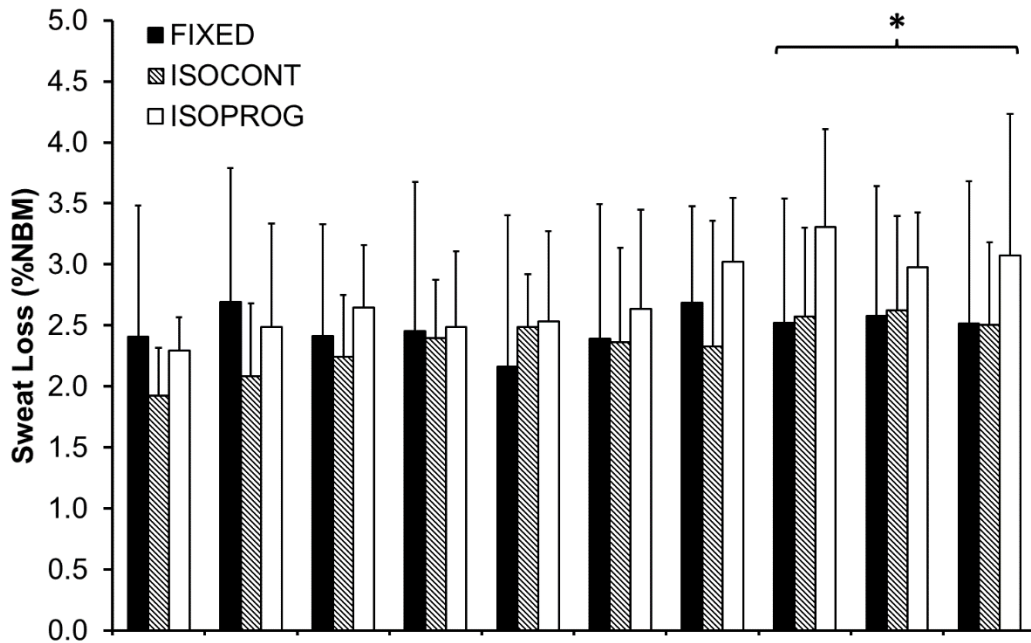


Figure 1. Experimental Schematic. See text for details.



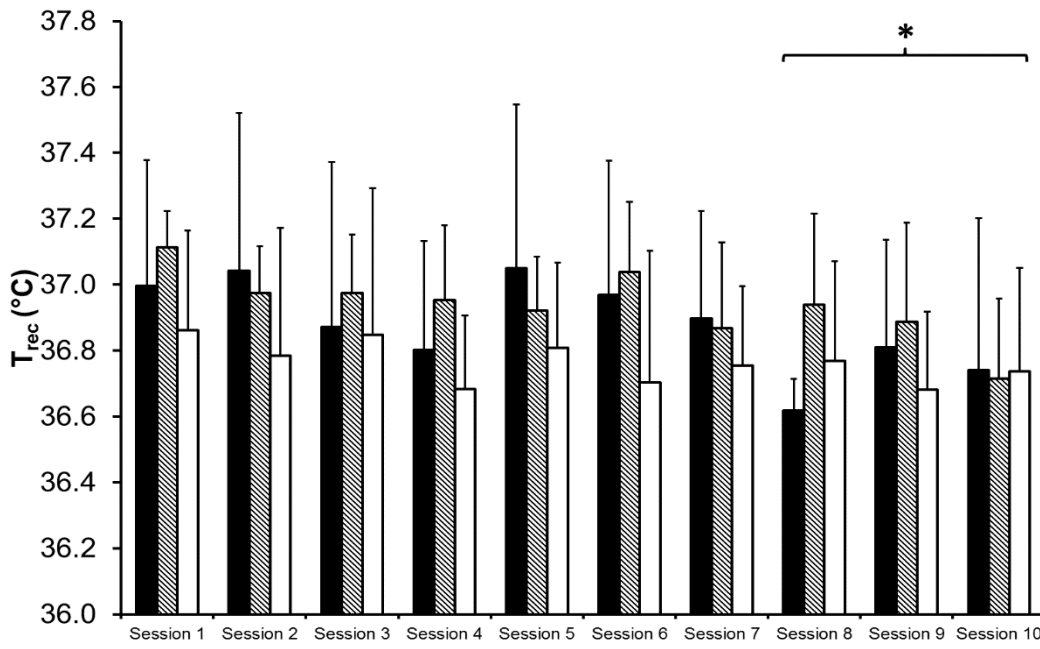


Figure 2. Mean  $\pm$  SD Sessional resting  $T_{rec}$ , resting HR and sweat loss adaptations to FIXED, ISO<sub>CONT</sub>, and ISO<sub>PROG</sub> HA methods. \* denotes difference from session one ( $p < 0.05$ ).

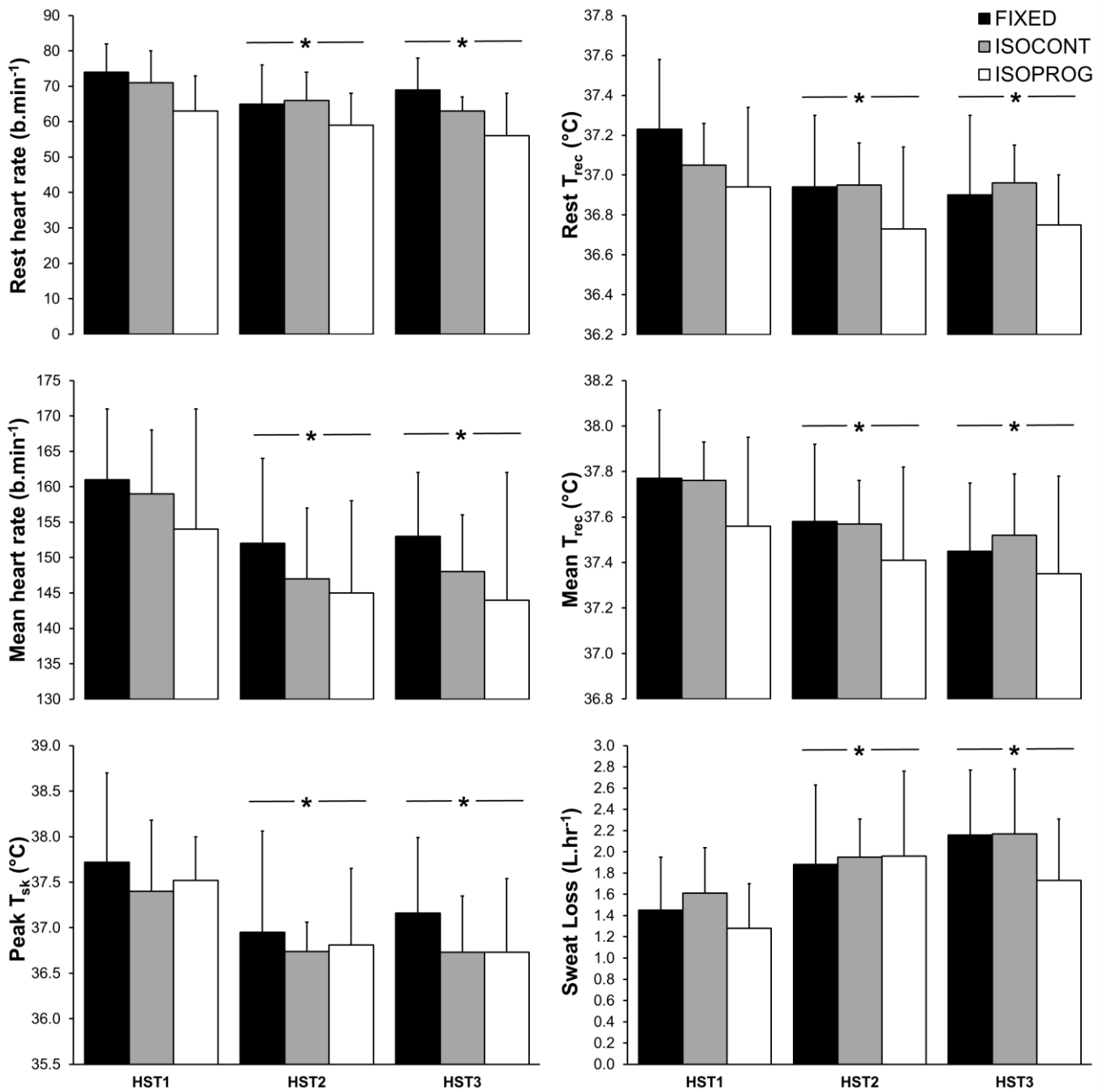


Figure 3. Mean  $\pm$  SD Physiological Heat Stress Test data at baseline (HST1), post STHA sessions (HST2) and post LTHA (HST3) of FIXED, ISO<sub>CONT</sub> and ISO<sub>PROG</sub>. \* denotes HST significantly different ( $p < 0.05$ ) from HST1 overall.

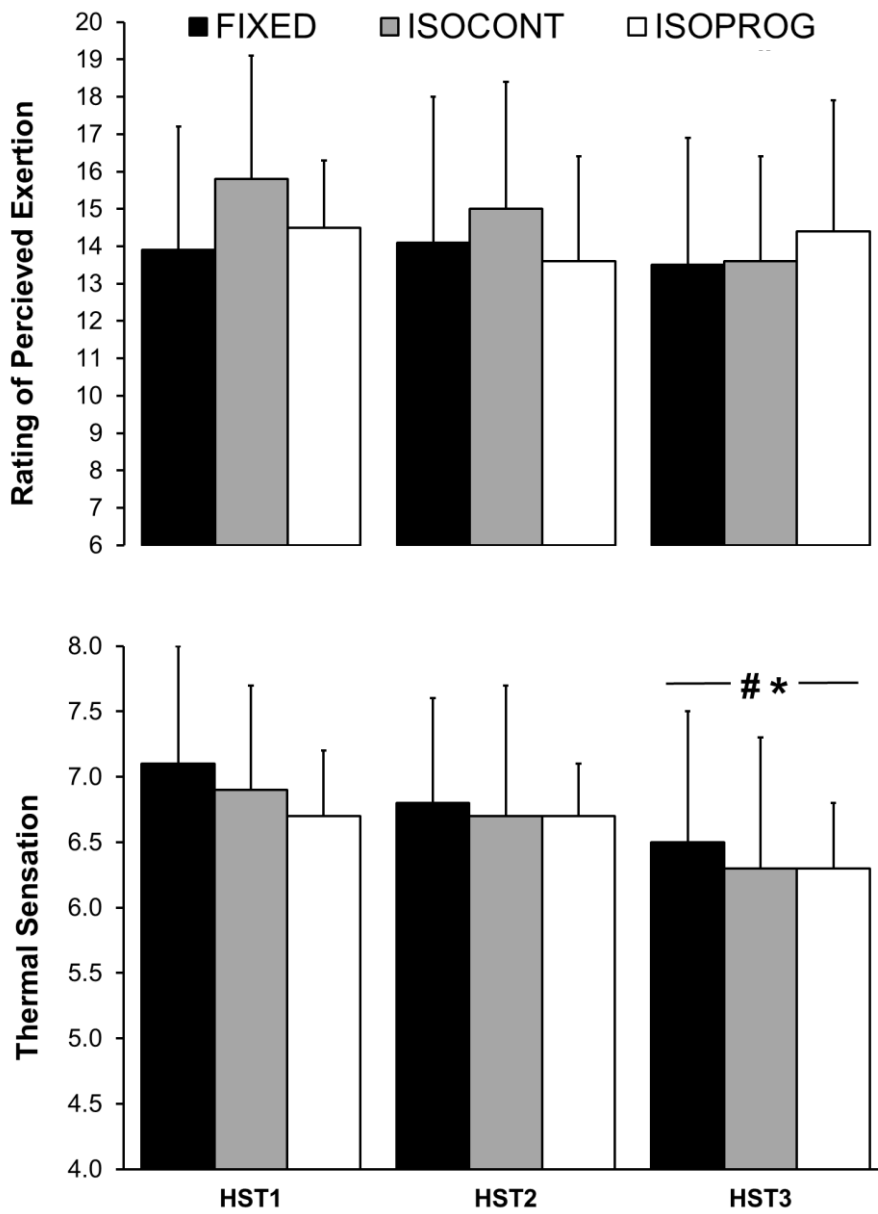


Figure 4. Mean  $\pm$  SD Perceptual Heat Stress Test data at baseline (HST1), post STHA sessions (HST2) and post LTHA (HST3) of FIXED, ISO<sub>CONT</sub> and ISO<sub>PROG</sub>. \* denotes HST significantly different ( $p < 0.05$ ) from HST1 overall. # denotes HST significantly different ( $p < 0.05$ ) from HST2 overall.



**Oliver Gibson** began his PhD at the University of Brighton in 2010 after receiving his master's degree in Applied Exercise Physiology in 2009 and undergraduate degree in Sport and Exercise Science in 2007. Oliver's PhD examines the cellular stress response to acute and chronic exercise heat stress. Oliver is a technical instructor for the undergraduate and postgraduate Sport and Exercise Science degrees at the University of Brighton, and combines his thermoregulatory doctoral research with research allied to intermittent sprint performance and training in hypoxia, and ergogenic aids to improve endurance performance.



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**Dr. James Tuttle** is a molecular exercise physiology PhD student and laboratory technician at the University of Bedfordshire. James' PhD investigated the contribution of heat and mechanical stress to heat shock protein (HSP) gene expression and mechanisms through which HSPs attenuate exercise induced muscle damage. James' other research interests include the applied exercise physiology of endurance sports particularly middle and long distance running.



**Dr. Lee Taylor** is a senior lecturer at the University of Bedfordshire. Lee is a member of the Institute of Sport and Physical Activity (ISPAR Bedford) and Muscle Cellular and Molecular Physiology (MCMP) Applied Sport and Exercise Physiology (ASEP) research groups. Lee's research interests are allied to the *in vivo* stress response to both environmental (hypoxia, hypothermia, hyperthermia and hyperbaria) and/or exercise stress. Specific "stress response" related interests include heat shock proteins (HSPs), disturbances to redox balance and other pro-inflammatory cytokine responses (IL-6, TNF- $\alpha$ , etc). Additionally Lee has published data implementing the use of various strategies (pre-cooling, pharmacological agents, acclimation strategies, etc) to ameliorate the negative impact such environments can have on exercise performance and occupational pursuits.



**Dr. Peter Watt** is a reader at the University of Brighton within the Centre for Sport and Exercise Science and Medicine (SESAME) and is the research theme champion for Exercise and Health. Peter's research primarily focuses on the application of stable isotope methods to measure metabolic and physiological changes occurring in humans during exercise, with application to health related problems, e.g. diabetes, obesity. Peter is also research active in areas including exercise and nutritional interactions which affect muscle growth and function, ammonia and fatigue, the effects of exercise and hypoxia on whole body metabolism in diabetes, and physiological responses to heat stress.



**Dr. Neil Maxwell** joined the University of Brighton as a lecturer in sport and exercise science in 1997. Neil continues to lecture undergraduate and postgraduate students, predominantly in the area of exercise and environmental physiology and research methods. Neil is research active and has published extensively in the international, scientific literature in areas allied to thermal and hypoxic stress and how the body tolerates each, particularly during exercise. He is an approved higher degrees supervisor with MPhil. /PhD. completions, external examination experience and a bank of existing postgraduate research students.

