Title: Once- and twice-daily heat acclimation confer similar heat adaptations, inflammatory responses and exercise tolerance improvements.

Running Title: Once- vs. twice-daily heat acclimation

Authors:

1A.G.B Willmott, 1M. Hayes, 1,2C.A James, 1J. Dekerle, 1,3O.R Gibson and 1N.S Maxwell

Address for Authors:

1Environmental Extremes Laboratory, University of Brighton, Eastbourne, UK, 2Institut Sukan Negara (National Sports Institute), National Sports Complex, Kuala Lumpur, Malaysia, 3Centre for Human Performance, Exercise and Rehabilitation (CHPER), Brunel University London, Uxbridge, UK

Details for the Corresponding Author:

Ashley Willmott – A.Willmott2@brighton.ac.uk

Word Count:

Abstract Word Count:

Tables: 5

Figures: 3
Abstract

This experiment aimed to investigate the efficacy of twice-daily, non-consecutive heat acclimation (TDHA) in comparison to once-daily heat acclimation (ODHA) and work matched once- or twice-daily temperate exercise (ODTEMP, TDTEMP) for inducing heat adaptations, improved exercise tolerance, and cytokine (immune) responses. Forty males, matched biophysically and for aerobic capacity, were assigned to ODHA, TDHA, ODTEMP or TDTEMP. Participants completed a cycling graded exercise test, heat acclimation state test and a time to task failure (TTTF) at 80% peak power output in temperate (TTTFTEMP: 22°C/40% RH) and hot conditions (TTTFHOT: 38°C/20% RH), before and after 10-sessions (60-min of cycling at ~2W.kg⁻¹) in 45°C/20% RH (ODHA and TDHA) or 22°C/40% RH (ODTEMP or TDTEMP). Plasma IL-6, TNF-α and cortisol were measured pre- and post-sessions 1, 5 and 10. ODHA and TDHA induced equivalent heat adaptations (P<0.05) (resting rectal temperature [-0.28±0.22, -0.28±0.19°C], heart rate [-10±3, -10±4 b.min⁻¹] and plasma volume expansion [+10.1±5.6, +8.5±3.1%]) and improved heat acclimation state (sweat setpoint [-0.22±0.18, -0.22±0.14°C] and gain [+0.14±0.10, +0.15±0.07g.sec⁻¹.°C⁻¹]). TTTFHOT increased (P<0.001) following ODHA (+25±4%) and TDHA (+24±10%), but not ODTEMP (+5±14%) or TDTEMP (+5±17%). TTTFTEMP did not improve (P>0.05) following ODHA (+14±4%), TDHA (14±8%), ODTEMP (9±10%) or TDTEMP (8±13%). Acute (P<0.05) but no chronic (P>0.05) increases were observed in IL-6, TNF-α or cortisol during ODHA and TDHA, or ODTEMP and TDTEMP. Once- and twice-daily heat acclimation conferred similar magnitudes of heat adaptation and exercise tolerance improvements, without differentially altering immune function, thus non-consecutive TDHA provides an effective, logistically flexible method of HA, benefitting individuals preparing for exercise-heat stress.

New and Noteworthy

- Greater heat adaptations and enhanced exercise performance in the heat were induced by 10-sessions of consecutive once-daily and non-consecutive twice-daily heat acclimation, compared with equivalent temperate exercise, without adverse inflammatory or stress responses.
- No difference in the magnitude of adaptation and enhanced exercise performance were observed between either non-consecutive twice-daily, or consecutive once-daily heat acclimation when protocols were matched for volume and intensity.
- Non-consecutive twice-daily heat acclimation provides an alternate method to consecutive once-daily heat acclimation to induce heat adaptation without requiring consecutive day training.

Glossary of terms

- Blood lactate concentration – [La]b
- Body surface area – BSA
- Body mass index – BMI
- Change – Δ
Cycling graded exercise test – GXT
Gross mechanical efficiency – GME
Heart rate – HR
Heat acclimation – HA
Heat acclimation state test – HAST
Interleukin-6 – IL-6
Lactate threshold – LT
Long-term heat acclimation – LTHA
Medium-term heat acclimation – MTHA
Metabolic heat production – $\dot{H}_{\text{prod}}$
Once-daily heat acclimation – ODHA
Onset of blood lactate accumulation – OBLA
Peak oxygen uptake – $\dot{V}O_2$
Peak power output – PPO
Plasma volume – PV
Rating of perceived exertion – RPE
Rectal temperature – $T_r$
Relative humidity – RH
Respiratory exchange ratio – RER
Short-term heat acclimation – STHA
Sodium concentration – $[Na^+]$
Thermal comfort – TC
Thermal sensation – TSS
Time to task failure – TTTF
Time to task failure in heat stress – TTTF\text{HOT}
Time to task failure in temperate conditions – TTTF\text{TEMP}
Tumour necrosis factor-alpha – TNF-$\alpha$
Twice-daily heat acclimation – TDHA
Urine colour – $U_{\text{col}}$
Urine osmolality – $U_{\text{osm}}$
Urine specific gravity – $U_{\text{sg}}$
Ventilation – $V_E$
Volume of oxygen uptake – $\dot{V}O_2$
Whole-body sweat loss – WBSL
Introduction

Heat acclimation (HA) is an important preparation strategy preceding exercise-heat stress (64,70) to alleviate physiological strain (61), attenuate heat related illness (HRI) (94), improve thermal perception (33) and exercise tolerance in hot (56), and possibly temperate conditions (50). A variety of HA strategies currently exist, predominantly differentiated by exercise-heat stress volume, and/or intensity (18,80). In this regard, HA may be applied within sporting and occupational settings (e.g. military), with current recommendations advocating the use of repeated, consecutive once-daily exertional heat exposures for 60-100-min, utilising an isothermic protocol (70). In spite of multiple manipulations of volume/intensity, the optimal frequency for HA remains largely unknown (83). Current recommendations for once-daily exposures are implied more readily than non-consecutive [e.g. 10-sessions in 21-days (32)] and twice-daily exposures [e.g. 100-min vs. 2x50-min (49)], due to the consistency of potentiating stimuli for adaptation e.g. daily elevations in rectal [Tre] and skin temperature alongside profuse sweating, which are required to evoke a multitude of physiological and perceptual adaptations (74). From a practical perspective, implementing consecutive-day protocols is challenging given access to hot-humid conditions is not ubiquitous, and the need for daily exposures is likely to interrupt sport/occupational-specific training, competition tapering and, or travel/recovery schedules. Medium- (MTHA: 10-14-days) and long-term (LTHA: >14-days) protocols which maximise adaptations exacerbate these challenges, a factor which may provide some explanation as to why, in spite of clear recommendations from the scientific community, only ~15% of athletes undertook HA prior to competition in heat stress (66).

We have previously shown that four HA sessions i.e. a short-term HA (STHA) intervention (89), administered over two consecutive days (i.e. twice-daily HA [TDHA]), demonstrated comparable adaptations to four consecutive once-daily HA (ODHA) sessions. However the magnitudes of adaptation using STHA are typically smaller than MTHA/LTHA interventions, thus the need to examine the efficacy of a twice-daily approach over longer periods exists. Furthermore given challenges associated with consecutive day interventions, completing TDHA intermittently (e.g. over non-consecutive days), over MTHA/LTHA timescales, may be desirable given an improved ability to integrate HA into complex training and travel schedules, potentially reducing disruption. For example, by administering the same number of HA sessions (i.e. the same dose) non-consectutively, athletes may be afforded recovery days during HA or have the ability to perform specific training on non-HA days. Whilst hypothetically beneficial, investigations are needed to assess the efficacy of this strategy, particularly given different markers of heat adaptation have differing timecourses for induction (67) and the associations between adaptation and performance enhancement are not ubiquitously reported. Previous research findings are equivocal, with sub-optimal adaptations reported during non-consecutive versus daily HA (32), attributable to heat decay (88) and insufficient physiological stimulus (4). Consequently, refining non-consecutive protocols so that the timescale, protocol and dose are in line
with best practice recommendations i.e. using an isothermic model of ~10-sessions over 10-14-days (70) and thus, ensuring twice-daily methods implement appropriate potentiating stimuli, may ameliorate current limitations and provide an alternative strategy for practitioners who pursue HA benefits but prioritise training quality and recovery schedules.

Whilst acute exercise-heat stress is unlikely to impair immune function (84, 85), few studies have investigated immunological biomarkers during HA despite the potential for immunological perturbations to culminate in exacerbated inflammatory (e.g. interleukin-6 [IL-6] and tumour necrosis factor-alpha [TNF-α]) and stress responses (e.g. cortisol) (14, 92), potentially increasing HRI susceptibility (48) and diminishing the application and efficacy of HA (34, 69). Investigation of inflammatory responses to once-daily isothermic HA reported few negative findings (14), however the immune response to our proposed twice-daily model of matched volume (dose), but altered frequency, remains unknown and maladaptation may be a concern. Therefore, investigation is required given the repeated exercise-induced hyperthermia, coupled with shorter recovery time during the ‘heat days’ of TDHA that may result in an overload of physiological strain, inducing residual stress between sessions (72).

This study investigated the efficacy of short- (i.e. 5-sessions) and medium-term (i.e. 10-sessions) HA, using non-consecutive TDHA and consecutive ODHA protocols, and compared these to temperate exercise groups (i.e. once-daily: ODTEMP and non-consecutive twice-daily: TDTEMP) as frequency and duration matched exercise controls. Secondly, this study investigated exercise tolerance through the determinants of aerobic performance, and subsequent performance in both hot and temperate conditions between interventions. Finally, this study also investigated the inflammatory and stress responses during interventions to determine whether a compromised immune function was an artefact of the twice-daily protocol. It was hypothesised that as the dose of HA was the same, TDHA would induce the same physiological and ergogenic benefits as ODHA, with both TDHA and ODHA superior to ODTEMP and TDTEMP. Given the alteration in frequency of the HA dose, it was hypothesised that the reduced duration between TDHA sessions would lead to undesirable inflammatory/stress responses in comparison to ODHA.

Methods

Participants and ethical approval

Forty moderately-trained [performance level 3 (62)] males provided informed consent to participate in the experiment, which was approved by the University of Brighton Institution’s Research Ethics and Governance Committee and conducted in accordance with Declaration of Helsinki (2013). Participants were matched for biophysical characteristics and aerobic capacity and assigned to; consecutive ODHA,
non-consecutive TDHA, consecutive ODTEMP or non-consecutive TDTEMP. No differences in participant characteristics were observed ($P>0.05$ [Table 1]),

***Add Table 1 near here***

**Experimental design**

Prior to group allocation, participants completed four tests comprising; cycling graded exercise test (GXT), heat acclimation state test (HAST) and time to task failure test in hot (TTTF$_{HOT}$) and temperate conditions (TTTF$_{TEMP}$), in a semi-randomised order, 48-hr apart with the GXT completed first. Interventions consisted of, 60-min exercise sessions performed in hot (45°C, 20% RH) or temperate conditions (22°C, 40% RH) over a 12-day period. Post-tests were repeated in the same order 48-hr apart (Figure 1). This study was completed during November-February, with trials occurring at the same time of day to minimise the effect of circadian variation on exercise tolerance (21) and thermoregulation (86). Participants avoided alcohol and caffeine 12-hr before experimentation, arrived in a euhydrated state (73) and replicated food intake the day of the each exercise trial (2).

***Add Figure 1 near here***

**Determinants of aerobic performance - Graded exercise test (GXT)**

Height (Detecto Scale Company, USA) and body mass (Adam Equipment Inc., USA) were measured, enabling the estimation of body surface area (BSA) (5). Skinfold thickness was measured (Harpenden, Baty International, UK) across four sites (22) to estimate body fat (%) (76). The GXT was completed on an electronically-braked stationary ergometer (SRM High performance model, Germany) within temperate conditions (22°C, 40% RH). Power output was initially set at 80 W and increased by 24 W every stage (3-min), with cadence kept at 80 rev.min$^{-1}$. Capillary blood lactate concentration ([La]$_b$) was sampled within the final 30-s of each stage and analysed immediately (2300 Plus, YSI, USA). Breath-by-breath metabolic gas data were continuously collected (Metalyzer 3B, Cortex, Germany). Lactate threshold (LT) was determined by an increase ($>1$ mmol.L$^{-1}$) in [La]$_b$ above resting level (15) and the test was terminated when the onset of blood lactate accumulation (OBLA) occurred ($>4$ mmol.L$^{-1}$) (93). Gross mechanical efficiency (GME) was calculated from steady-state oxygen consumption and respiratory exchange ratio (RER $<1.0$) values collected during the final 30-s of each stage of the LT test (25). Following 15-min rest, participants performed a second test with an initial power output 48 W below OBLA that was increased by 20 W.min$^{-1}$ until volitional exhaustion (39). Peak oxygen uptake (VO$_{2peak}$) and power output (PPO) were determined as the highest average VO$_2$ and power output during the final 30-s of each stage. Following 15-min rest, participants were familiarized to the TTTF at 80% of their PPO.

**Aerobic performance - Time to task failure (TTTF)**
TTTFTEMP (22°C, 40% RH) and TTTFHOT (38°C, 40% RH) were completed at 80% of PPO (51) on a modified cycle ergometer (SRM crankset and wireless PowerControl meter on a Monark 874E, Sweden). Following a standardised warm-up (2-min seated rest, 5-min at 90% of LT, 3-min rest and then 3-min of unloaded pedalling at 80 rev.min⁻¹), power output was increased to 80% PPO. HR, Tref and metabolic gas data were collected every minute and RPE was recorded at task failure (i.e. when cadence failed <77 rev.min⁻¹ for >3-s following a warning). Power output, HR and time were obscured with only cadence displayed.

Heat acclimation state test (HAST)

HASTs were completed in hot-dry conditions (45°C, 15% RH) within an environmental chamber (TISS, UK) on a cycle ergometer (Monark 620, Sweden). HASTs simulated Havenith and Middendorp (1986) protocol, but prescribed exercise intensities at given rates of Ḥprod relative to body mass (3.0, 4.5 and 6.0 W.kg⁻¹) (91). Heat acclimation state was identified via sweat setpoint and sweat gain measures (37). Metabolic energy expenditure was estimated from known values of VO₂ and RER below LT during the GXT (58). Ḥprod was subsequently calculated and associated exercise intensities prescribed (Cramer and Jay 2014) during the HAST, which were re-calculated post-intervention.

Heat acclimation and temperate exercise protocols

Participants completed ten 60-min exercise sessions over 12-days. Once-a-day groups (ODHA, ODTEMP) exercised on days 1-5 and 8-12 at 08:00-hr, whereas twice-daily groups (TDHA, TDTEMP) exercised twice on days 1, 3, 8 and 10 at 08:00-hr and 16:00-hr, and then once on days 5 and 12 at 08:00-hr (Figure 1). Exercise commenced at 2.3 W.kg⁻¹ (~65% VO₂peak) for 15-min at 80 rev.min⁻¹, in line with recommended guidelines to rapidly attain the desired change in core temperature (31, 41). Power output was subsequently altered depending on changes in Tref (∆Tref) and perceived effort (55), to target a Tref of ≥38.5°C for the remainder of the session (80) (see Table 2 for actual training data). To amplify ∆Tref, upper-body sauna suits (Everlast, London, UK) (90) were worn during the initial 15-min of exercise. This method has been applied prior to HA (53) to increase physiological strain without increasing exercise intensity or volume (19, 90). Physiological and perceptual measures were recorded at rest and every 5-min during exercise for all 10 sessions. During sessions 1, 5 and 10, fluid ingestion was prohibited for accurate estimation of sweat loss. Participants were permitted to drink ab libitum during the remaining sessions (55). Euhydration was determined on arrival to each session by collection of mid flow urine; colour <3 (Ucol), osmolality <700 mOsmol.kg⁻¹ (Uosm) (Osmocheck, Vitech Scientific Ltd., Japan) and specific gravity <1.020 (Usg) (hand refractometer, Atago, Japan) (73). HR was manually recorded (Polar Electro, Oy, Finland) and Tref was continuously monitored using a thermistor probe (Henleys Medical Supplies, UK) self-inserted 10 cm past the anal sphincter. Whole-body sweat loss (WBSL) was estimated for each session from towel dried nude body mass differences pre- to post-exercise. Sweat samples (~2 mL) were collected in a Tegaderm+Pad (3M™, USA) placed on the
midpoint of the trapezius before being analysed for sodium concentration ([Na⁺]) using a Sweat-Chek™ (Eli Tech Group, Wescor Inc., USA) for sessions 1, 5 and 10.

225 Phlebotomy and biochemistry

Following 10-min of seated rest immediately before and after sessions 1, 5 and 10, fingertip capillary blood (~200 µL) was sampled for haemoglobin (HemoCue, Ltd., Sweden) and haematocrit (Hawksley and Sons Ltd., England) to estimate ΔPV (20). A 10 mL venepuncture sample was also collected from the antecubital fossa, transferred into two 5 mL tubes (EDTA Sarstedt, Akteingesellschaft and Co, Germany), centrifuged (Eppendorf 5702 R Centrifuge, UK) for 10-min at 5000 rev.min⁻¹, and then plasma stored at -86°C. Upon analysis, commercially available ELISA kits were used to measure IL-6 and TNF-α (Ready Set Go!®, eBioscience, Affymetrix Inc., USA) and cortisol (Sigma-Aldrich, USA) in duplicate and corrected for ΔPV.

234 Perceptual measures

RPE (6) from 6 (No exertion) to 20 (Maximal Exertion), thermal sensation scale [TSS (82) from 0 (Very Very Cold), 4 (Neutral) to 8 (Very Very Hot)] and thermal comfort [TC (95) from 0 (Very Comfortable) to 5 (Very Uncomfortable)], were collected during exercise sessions every 5-min following familiarisation.

239 Data and statistical analyses

All data are reported as mean ± SD, with statistical significance set at \( P<0.05 \). Data were assessed and conformed to normality and sphericity prior to further statistical analysis. Within-group differences for pre-intervention data sets were analysed using a one-way ANOVA. To assess intervention efficacy, physiological, performance and perceptual data were analysed using a three-way mixed design ANOVA (Time*Condition*Frequency), for time (pre- to post-intervention), condition (HA and TEMP) and frequency (once- and twice-daily exercise). Following a significant F-value, follow up Bonferroni-corrected post-hoc comparisons were used. Predefined analytical limits to highlight meaningful heat adaptations were; \( \Delta T_{re}>0.20°C \), \( \Delta HR>5 \text{ b.min}^{-1} \), \( \Delta WBSL>200 \text{ mL} \), \( \Delta PV>5\% \) and >1 in perceptual scales (RPE, TSS and TC) (92). Typical error of measurement (TEM) were used to determine meaningful differences for sweat setpoint (0.21°C), sweat gain (0.09 g.sec⁻¹.°C⁻¹), TTTF test (15%), \( \dot{V}O_{peak} \) test (4.8%), IL-6 (2 pg.mL⁻¹), TNF-α (1 pg.mL⁻¹) and cortisol (57 nmol.L⁻¹). Isotime data (i.e. task failure time-point pre-intervention compared to the corresponding time-point post-intervention) was also analysed.

253 Results

254 Heat adaptations
During both ODHA and TDHA interventions, resting Tre, resting HR, and sweat [Na⁺] were reduced, while WBSL and PV were increased within session 5 (STHA) and 10 (MTHA) \((P<0.05)\) compared to session 1 (Table 2). The highest recorded perceptual measures (i.e. peak RPE, TSS and TC) were also lower \((P<0.05)\) from session 1-5 (STHA), and 1-10 (MTHA) during ODHA and TDHA. These physiological and perceptual adaptations were greater following session 10 (MTHA) compared to session 5 (STHA) \((P<0.05)\). Adaptations did not differ between HA groups (all \(P>0.05)\), but larger magnitudes in adaptations were observed compared to both TEMP interventions \((P<0.05)\) (Table 2).

There were no differences \((P>0.05)\) between groups for exercise time, intensity or work completed during the HA or TEMP sessions. However, as expected physiological strain (i.e. time >38.5°C and \(\Delta T_{re}\)) was larger \((P<0.05)\) during HA compared to TEMP (Table 3). Exercise time and work completed during exercise sessions were greater \((P<0.05)\) between session 1-10 (MTHA) and session 1-5 (STHA) for each group (Table 3).

Post-intervention HASTs demonstrated reductions in sweat setpoint, HR\(_{peak}\) and TC\(_{peak}\), and improvements in sweat gain and WBSL \((P<0.05)\) for ODHA and TDHA groups, with greater improvements compared to TEMP \((P<0.05)\), yet no differences were found between HA protocols \((P>0.05)\) (Table 2).

Exercise tolerance

Determinants of aerobic performance - GXT

A main effect was found for power output at LT and VO\(_{2peak}\) \((P<0.05)\), with a greater \((P<0.05)\) improvement following HA (ODHA and TDHA), compared to TEMP (ODTEMP and TDTEMP; Table 4). No Time\(^*\)Condition\(^*\)Frequency interaction \((P>0.05)\) was found for any GXT data. No improvements \((P>0.05)\) were found in PPO or GME.

Aerobic performance - TTTF

Pre-intervention TTTF\(_{HOT}\) was shorter (all \(P<0.001)\) compared to TTTF\(_{TEMP}\) for all groups, with no between-group differences \((P>0.05)\).

TTTF\(_{HOT}\) improved \((P<0.001)\) following ODHA and TDHA, but not ODTEMP or TDTEMP \((P>0.05)\), whereas TTTF\(_{TEMP}\) did not improve \((P>0.05)\) following any intervention (Table 4). Following TDHA and ODHA only, Tre and HR were lower at isotime \((P<0.05)\) during TTTF\(_{HOT}\) and TTTF\(_{TEMP}\) (Table 5).

Biomarkers

***Add Table 2 and 3 near here***

Exercise tolerance

Determinants of aerobic performance - GXT

A main effect was found for power output at LT and VO\(_{2peak}\) \((P<0.05)\), with a greater \((P<0.05)\) improvement following HA (ODHA and TDHA), compared to TEMP (ODTEMP and TDTEMP; Table 4). No Time\(^*\)Condition\(^*\)Frequency interaction \((P>0.05)\) was found for any GXT data. No improvements \((P>0.05)\) were found in PPO or GME.

Aerobic performance - TTTF

Pre-intervention TTTF\(_{HOT}\) was shorter (all \(P<0.001)\) compared to TTTF\(_{TEMP}\) for all groups, with no between-group differences \((P>0.05)\).

TTTF\(_{HOT}\) improved \((P<0.001)\) following ODHA and TDHA, but not ODTEMP or TDTEMP \((P>0.05)\), whereas TTTF\(_{TEMP}\) did not improve \((P>0.05)\) following any intervention (Table 4). Following TDHA and ODHA only, Tre and HR were lower at isotime \((P<0.05)\) during TTTF\(_{HOT}\) and TTTF\(_{TEMP}\) (Table 5).

***Add Table 4 and 5, and Figure 2 near here***

Biomarkers
Increased plasma [IL-6], [TNF-α] and [cortisol] \((P<0.05)\) were observed from pre- to post-session 1, 5 and 10 during both HA and TEMP protocols (Figure 3). Inflammatory and stress responses were greater for HA compared to TEMP with larger mean: \(\Delta\text{IL-6} \) values following session 1, 5 and 10 \((P<0.001)\); \(\Delta\text{TNF-α} \) following session 1 and 10, but not 5 when comparing HA to ODTEMP only \((P<0.05)\); and \(\Delta\text{cortisol} \) following session 5 for ODHA vs. TEMP, and following session 5 and 10 for TDHA vs. TEMP (Figure 3). No differences in inflammatory or stress responses were observed between the HA protocols at any time point \((P<0.05)\). Interestingly, there was no evidence of chronic effects over the course of HA or TEMP \((P>0.05)\), however there was a trend \((P<0.10)\) for the \(\Delta\text{IL-6} \) and \(\Delta\text{cortisol} \) to be lower and \(\Delta\text{TNF-α} \) to be higher for session 10 when compared to the other sessions for ODHA and TDHA only.

***Add Figure 3 near here***

**Discussion**

In agreement with our hypothesis, ODHA and TDHA induced comparable heat adaptations to one another, thus demonstrating an improved heat acclimation state compared to ODTEMP and TDTEMP. Improvements in power at LT and \(\dot{V}_\text{O}_2\text{peak} \) were found following HA, in addition to both ODHA and TDHA enhancing performance (TTTF) in hot, but not temperate conditions, an improvement that was not observed by either TEMP group. Inflammatory responses increased acutely following single sessions in all groups, with larger responses during HA vs TEMP. However, contrary to our hypothesis, no difference was observed between ODHA and TDHA groups. These data highlight that non-consecutive TDHA presents no difference to ODHA, inducing similar heat adaptation and improvements in exercise tolerance during heat stress, without compromising immune status. These findings suggest the dose of HA (e.g. matched weekly exposure and intensity) is most important for the mechanisms which underpin adaptation, as opposed to the structure of HA (e.g. frequency [once- or twice-daily] and timing [morning or afternoon]).

**Heat adaptations**

HA efficacy was confirmed by the acquisition of key physiological heat adaptations including reductions in resting \(T_e\) (-0.3°C) and HR (-10 b.min\(^{-1}\)), \([\text{Na}^+]\) retention (-14 to -27 mmol.L\(^{-1}\)) and, increased WBSL (+398 to +533 mL) and PV expansion (+8.5 to +10.1) (Table 2). Proportional improvements were also observed following just 5-sessions (i.e. STHA). Reductions in RPE (-2) and TSS (-0.7 to -0.9), and an improved TC (-1), also demonstrate positive perceptual improvements following 10-sessions of both ODHA and TDHA. Collectively, these adaptations are in line with a recent meta-analysis on HA (83) and, whilst direct comparisons across studies are difficult due to differences in HA exercise protocols, MTHA studies (i.e. once-daily) do report equivalent magnitudes of adaptation to the present study [e.g. resting \(T_e\): -0.17°C, and HR: -5 b.min\(^{-1}\), \([\text{Na}^+]\) retention: -
 adaptation superior to our predefined analytical limits ($\Delta T_{re}>0.20^\circ C$, $\Delta HR>5\ b.min^{-1}$, $\Delta WBSL>200\ mL$, $\Delta PV>5\%$ and $>1$ in perceptual scales [RPE, TSS and TC] (92)) highlighting meaningful heat adaptations, a critical factor when assessing intervention strategies.

Both HA strategies improved heat acclimation state, as indicated by a lower sweat setpoint (-0.22°C) and a larger sweat gain (+0.14 to +0.15 g.sec$^{-1}.^\circ C^{-1}$) during the post-intervention HAST (Table 2). Whilst no reductions in $\Delta T_{re}$, $T_{repeak}$, RPE$_{peak}$ or TSS$_{peak}$ occurred following HA, this can be explained by the re-prescription of exercise intensities, thus, controlling for $\dot{H}_{prod}$ post-intervention and providing confidence in our adaptations. The unchanged $T_{re}$ but larger WBSL (both +35%) shows thermosensitivity is enhanced via increased sweat gain for ODHA (+48%) and TDHA (+49%). Though these changes are superior to the meta-analysis findings (+25% (83)) and TEM (0.09 g.sec$^{-1}.^\circ C^{-1}$ (91)) the authors accept that an oesophageal core temperature and real-time local sweat rate measurements would offer superior assessment of these data given a more rapid response in comparison to our rectal thermistor (81). Parallel reductions in resting $T_{re}$ and sweat setpoint, following ODHA (-0.28 and -0.22°C) and TDHA (-0.28 and -0.22°C), respectively, agree with meta-analysis findings (-0.28 (83)) and are larger than the TEM (0.21°C (92)). MTHA (e.g. 10-sessions) induced greater magnitudes of physiological and perceptual heat adaptation compared to STHA (e.g. 5-sessions [Table 2 and 3]). Though not in agreement with all experimental data (27, 28), these findings agree with consensus recommendations that longer-term HA (e.g. $\geq$10-days) is preferable to induce greater physiological heat adaptations (67, 70) achieved in this study through the maintained physiological strain imposed using isothermic prescription (80). These data provide supporting evidence that medium- to long-term HA could be prescribed immediately before, or potentially several weeks before major athletic competition or military deployment in heat stress (18) to induce greater initial adaptations, as opposed to solely implementing STHA during the final training microcycle. This notion, alongside the decay of these aforementioned adaptations (18), should be experimentally examined as this strategy would allow alternate approaches (e.g. intermittent ‘top up’ exposures in the days preceding exposure) to be implemented to maintain the enhanced heat acclimation state (8).

Seminal work by Lind and Bass (49) demonstrated the benefits of continuous, once-daily HA (i.e. 100-min sessions), as opposed to longer and shorter intermittent times (e.g. twice-daily, 2x50-min), which contributed to duration recommendations for optimal heat adaptations (70). Our data indicate no advantage but more importantly, no disadvantage of non-consecutive TDHA over consecutive ODHA, agreeing with our previous STHA investigation (89). Further to this, as outlined above, these observations are true even when the session duration is 60 min (this study), as opposed to 90 – 100 min which has been previously described as preferable (70). Our novel findings are in contrast to others which have not demonstrated efficacy of TDHA (32), but may be explained by a) the use of an isothermic model, b) the matching of exercise-heat dose (e.g. duration, intensity and total number of
exposures) to induce equivalent heat adaptations and improved exercise tolerance, and/or c) more significant heat strain i.e. maximising time spent at the targeted $T_{re}$. This non-consecutive twice-daily structure is likely to be appealing to coaches and practitioners with upcoming competitions in challenging, hot conditions (e.g. Tokyo 2020 Olympic and Paralympic Games) for whom scheduling HA around sport-specific training, competition tapering, rest and travel is challenging. This study is the first to demonstrate equivalent heat adaptations following both TDHA and ODHA, with greater adaptations for longer interventions (i.e. 5- vs. 10-days) suggesting the dose of HA (i.e. attaining key physiological responses to a greater extent) is the primary factor that underpins adaptation.

### Exercise tolerance

#### Determinants of aerobic performance

Our study provides a holistic overview of the changes in exercise tolerance following non-consecutive TDHA, in comparison to consecutive ODHA and matched TEMP interventions. $\dot{V}O_{2}\text{peak}$ improved following HA (ODHA: +4.6%; TDHA: +3.7%), with this change greater than TEMP changes (ODTEMP: +2.6%; TDTEMP: +1.4%). This is likely due to hypervolemia following HA and potential increments in cardiac output (50) however it must be acknowledged that participants were not elite athletes whom as a cohort may be less responsive to this mechanism (59). Nonetheless, previous studies report ergogenic benefits of HA on $\dot{V}O_{2}\text{peak}$ and PPO in temperate conditions (23, 24, 50, 71, 75, 79) whilst others present no changes (43, 44, 55). Power at LT also improved significantly following HA, in agreement with previous findings (9, 10, 42, 45, 50, 55, 68, 71) however improvements following ODHA (+7±10 W) and TDHA (+7±8 W) were of a lower magnitude than those reported in well-trained cyclists in 13°C (+12-15 W (50)) and 22°C (+16 W [(55)] and +15 W [(71)]. Furthermore, GME did not change following interventions, in agreement with previous LTHA (43). Whilst the ergogenic benefits of HA remain disputed between research groups, potentially as a result of insufficient potentiating stimuli or inter-individual differences (13), our data are the first to demonstrate that implementing a non-consecutive twice-daily intervention does not induce differential ergogenic effects to that of a matched dose once-daily protocol, for the determinants of aerobic performance (e.g. $\dot{V}O_{2}\text{peak}$ and power at LT) in temperate conditions.

#### Aerobic performance - Time to task failure

Determinants of aerobic performance

$T_TTF_{HOT}$ improved following ODHA (+25%) and TDHA (+24%), but not ODTEMP and TDTEMP (both +5%), agreeing with previous reports following MTHA (+67% [(56)], +17% [(57)] and +24% [(17)]), which appear to exceed STHA (+14% [(26)] and +7% [(11)]) likely due to greater physiological adaptation. Evidence for $T_TTF_{HOT}$ improvements likely reflecting the magnitude HA adaptations (e.g. PV expansion improving cardiac output (50, 56), leading to increased $\dot{V}O_{2}\text{peak}$ and power at LT, resulting in a lessened physiological strain (67), is indicated by a lower mean $T_{re}$ (-0.26°C) and HR (-8 b.min⁻¹)
at isotime (Table 5). Consequently, non-consecutive TDHA appears equally effective as ODHA for improving aerobic performance (e.g. extending exercise tolerance time) in sub-elite athletes within the severe-intensity domain under heat stress. It is likely adaptations contributed to the improved TTTFTEMP following ODHA and TDHA (both +14%). However, these data describe that HA (irrespective of once- or twice-daily frequency) provided only moderate ergogenic benefits for performance in temperate conditions, opposing significant time trial improvements in 13°C (50) and 22°C (71) but agreeing with recent studies which suggest these physiological constructs are not limiting (43, 44, 55). Nonetheless, this is the first study to collectively assess TTTF in both hot and temperate conditions which, whilst demonstrating some inter-individual differences (Figure 3), describes ODHA and TDHA as providing ergogenic benefits for enhanced performance in hot conditions with data in temperate conditions encouraging, albeit not unequivocal (54, 60).

**Inflammatory and stress responses**

Agreeing with previous literature, larger ∆IL-6 and ∆TNF-α were observed during HA compared to TEMP (Figure 2) (3, 34, 35, 46, 47, 63, 77). The larger responses observed for ∆cortisol for session 5 and 10, but not 1 (1, 7, 12, 36, 40, 63, 77) during HA are a response to increased physiological strain due to the heat stress for the same absolute exercise intensity (e.g. higher T_r, ∆T_r, and HR) (77). Our changes in IL-6 (+55%), TNF-α (+45%) and cortisol (+34%) during HA were comparable in ODHA and TDHA, and are less than, or comparable to, responses published elsewhere (IL-6: +20-2000%, TNF-α: +15-65% and cortisol: +20-70%) (1, 3, 7, 14, 34, 35, 40, 46, 63, 77). Our findings also agree with reported transient ∆IL-6 during MTHA (14, 34) alongside induced heat adaptations (71) and no evidence of chronic inflammatory effects or signs of exaggerated ∆TNF-α (e.g. possible endotoxemia) (34). The absence of augmented ∆cortisol as HA progresses, conforms to previous literature describing the sensitivity of this biomarker to various stressors (1, 14, 26, 78, 87). In summary, our data indicates no chronic inflammatory effects or stress responses during ODHA and, for the first time during non-consecutive TDHA, which is likely due to the equivalent acquisition in physiological heat adaptation. These novel data provide confidence that our TDHA protocol did not induce unexpected inflammatory or stress responses which could compromise immune status in subsequent heat exposures to any greater extent than ODHA. This further strengthens the argument for TDHA when ODHA is impractical.

**Application**

The similarity of the responses to non-consecutive TDHA and ODHA, may be of particular interest to sporting and occupational organisations that require heat adaptations to lessen the physiological strain and HRI risk, and improve exercise performance in heat stress. Non-consecutive TDHA provides an alternate and flexible strategy, providing the potential to half the number of interrupted training days, thus maximising an individual’s time to complete specific (e.g. non-heat) training or rest/recover
without compromising the magnitude of adaptation. Logistically, the non-consecutive TDHA is appealing given the cost and time associated with athletes or workers travelling to specialist heat training facilities in cool climates, may be reduced if multiple heat sessions can be completed on one day. The transient nature of heat adaptations requires STHA during crucial preparation periods, where training is predominantly sport-specific with volume often adjusted to optimise recovery, thus resulting in training that opposes targetted physiological adaptations. It is unsurprising therefore, that repeated steady-state exercise during consecutive day HA, do not appear to be widely embraced by competitive athletes (65). Prescribing TDHA and specifically afternoon sessions, may also increase HA efficiency as time spent at the desired isothermic $T_e$ of $>38.5^\circ C$ was extended during afternoon compared to morning sessions (+14 vs. +6 min), yet $\Delta T_e$ were lower (+1.3°C vs. +1.6°C), thus requiring less exercise time to reach target temperatures due to circadian rhythm and higher resting $T_e$. Ultimately, shorter duration HA (~60 min) that provides sufficient physiological strain to evoke meaningful phenotypic adaptations irrespective of daily frequency and consecutive scheduling is desirable, with non-consecutive TDHA providing greater flexibility than a consecutive day protocol.

Limitations and future direction

Despite our biomarker data indicating TDHA does not induce excessive inflammatory/immune responses, our mechanistic insights are limited due to the number and timing of blood sampling. Collecting additional biomarker measures and across more time-points during the recovery phase (e.g. 1-24-hr) would provide further insight into the inflammatory responses and potential maladaptive influences on the magnitude and kinetics of heat adaptation. An extension of this work would also examine intracellular heat shock proteins (46) and the relevant gene transcripts (27) to elucidate the impact of TDHA vs. ODHA on attaining thermotolerance (46), and potential benefits across environmental stressors (29, 30). We also highlight a need to investigate the precise effect of consecutive and non-consecutive TDHA in females, who experience different thermoregulatory adaptation kinetics to males (52). Moreover, the effect of HA duration should be considered (e.g. 60-vs. 90/100-min sessions) given an extended heat dose may impact the kinetics and magnitude of both adaptation and the inflammatory responses. A paucity of data still exists to effectively characterise the rate of heat decay and re-induction of HA at a physiological and molecular level, which is critical for the implementation of all HA protocols including TDHA. Finally, we highlight the need for investigations regarding the feasibility and appropriateness of HA and other concurrent training (e.g. interval or competition specific intensity sessions) for elite athletes.

Conclusion

This is the first study to investigate the efficacy of non-consecutive twice-daily HA compared to daily HA for adaptations, biomarkers and exercise tolerance. Greater heat adaptations were induced by both once- and twice-daily HA protocols, compared with equivalent temperate exercise, without adverse
effects on inflammatory or stress responses. Exercise tolerance in heat stress was improved following
both HA protocols, yet no effect was found for matched-volume TEMP, nor were improvements found
for exercise tolerance in temperate conditions for all interventions. The concomitant increase in power
at LT and VO$_{2\text{peak}}$ following HA, reaffirms the erogenicity of HA on aerobic performance within heat
stress, although our data do not provide supportive evidence for HA to enhance aerobic performance in
temperate conditions.

Acknowledgments

The authors would like to thank all the participants who volunteered for this study.

Conflict of interest

The authors confirm there are no conflict of interest.

References

1. **Armstrong LE, De Luca JP, Hubbard RW.** Time Course of Recovery and Heat Acclimation

2. **Bailey TG, Jones H, Gregson W, Atkinson G, Cable NT, Thijssen DHJ.** Effect of ischemic
   preconditioning on lactate accumulation and running performance. *Med Sci Sports Exerc* 44:

3. **Barberio MD, Elmer DJ, Laird RH, Lee KA, Gladden B, Pascoe DD.** Systemic LPS and
   270, 2015.

4. **Barnett A, Maughan RJ.** Response of unacclimatized males to repeated weekly bouts of

5. **Du Bois D, Du Bois EF.** A formula to estimate the approximate surface area if height and weight


7. **Brenner IKM, Zamecnik J, Shek PN, Shephard RJ.** The impact of heat exposure and
   454, 1997.

8. **Casadio JR, Kilding AE, Cotter JD, Laursen PB.** From Lab to Real World : Heat Acclimation


44. **Keiser S, Flück D, Hüppin F, Stravs A, Hilty MP, Lundby C.** Heat training increases exercise
capacity in hot but not in temperate conditions: a mechanistic counter-balanced cross-over study.


69. Pyne DB, Guy JH, Edwards AM. Managing heat and immune stress in athletes with evidence-


82. Toner MM, Drolet LL, Pandolf KB. Perceptual and physiological responses during exercise


93. Winter EM, Andrew M., Davison RCR, Bromley PD, Mercer TH. Sport and exercise physiology testing guidelines: the British Association of Sport and Exercise Sciences guide.
Figure Legends

Figure 1. Schematic design of the study. Note. HAST, TTTF_{HOT} and TTTF_{TEMP} performed in randomised order. GXT = graded exercise test, HAST = heat acclimation state test, TTTF = time to task failure in hot (HOT) or temperate (TEMP) conditions, ODHA = once daily heat acclimation, TDHA = twice daily, non-consecutive day heat acclimation, ODTEMP = once daily temperate training, TDTEMP = twice daily, non-consecutive day temperate training.

Figure 2. Mean ± SD Mean ± SD changes in the determinants of aerobic performance and aerobic performance in hot and temperate conditions. *represents a significant ($P<0.05$) within-group difference pre- to post-session. ‡represents a significant ($P<0.05$) between-group difference with (HA vs. TEMP). Shapes denote individual participants within group.

Figure 3. Mean ± SD changes in cortisol, TNF-$\alpha$ and IL-6 for session 1, 5, 10. *represents a significant ($P<0.05$) within-group difference pre- to post-session. †represents a significant ($P<0.05$) between-group difference with ODTEMP. ‡represents a significant ($P<0.05$) between-group difference with TDTEMP. Shapes denote individual participants within group.
Table 1. Mean ± standard deviation (SD) participant characteristics.

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (years)</th>
<th>Body mass (kg)</th>
<th>Height (m)</th>
<th>BMI (kg.m²)</th>
<th>BSA (m²)</th>
<th>Sum of skinfolds (mm)</th>
<th>Body fat (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ODHA</strong></td>
<td>23±6</td>
<td>77.2±10.0</td>
<td>1.78±0.08</td>
<td>24.4±2.1</td>
<td>1.95±0.16</td>
<td>34.5±7.3</td>
<td>14.9±2.7</td>
</tr>
<tr>
<td><strong>TDHA</strong></td>
<td>25±7</td>
<td>75.3±9.5</td>
<td>1.79±0.04</td>
<td>23.4±2.5</td>
<td>1.94±0.13</td>
<td>33.4±9.9</td>
<td>14.3±3.7</td>
</tr>
<tr>
<td><strong>ODTEMP</strong></td>
<td>22±1</td>
<td>77.3±8.6</td>
<td>1.77±0.04</td>
<td>25.5±3.0</td>
<td>1.92±0.10</td>
<td>35.7±6.4</td>
<td>15.0±1.7</td>
</tr>
<tr>
<td><strong>TDTEMP</strong></td>
<td>22±1</td>
<td>75.2±7.8</td>
<td>1.78±0.07</td>
<td>23.8±1.5</td>
<td>1.93±0.14</td>
<td>33.8±7.5</td>
<td>14.6±2.9</td>
</tr>
</tbody>
</table>

Table 2. Mean ± SD changes (Δ) in heat adaptations over days 1-5 (short-term) and days 1-10 (medium-term) and during the heat acclimation state pre-post intervention.

<table>
<thead>
<tr>
<th>Group</th>
<th>ODHA</th>
<th>TDHA</th>
<th>ODTEMP</th>
<th>TDTEMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Session</td>
<td>1-5</td>
<td>1-10</td>
<td>1-5</td>
<td>1-10</td>
</tr>
<tr>
<td>Heat adaptations</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔRest T&lt;sub&gt;rc&lt;/sub&gt; (°C)</td>
<td>-0.18±0.27*</td>
<td>-0.28±0.22*</td>
<td>-0.22±0.17*</td>
<td>-0.28±0.19*</td>
</tr>
<tr>
<td>ΔRest HR (b.min&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>-5±1*</td>
<td>-10±3*</td>
<td>-5±5*</td>
<td>-10±4*</td>
</tr>
<tr>
<td>ΔPV (%)</td>
<td>+6.3±4.0</td>
<td>+10.1±5.6*</td>
<td>+5.4±4.0</td>
<td>+8.5±3.1*</td>
</tr>
<tr>
<td>ΔWBSL (mL)</td>
<td>+230±207*</td>
<td>+533±261*†+</td>
<td>+178±142*†‡+</td>
<td>+398±97*†‡+</td>
</tr>
<tr>
<td>Δ [Na&lt;sup&gt;+&lt;/sup&gt;] (mmol.L&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>-13±13*†‡</td>
<td>-27±19*†‡+</td>
<td>-7±6</td>
<td>-14±5*</td>
</tr>
<tr>
<td>ΔRPE&lt;sub&gt;peak&lt;/sub&gt;</td>
<td>-1±1</td>
<td>-2±1*+</td>
<td>-1±1</td>
<td>-2±1*+</td>
</tr>
<tr>
<td>ΔTSS&lt;sub&gt;peak&lt;/sub&gt;</td>
<td>-0.3±0.4</td>
<td>-0.7±0.5*+</td>
<td>-0.5±0.5</td>
<td>-0.9±0.5*+</td>
</tr>
<tr>
<td>ΔTC&lt;sub&gt;peak&lt;/sub&gt;</td>
<td>-1±1</td>
<td>-1±1*+</td>
<td>0±1</td>
<td>-1±1*+</td>
</tr>
</tbody>
</table>

Heat acclimation state (1-10)

<p>| | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔSweat setpoint (°C)</td>
<td>-0.22±0.18*†</td>
<td>-0.22±0.14*†‡+</td>
<td>-0.14±0.18*</td>
<td>-0.11±0.10*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔSweat gain (g.sec&lt;sup&gt;-1&lt;/sup&gt;.°C&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>+0.14±0.10*</td>
<td>+0.15±0.07*</td>
<td>+0.05±0.07</td>
<td>+0.06±0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔWBSL (mL)</td>
<td>+262±180*</td>
<td>+278±211*</td>
<td>+68±118</td>
<td>+68±112</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔT&lt;sub&gt;rc&lt;/sub&gt;peak (°C)</td>
<td>-0.25±0.11*</td>
<td>-0.28±0.11*</td>
<td>-0.15±0.27</td>
<td>-0.08±0.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔHR&lt;sub&gt;peak&lt;/sub&gt; (b.min&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>-13±9*</td>
<td>-14±10*</td>
<td>-4±1</td>
<td>-2±6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔRPE&lt;sub&gt;peak&lt;/sub&gt;</td>
<td>-3±2*</td>
<td>-3±1*</td>
<td>-3±2*</td>
<td>-2±2*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔTSS&lt;sub&gt;peak&lt;/sub&gt;</td>
<td>-0.7±0.5</td>
<td>-0.6±0.7</td>
<td>-0.4±0.9</td>
<td>-0.3±0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔTC&lt;sub&gt;peak&lt;/sub&gt;</td>
<td>-1±1*†‡</td>
<td>-1±1*†‡</td>
<td>0±1</td>
<td>0±1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*represents a significant (P<0.05) within-group difference, †represents a significant (P<0.05) between-group difference with ODTEMP, ‡represents a significant (P<0.05) between-group difference with TDTEMP, and +represents a significant difference (P<0.05) between 1-5 and 1-10 adaptations. ODHA: once-daily heat acclimation, TDHA: twice-daily heat acclimation, ODTEMP: once-daily temperate exercise, TDTEMP: twice-daily temperate exercise, T<sub>rc</sub>: rectal temperature, HR: heart rate, PV: plasma volume, WBSL: whole-body sweat loss, [Na<sup>+</sup>]: sodium concentration, RPE: rating of perceived exertion, TSS: thermal sensation and TC: thermal comfort.
Table 3. Mean ± SD exercise data for sessions 1-5 (short-term) and 1-10 (medium-term).

<table>
<thead>
<tr>
<th>Group</th>
<th>Session</th>
<th>ODHA (44.4±1.2°C, 21.1±2.4 % RH)†‡</th>
<th>TDHA (44.3±1.3°C, 22.2±3.9 % RH)†‡</th>
<th>ODTEMP (21.6±1.1°C, 40.9±4.2 % RH)†‡</th>
<th>TDTEMP (21.8±1.0°C, 38.6±4.7 % RH)†‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1-5</td>
<td>1-10</td>
<td>1-5</td>
<td>1-10</td>
<td>1-5</td>
</tr>
<tr>
<td>Time (min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>300±0</td>
<td>600±0*+</td>
<td>300±0</td>
<td>600±0*+</td>
<td>300±0*</td>
</tr>
<tr>
<td>Total work (kJ)</td>
<td>2378±280</td>
<td>4838±573*+</td>
<td>2338±211</td>
<td>4751±374*+</td>
<td>2419±199</td>
</tr>
<tr>
<td>Mean power (W.kg⁻¹)</td>
<td>1.7±0.1</td>
<td>1.7±0.2</td>
<td>1.7±0.1</td>
<td>1.7±0.1</td>
<td>1.7±0.1</td>
</tr>
<tr>
<td>Mean power (% PPO)</td>
<td>49±4</td>
<td>49±3</td>
<td>47±5</td>
<td>47±5</td>
<td>47±5</td>
</tr>
<tr>
<td>Tₚₑₚₑ (°C)</td>
<td>38.45±0.33</td>
<td>38.39±0.36</td>
<td>38.52±0.24</td>
<td>38.44±0.21</td>
<td>38.39±0.32</td>
</tr>
<tr>
<td>ΔTₑₑ (°C)</td>
<td>1.29±0.21†‡‡</td>
<td>1.52±0.23*†‡‡+</td>
<td>1.48±0.22†‡‡</td>
<td>1.68±0.28*†‡‡+</td>
<td>0.70±0.17</td>
</tr>
<tr>
<td>Time &gt;38.5°C (min)</td>
<td>62.8±61.2†‡‡</td>
<td>118.6±118.11*†‡‡+</td>
<td>50.2±30.3†‡‡</td>
<td>90.9±49.5*†‡‡+</td>
<td>21.3±24.5</td>
</tr>
<tr>
<td>HRₚₑₚₑ (b.min⁻¹)</td>
<td>167±15</td>
<td>163±13</td>
<td>163±11</td>
<td>155±11+</td>
<td>169±19</td>
</tr>
<tr>
<td>Sweat loss (mL)</td>
<td>980±287</td>
<td>1513±504*†‡‡</td>
<td>1146±429†‡‡</td>
<td>1545±375*†‡‡</td>
<td>655±95</td>
</tr>
<tr>
<td>ΔPV (%)</td>
<td>-7.9±4.0</td>
<td>-4.4±2.8+</td>
<td>-9.4±5.5</td>
<td>-4.6±3.3+</td>
<td>-3.2±2.6</td>
</tr>
<tr>
<td>RPEₚₑₚₑ</td>
<td>15±1†‡</td>
<td>13±1*†‡‡</td>
<td>15±1†</td>
<td>14±1*†‡‡</td>
<td>17±1</td>
</tr>
<tr>
<td>TSSₚₑₚₑ</td>
<td>6.8±0.4†‡‡</td>
<td>6.1±0.6*</td>
<td>7.0±0.4†‡‡</td>
<td>6.1±0.4*‡‡</td>
<td>5.8±0.5</td>
</tr>
<tr>
<td>TCₚₑₚₑ</td>
<td>4±1</td>
<td>2±1*</td>
<td>4±1</td>
<td>3±1*</td>
<td>3±0</td>
</tr>
</tbody>
</table>

*represents a (P<0.05) within-group difference, †represents a (P<0.05) between-group difference with ODTEMP, ‡represents a (P<0.05) between-group difference with TDTEMP, †‡represents a significant difference (P<0.05) between 1-5 and 1-10 adaptations and ‡represents a significant (P<0.05) between-intervention difference (e.g. HA vs. TEMP). ODHA: once-daily heat acclimation, TDHA: twice-daily heat acclimation, ODTEMP: once-daily temperate exercise, TDTEMP: twice-daily temperate exercise, Tₑₑ: rectal temperature, HR: heart rate, PV: plasma volume, Δ: change, RPE: rating of perceived exertion, TSS: thermal sensation and TC: thermal comfort.
Table 4. Mean ± SD changes (Δ) in exercise tolerance (determinants of aerobic performance and aerobic performance).

<table>
<thead>
<tr>
<th>Group</th>
<th>ODHA</th>
<th></th>
<th></th>
<th>TDHA</th>
<th></th>
<th></th>
<th>TDTEMP</th>
<th></th>
<th></th>
<th>ODTEMP</th>
<th></th>
<th></th>
<th>TDTEMP</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Δ (% Δ)</td>
<td>Pre</td>
<td>Post</td>
<td>Δ (% Δ)</td>
<td>Pre</td>
<td>Post</td>
<td>Δ (% Δ)</td>
<td>Pre</td>
<td>Post</td>
<td>Δ (% Δ)</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Power at LT (W)</td>
<td>159±20</td>
<td>166±26</td>
<td>+7±10a</td>
<td>163±30</td>
<td>170±28</td>
<td>+7±8a</td>
<td>157±21</td>
<td>160±23</td>
<td>+3±5</td>
<td>159±17</td>
<td>160±13</td>
<td>+1±6</td>
<td>161±14</td>
<td>161±12</td>
</tr>
<tr>
<td>GME (%)</td>
<td>19.9±1.0</td>
<td>21.0±2.0</td>
<td>+1.0±2.2</td>
<td>20.5±1.7</td>
<td>20.8±1.4</td>
<td>+0.2±1.6</td>
<td>19.3±1.7</td>
<td>19.2±1.6</td>
<td>-0.1±1.5</td>
<td>19.7±1.9</td>
<td>19.7±2.0</td>
<td>+0.1±1.2</td>
<td>18.9±1.0</td>
<td>19.0±1.2</td>
</tr>
<tr>
<td>VO_{2peak} (L.min⁻¹)</td>
<td>3.76±0.46</td>
<td>3.95±0.52</td>
<td>+0.18±0.12</td>
<td>3.74±0.50</td>
<td>3.89±0.45</td>
<td>+0.13±0.09</td>
<td>3.73±0.43</td>
<td>3.83±0.45</td>
<td>+0.10±0.09</td>
<td>3.69±0.34</td>
<td>3.73±0.31</td>
<td>+0.05±0.07</td>
<td>3.60±0.29</td>
<td>3.70±0.38</td>
</tr>
<tr>
<td>PPO (W)</td>
<td>291±39</td>
<td>304±48</td>
<td>+13±18</td>
<td>296±50</td>
<td>308±56</td>
<td>+11±8</td>
<td>288±27</td>
<td>291±31</td>
<td>+3±14</td>
<td>287±18</td>
<td>296±18</td>
<td>+6±11</td>
<td>292±14</td>
<td>299±15</td>
</tr>
</tbody>
</table>

Determinants of Aerobic Performance

<table>
<thead>
<tr>
<th>Group</th>
<th>ODHA</th>
<th></th>
<th></th>
<th>TDHA</th>
<th></th>
<th></th>
<th>TDTEMP</th>
<th></th>
<th></th>
<th>ODTEMP</th>
<th></th>
<th></th>
<th>TDTEMP</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>TTTF_TEMP (s)</td>
<td>519±151</td>
<td>588±153</td>
<td>+68±11</td>
<td>553±74</td>
<td>631±82</td>
<td>+78±47</td>
<td>510±102</td>
<td>553±106</td>
<td>+42±51</td>
<td>532±116</td>
<td>579±161</td>
<td>+47±62</td>
<td>503±124</td>
<td>550±169</td>
</tr>
<tr>
<td>TTTF_HOT (s)</td>
<td>412±111</td>
<td>516±140*</td>
<td>+104±31</td>
<td>450±85</td>
<td>558±117*</td>
<td>+109±57</td>
<td>416±131</td>
<td>435±149</td>
<td>+19±58</td>
<td>430±91</td>
<td>444±97</td>
<td>+15±77</td>
<td>410±104</td>
<td>437±132</td>
</tr>
</tbody>
</table>

*represents a significant (P<0.05) within-group difference and a represents a significant (P<0.05) between-intervention difference (e.g. HA vs. TEMP). ODHA: once-daily heat acclimation, TDHA: twice-daily heat acclimation, ODTEMP: once-daily temperate exercise, TDTEMP: twice-daily temperate exercise, LT: lactate threshold, GME: gross mechanical efficiency, VO_{2peak}: peak oxygen uptake, PPO: peak power output, TTTF\_TEMP: time to task failure in temperate condition and TTTF\_HOT: time to task failure in heat stress.
Table 5. Mean ± SD changes (Δ) in physiological measures compared to pre-intervention time to task failure in temperate (TTTFTEMP) and hot conditions (TTTFHOT).

<table>
<thead>
<tr>
<th>Group</th>
<th>ODHA</th>
<th>TDHA</th>
<th>ODTEMP</th>
<th>TDTEMP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TTTFTEMP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔT&lt;sub&gt;re&lt;/sub&gt; (°C)</td>
<td>-0.21±0.12*</td>
<td>-0.29±0.24*</td>
<td>-0.14±0.16</td>
<td>-0.14±0.28</td>
</tr>
<tr>
<td>ΔHR (b.min&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>-6±8*</td>
<td>-6±4*</td>
<td>+1±7</td>
<td>-3±10</td>
</tr>
<tr>
<td>ΔVO&lt;sub&gt;2&lt;/sub&gt; (L.min&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>-0.02±0.21</td>
<td>0.00±0.17</td>
<td>-0.03±0.32</td>
<td>-0.02±0.28</td>
</tr>
<tr>
<td>ΔRER</td>
<td>-0.08±0.15</td>
<td>-0.01±0.10</td>
<td>-0.06±0.05</td>
<td>-0.07±0.08</td>
</tr>
<tr>
<td>ΔH&lt;sub&gt;prod&lt;/sub&gt; (W)</td>
<td>-26±73</td>
<td>-28±72</td>
<td>+36±127</td>
<td>+12±170</td>
</tr>
<tr>
<td>ΔV&lt;sub&gt;E&lt;/sub&gt; (L.min&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>-8.4±20.1</td>
<td>+5.2±16.0</td>
<td>+8.1±16.3</td>
<td>-6.2±21.4</td>
</tr>
<tr>
<td><strong>TTTFHOT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔT&lt;sub&gt;re&lt;/sub&gt; (°C)</td>
<td>-0.26±0.26*</td>
<td>-0.26±0.27*</td>
<td>-0.14±0.28</td>
<td>-0.16±0.33</td>
</tr>
<tr>
<td>ΔHR (b.min&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>-6±5*</td>
<td>-8±6*</td>
<td>0±6</td>
<td>-3±7</td>
</tr>
<tr>
<td>ΔVO&lt;sub&gt;2&lt;/sub&gt; (L.min&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>+0.01±0.29</td>
<td>-0.09±0.20</td>
<td>+0.03±0.22</td>
<td>-0.05±0.12</td>
</tr>
<tr>
<td>ΔRER</td>
<td>-0.01±0.08</td>
<td>+0.02±0.06</td>
<td>-0.04±0.10</td>
<td>-0.07±0.08</td>
</tr>
<tr>
<td>ΔH&lt;sub&gt;prod&lt;/sub&gt; (W)</td>
<td>-17±104</td>
<td>-11±99</td>
<td>+16±179</td>
<td>+20±209</td>
</tr>
<tr>
<td>ΔV&lt;sub&gt;E&lt;/sub&gt; (L.min&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>-5.5±20.4</td>
<td>-2.3±16.0</td>
<td>+5.7±20.7</td>
<td>+1.9±16.0</td>
</tr>
</tbody>
</table>

*represents a significant (P<0.05) within-group difference. ODHA: once-daily heat acclimation, TDHA: twice-daily heat acclimation, ODTEMP: once-daily temperate exercise, TDTEMP: twice-daily temperate exercise, T<sub>re</sub>: rectal temperature, HR: heart rate, VO<sub>2</sub>: oxygen uptake, RER: respiratory exchange ratio, H<sub>prod</sub>: metabolic heat production, V<sub>E</sub>: ventilation, Δ: change, TTTFTEMP: time to task failure in temperate condition and TTTFHOT: time to task failure in heat stress.
### Week 1

<table>
<thead>
<tr>
<th>Monday</th>
<th>Tuesday</th>
<th>Wednesday</th>
<th>Thursday</th>
<th>Friday</th>
<th>Saturday</th>
<th>Sunday</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preliminary and GXT (22°C, 40% R.H.)</td>
<td>Rest</td>
<td>HAST (45°C, 20% R.H.)</td>
<td>Rest</td>
<td>TTTF&lt;sub&gt;HOT&lt;/sub&gt; (38°C, 40% R.H.)</td>
<td>Rest</td>
<td>TTTF&lt;sub&gt;TEMP&lt;/sub&gt; (22°C, 40% R.H.)</td>
</tr>
</tbody>
</table>

### Week 2

<table>
<thead>
<tr>
<th>Morning (08:00)</th>
<th>Afternoon (16:00)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ODHA, TDHA, ODTEMP, TDTEMP</td>
<td>ODHA, TDHA, ODTEMP</td>
</tr>
<tr>
<td>ODHA, ODTEMP</td>
<td>ODHA, ODTEMP</td>
</tr>
<tr>
<td>Rest</td>
<td>Rest</td>
</tr>
</tbody>
</table>

### Week 3

<table>
<thead>
<tr>
<th>Morning (08:00)</th>
<th>Afternoon (16:00)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ODHA, TDHA, ODTEMP, TDTEMP</td>
<td>ODHA, TDHA, ODTEMP</td>
</tr>
<tr>
<td>ODHA, ODTEMP</td>
<td>ODHA, ODTEMP</td>
</tr>
<tr>
<td>Rest</td>
<td>Rest</td>
</tr>
</tbody>
</table>

### Week 4

<table>
<thead>
<tr>
<th>Monday</th>
<th>Tuesday</th>
<th>Wednesday</th>
<th>Thursday</th>
<th>Friday</th>
<th>Saturday</th>
<th>Sunday</th>
</tr>
</thead>
<tbody>
<tr>
<td>GXT (22°C, 40% R.H.)</td>
<td>Rest</td>
<td>HAST (45°C, 20% R.H.)</td>
<td>Rest</td>
<td>TTTF&lt;sub&gt;HOT&lt;/sub&gt; (38°C, 40% R.H.)</td>
<td>Rest</td>
<td>TTTF&lt;sub&gt;TEMP&lt;/sub&gt; (22°C, 40% R.H.)</td>
</tr>
</tbody>
</table>
Figure 2. Mean ± SD changes in the determinants of aerobic performance and aerobic performance in hot and temperate conditions. *represents a significant (P<0.05) within-group difference pre- to post-session. δ represents a significant (P<0.05) between-group difference with (HA vs. TEMP). Shapes denote individual participants within group.
Figure 3. Mean ± SD changes in cortisol, TNF-α and IL-6 for session 1, 5, 10. *represents a significant (P<0.05) within-group difference pre- to post-session. †represents a significant (P<0.05) between-group difference with ODTEMP. ‡represents a significant (P<0.05) between-group difference with TDTEMP. Shapes denote individual participants within group.