Temperate performance benefits after heat, but not combined heat and hypoxic training

Erin L. McCleave[1,2], Katie M. Slattery[2], Rob Duffield[1], Philo U. Saunders[3,4], Avish P. Sharma[3,4], Stephen J. Crowcroft[1,2] and Aaron J. Coutts[1].

[1]Sport and Exercise Science Discipline Group, Faculty of Health, University of Technology Sydney (UTS), Moore Park, AUSTRALIA; [2]New South Wales Institute of Sport, Sydney Olympic Park, AUSTRALIA, [3]Department of Physiology, Australian Institute of Sport (AIS), Canberra, AUSTRALIA, [4]University of Canberra Research Institute for Sport and Exercise (UCRISE), Canberra, AUSTRALIA.

Corresponding author:

Erin L. McCleave

6B Figtree Drive, Sydney Olympic Park, 2127, AUSTRALIA

Phone: (02) 9876 0650

Email: erin.mccleave@nswis.com.au

Running head: Heat interval training improves performance

Abstract

Introduction: Independent heat and hypoxic exposure can enhance temperate endurance performance in trained athletes, although their combined effects remain unknown. This study examined whether the addition of heat interval training during 'Live High, Train Low' (LHTL) hypoxic exposure would result in enhanced performance and physiological adaptations as compared to heat or temperate training.

Methods: Twenty-six well-trained runners completed three weeks of interval training assigned to one of three conditions: 1) LHTL hypoxic exposure plus heat training (H+H; 3000 m for 13 h·day^{-1}, train at 33ºC, 60% RH), 2) heat training with no hypoxic exposure (HOT, live at <600m and train at 33ºC, 60% RH), or 3) temperate training with no hypoxic exposure (CONT; live at <600m and train at 14ºC, 55% RH). Performance 3-km time-trials (3-km TT), running economy (RE), haemoglobin mass (Hb_{mass}) and plasma volume (PV) were assessed utilising magnitude based inferences statistical approach before (Baseline), after (Post), and three weeks (3wkP) following exposure.

Results: Compared to Baseline, 3-km TT performance was likely increased in HOT at 3wkP (-3.3%; ±1.3% (mean; ±90% CL)), with no performance improvement in either H+H or CONT. Hb_{mass} increased by 3.8%; ±1.8% at Post in H+H only. PV in HOT was possibly elevated above H+H and CONT at Post but not at 3wkP. Correlations between changes in 3-km TT performance and physiological adaptations were unclear.

Conclusion: Incorporating heat-based training into a three week training block can improve temperate performance at three weeks following exposure, with athlete psychology, physiology and environmental dose all important considerations. Despite haematological adaptations, the addition of LHTL to heat interval training has no greater 3-km TT performance benefit than temperate training alone.

Key words: Heat acclimation · Hypoxia · Plasma volume · Endurance · Haemoglobin mass

INTRODUCTION

Paragraph 1: Substantial training loads are undertaken by endurance athletes to maximise physiological adaptations and physical performance. However, both high and/or unaccustomed loads can increase risks of overreaching and injury, which are counterproductive to maximizing performance (9, 14). Therefore, interventions that enhance the physiological and performance outcomes in the absence of increased training volume are attractive to coaches and athletes. Accordingly, considerable interest exists on the effects of living and training in altered environments (i.e. heat and hypoxia). This approach can be used to increase the physiological stress without the need for large increases in external training load (23). Whilst studies have examined the performance benefits of independent heat (21, 33) and hypoxic exposure (4, 20), the combined effects of heat and hypoxia are not yet well understood (5, 8).

Paragraph 2: Repeated exposure to hypoxia can have both ergogenic effects on endurance performance and amplify systemic physiological adaptations (23). The Live High, Train Low (LHTL) model traditionally incorporates 12-14 $h \cdot day^{-1}$ of altitude exposure (i.e. >2000 m), with training conducted at low-moderate altitude (i.e. <1250 m) to allow the maintenance of training intensity (23). This model has been shown to improve sea-level endurance performance (4, 28), haemoglobin mass (Hb_{mass}) and maximal aerobic capacity (VO_{2max}) in well-trained endurance athletes (20). Several studies have demonstrated small but significant improvements in run time-trial performance over 3-km (28, 37) and 5-km (20) following 2-4 weeks of LHTL. However, not all studies have shown improvements over similar distances (13, 27). This lack of consistent improvement is suggested to be related to a number factors, not limited to the extent of physiological adaptation incurred, the hypoxic dose and training status of the athletes (4).

Paragraph 3: In addition to hypoxia, repeated heat exposure has been shown to have a positive ergogenic benefit in hot (19, 21) and temperate environments INSERT SCOON (3) (21, 26). However, a recent debate in the literature highlights the uncertainty surrounding the capacity of heat to improve temperate performance (CROSS TALK DEBATE). The proposed mechanisms for heat exposure improving temperate performance are not clearly understood, but are suggested to be related to elevated plasma volume (PV), reduced cardiovascular and thermoregulatory strain, enhanced lactate threshold and VO_{2max} (21, 32). In addition, lower perceptions of heat stress are also evident after heat exposure, which may also be related to performance improvements (35).

Paragraph 4: In a previous study investigating concurrent heat and intermittent hypoxic exposure in untrained individuals, it was apparent that the combined stimuli elevated PV but had no impact on VO_{2peak} (Takeno, 2001). However, the combination of LHTL hypoxia and heat training has suggested possible positive physiological and temperate performance adaptations. Buchheit et al. (5), conducted a two week pre-season training camp incorporating LHTL plus heat training in team sport athletes. Compared to training in a hot environment alone, the LHTL plus heat group had a greater Hb_{mass} increase, with no difference between groups in PV or Yo-Yo Intermittent Recovery Test 2 performance. Interestingly, four weeks later there was a better maintenance of performance, PV and Hb_{mass} in the combined LHTL plus heat training group (5). The possibility of greater and longer lasting adaptations following concurrent heat and hypoxic exposure makes it an attractive training method. However, this study was limited by the lack of a control group and the early pre-season training status of the athletes. Given these limitations, the impact of combined heat and hypoxic training remains equivocal, and is yet to be examined on well-trained endurance athletes.

Paragraph 5: Accordingly, the aim of this study was to examine performance and physiological adaptations to three weeks of LHTL combined with heat interval training in well-trained runners. In addition, we aimed to assess the time course of these adaptations in the three weeks following exposure. It was hypothesised that LHTL combined with heat interval training would elicit greater and longer lasting physiological adaptations and 3-km time-trial performance improvements than training in the heat alone or temperate conditions.

METHODS

Participants

Paragraph 6: Twenty-eight well-trained male and female middle distance runners were recruited for the study, with twenty-six included for final analyses. Of the excluded participants, one did not complete all testing requirements and one participant reported illness during the study. Participants were matched based on prior training load, peak oxygen uptake (VO_{2peak}) and associated velocity (vVO_{2peak}) obtained during preliminary testing. After taking into account the participants geographic proximity to the testing centres, they were randomly assigned (coin toss/number) by an independent associate to one of three groups; 1) LHTL hypoxic exposure plus training in a hot environment (H+H; F_iO_2 =14.8% (3000 m) for 13 $h \cdot day^{-1}$; train at <600 m, 33ºC, 60% RH); 2) heat training with no hypoxic exposure (HOT; live and train at <600 m, 33ºC, 60% RH); or 3) temperate training with no hypoxic exposure (CONT; live and train at <600 m, 14ºC, 55% RH). Participants had ≥2 y running experience and regularly completed 10–20 h of training each week. All groups contained a mix of male and female athletes, and no participants had heat or hypoxic exposure in the four weeks prior. All differences in baseline characteristics between training groups were unclear (Table 1). Prior to the study, all participants were informed of all procedures and potential risks involved in the study and a written informed consent was obtained. The study was approved

by the Human Research Ethics Committee of the University of Technology Sydney (Trial no. UTS HREC 2014000203).

INSERT TABLE 1

Experimental Overview

Paragraph 7: This study was a multicentre, parallel, matched group design, with all training and testing conducted during winter and early spring months in Sydney or Canberra, Australia (June – November, 2014). The study included a three week period (exposure), whereby participants lived and trained in their assigned environmental conditions. This was followed by a three week period (non-exposure), in which all individuals lived and trained in temperate, normoxic conditions. During the exposure period, individuals in the H+H group spent 21 days, (13 $h \cdot day^{-1}$, F_iO_2 = 14.8%,) in a normobaric hypoxic facility at the Australian Institute of Sport (AIS, Canberra). All participants completed 3 x 90 min treadmill sessions per week, including two interval sessions and one moderate continuous run (9 total sessions). H+H and HOT participants completed heat sessions in a climate-controlled chamber (Altitude Training Systems, Lidcombe, Australia). Canberra-based participants trained at the University of Canberra (32.5 ±0.7°C; 59 ±7% RH), while Sydney-based participants trained at the New South Wales Institute of Sport (NSWIS, 32.9 ±0.5°C, 56 ±3% RH). Sydney-based participants assigned to the CONT group completed treadmill sessions in an air-conditioned room (14.4 ±1.9°C, 51 ±13% RH), while Canberra-based participants trained in a covered, outdoor covered area (12.6 ±4°C, 56 ±13% RH). In addition to the treadmill

sessions, all participants maintained aerobic training in a temperate, normoxic environment during the study in order to maintain aerobic conditioning. As part of additional testing not described in the current study, each participant undertook a heat tolerance test with 75 min exposure to 33°C at the start and end of each three week period (2 in exposure, 2 in non-exposure, data not reported here). Core temperature was assessed via a temperature probe (Mon-a-therm, Mansfield, USA) inserted 10 cm beyond the anal sphincter, with temperature elevated to an average of 38.3 ±0.4°C across all groups (average peak 39.1 ±0.5°C), suggesting that the heat dose was sufficient to elicit an adaptive response (Racinais consensus statement). Performance tests were completed a minimum of 4 days after any heat exposure, and the control group received no more than one 75 min heat exposure within a 7 day period. Thus, this testing was not expected to induce any heat acclimation adaptations (2).

Paragraph 8: Within two weeks prior to the exposure period, participants undertook an incremental treadmill test for assessment of running economy (RE) and VO_{2peak}. A double baseline measure of Hb_{mass} was assessed during the same period, along with a resting venous blood sample for measurement of ferritin concentration. Approximately five days prior to the exposure period, performance was assessed via a 3-km run time-trial (3-km TT) (Baseline). Running economy, Hb_{mass} and the 3-km TT were repeated immediately (Post) and three weeks following (3wkP) the exposure period. An additional Hb_{mass} test was conducted one week (1wkP) following the exposure period in order to further quantify the decay timeline of adaptations (as shown in Figure 1). All equipment was matched between locations, with participants completing testing and treadmill sessions at the same location and at a similar time of day.

INSERT FIGURE 1 HERE

Incremental treadmill test

Paragraph 9: Participants completed a progressive 4 x 4 min incremental run (0% gradient, 1 min recovery between stages) on a motorised treadmill (Canberra; custom-built motorised treadmill, AIS. Sydney: Payne Treadmill, Stanton Engineering, Girraween, Australia). Starting speed was determined based on participant's ability (between 11–17 $km \cdot h^{-1}$) with each stage increased by 1 $km \cdot h^{-1}$. Heart rate (HR; Suunto T6, Vantaa, Finland) and oxygen consumption (VO_2) were measured continuously throughout the test (Canberra: in-house automated metabolic system as described previously (29); Sydney: Moxus Modular Metabolic System, AEI Technologies, Pittsburgh, USA). Running economy was determined as the mean VO_2 during the last minute of the first two submaximal stages (17). Following Baseline testing only, participants completed an incremental run to maximal volitional fatigue for determination of VO_{2peak}, corresponding velocity at VO_{2peak} (vVO_{2peak}) and maximal heart rate (HR_{max}) (38).

Performance Time Trial

Paragraph 10: In both training locations, 3-km TT's were conducted on a 400-m outdoor athletics track (MONDO synthetic track, Mondo S.p.A., Italy). Participants completed a self-selected warm up that was replicated at each 3-km TT. Participants were blinded to all pacing and timing information, with verbal feedback given only to notify when one lap remained. Time splits were recorded via hand held stopwatch (Seiko, Tokyo, Japan), with Rating of Perceived Exertion (RPE CR-10) (10) collected immediately after. Environmental temperature, relative humidity and wind speed (Kestrel 3500 Delta T Meter, Nielsen-

Kellerman, Boothwyn, USA) were recorded during each 3-km TT (Canberra: 13.5 ±4.3°C, 55.2 ±18% RH, 1.0 ±1.0 $m \cdot s^{-1}$ wind speed; Sydney: 19.5 ±3.4°C, 53.3 ±16% RH, 1.5 ±0.9 $m \cdot s^{-1}$). To minimise the effects of diet on physical performance, participants recorded their diet for the 24 hours prior to the Baseline 3-km TT, and replicated this diet for each subsequent test. Further, prior to each 3-km TT, participants completed a series of questions pertaining to muscle soreness, general fatigue and motivation (5-point Likert scale) (36). In addition, participants were asked the specific question of 'how important is this upcoming 3-km TT to you?', with answers scaled on a 10-point Likert Scale (1), ranging from not important at all' (0) through to 'highly important' (10). Participants also rated 'What percentage (0 – 100) of your full potential do you think you can run today?"

Training Monitoring

Paragraph 11: Daily training load (AU) was monitored using the session rating of perceived exertion (sRPE) method, calculated as the product of training duration (min) and the mean training intensity (RPE CR-10). Treadmill interval sessions were conducted on motorised treadmills (Canberra: Trackmaster TMX58, Newtown, USA; Sydney: Life Fitness 9500HR, Brunswick Corporation, Illinois, USA), with participants completed a standardised and individualised 20 min warm-up prior to each session. An outline of the treadmill sessions is presented in Table 2. Interval intensities were matched across all groups based on a percentage of vVO_{2peak} as determined from Baseline testing. Intensities ranged from 80-100% vVO_{2peak}, with the only exceptions being sessions 1, 5 and 9, which were conducted as 45 min continuous running at 65% $vVO_{2peak.}$ Participants completed their own standardised warm-down and remained in the heat chamber or air-conditioned room until 90 min of exposure was completed. HR was recorded continuously, with sRPE recorded at the

conclusion of each session. Participants were allowed to drink water *ad libitum* during training sessions.

INSERT TABLE 2 HERE

Paragraph 12: Participants recorded all training throughout the study, commencing two weeks prior to the exposure period to capture participants' habitual training programs. Participants were instructed to continue with their normal aerobic training during the study in temperate normoxic conditions, in addition to the prescribed three weekly treadmill sessions, and were instructed to replace regular high intensity sessions with the treadmill sessions. As part of this additional aerobic temperate training during the exposure period, all participants reported completing one long duration, and one aerobic interval session per week. During the non-exposure period, participants were prescribed an individualised training program based on their prior TL.

Haemoglobin Mass

Paragraph 13: Hb_{mass} was measured via the optimized carbon monoxide (CO) rebreathing method (34). Briefly, a CO dose of 1.2 $ml \cdot kg^{-1}$ body mass was rebreathed for 2 min through a glass spirometer. Capillary fingertip blood samples (200 µL) were obtained prior to CO administration and 7 min after CO inhalation. An average of five blood samples were used for measurement of percent carboxyhemoglobin (%HbCO) via a CO-oximeter (OSM3, Radiometer, Copenhagen, Denmark), with Hb_{mass} determined as the mean change in %HbCO (11). Duplicate measures were obtained at Baseline on twenty-three out of twenty-six

participants, with the typical error of measurement (TE) for Hb_{mass} calculated at 1.8% (1.4–2.4%, 90% confidence limits). The duplicate measures were obtained with a minimum of 48 hours between tests (maximum 2 weeks), with these values averaged into a single time point for analysis. PV and BV were indirectly calculated by the optimized CO rebreathing procedure as described above. All measures were performed by three experienced researchers, with the same tester completing tests on the same participants where possible.

Blood Biochemistry

Paragraph 14: Venous blood was collected from the antecubital vein 2-3 weeks prior to commencement of the study for determination of blood ferritin levels. Blood was collected into serum separation tubes (SST; Vacuette®, Greiner Bio-One, Frickenhausen, Germany), centrifuged at 3000 rpm and 4°C for 10 min (2-16K, Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany) and sent to the laboratory for same day analysis (Sydney: Douglass Hanly Moir Pathology, Macquarie Park, Australia; Canberra: AIS Biochemisty Lab). Sydney samples were assessed on an Abbott i2000 (Abbott Diagnostics, Lake Forest, Illinois, USA) and Canberra on a Cobas Integra 400 plus analyser (Roche Diagnostics Ltd., Forrenstrasse, Switzerland). Any participants with ferritin levels <100 $ug \cdot L^{-1}$ were provided a daily oral iron supplement to take throughout the duration of the study in order to maintain adequate iron levels required for accelerated erythropoiesis (Ferrograd C, 325 mg dried ferrous sulphate + 562.4 mg sodium ascorbate; Abbott, Botany, Australia).

Statistical Analyses

Paragraph 15: Data are presented as means and standard deviation (±SD) unless otherwise stated. Data were log-transformed to reduce bias from any non-uniformity of error, and

assessed for practicality according to magnitude based-inferences (3). Effects were deemed unclear if the confidence limits overlapped the thresholds for both the smallest positive and negative effects (>5%), with clear effects assessed as the following: <1%, almost certainly note; 1–5%, very unlikely; >5–25%, unlikely; >25–75%, possibly; >75–95%, likely; >95–99%, very likely; >99%, almost certainly (15). The smallest worthwhile change in performance was half the typical within-athlete coefficient of variation (CV), or 1.0% in elite runners (16). For measures not directly related to performance, the smallest worthwhile change was calculated as a standardised small effect size (0.20) multiplied by the pre-test between-subject standard deviation (6). Effect Size (ES) = 0.20, 0.50, and 0.80 were considered as small, medium, and large, respectively. The TE for outcome measures was calculated from the SD of the change scores divided by the mean and presented as a coefficient of variation (%). Pearson product-moment correlation analyses were calculated to assess the relationship between 3-km TT and physiological parameters. The following thresholds were used to assess the magnitude of correlation (r (90% CL)) between measures: <0.30, trivial to small; 0.30–0.49, moderate; 0.50–0.69, large; 0.70–0.89, very large and 0.90–1.00, almost perfect. If the 90% CL overlapped the positive and negative values, the magnitudes were deemed unclear. An *a priori* power analysis was completed using G*Power (G*Power version 3.1.9.2, Universität Kiel, Germany) based on time-trial data obtained from previous similar studies demonstrated 10 subjects per group is the minimum required to achieve a power of 0.8, and as such we recognize the potential limitation of reduced power of this study.

RESULTS

Training Load

Paragraph 16: During the exposure period, HOT and H+H received 13.5 h total heat exposure, with control receiving 2.5 h. Both groups had an additional 2.5 h heat during the non-exposure period (heat response testing, data is not presented here). Participants in H+H spent 291.0 ±13.4 h in normobaric hypoxia, averaging of 13.9 ±0.6 $h \cdot day^{-1}$.

Paragraph 17: During the Baseline period, there were no clear differences between groups in weekly training load (TL) as determined from sRPE (H+H vs. HOT: ES = -0.44 (-1.22; 0.34), H+H vs. CONT: ES = -0.17 (-1.04; 0.70), HOT v. CONT: ES = -0.21 (-1.05; 0.63) (Figure 2). Across the entire 6 weeks of the study, no clear TL differences existed between groups (HOT vs. H+H: ES = 0.02 (-0.76; 0.80), CONT vs. H+H: ES = 0.20 (-0.63; 1.02), HOT vs. CONT: ES = -0.11 (-0.93; 0.71)). However, when comparing the exposure to non-exposure period, HOT and H+H had a within-group reduction in TL during the non-exposure period (HOT: ES = -0.31 (-0.53; -0.08) likely, H+H: ES = -1.75 (-2.12; 1.37) most likely, CONT: ES = -0.08 (-0.5; 0.33) unclear). During the same period, H+H had a very likely TL reduction in H+H compared to both CONT and HOT (H+H vs. CONT: ES = -1.26 (-2.00; -0.53), H+H vs. HOT: ES = -0.8 (-1.19; -0.40)), with unclear differences between HOT and CONT (ES = -0.26 (-0.80; 0.28)).

INSERT FIGURE 2 HERE

Time-trial Performance

306 *Paragraph 18:* Improvement in 3-km TT performance occurred only in HOT, with a likely
307 faster completion time by -3.3%; ±1.3% (mean; ±90% CL) from Baseline to 3wkP (652 ±76
308 vs. 629 ±67 s; ES = -0.26 (-0.36; -0.16), Figure 3). This improvement was possibly greater
309 when compared to both H+H (643 ± 72 vs. 639 ± 74 s; ES = -0.24 (-0.40; -0.08)) and CONT
310 (651 ± 118 vs. 649 ± 127 s; ES = -0.19 (-0.32; -0.07), Figure 3). There were no substantial
311 changes from Baseline in performance in any group at POST, and also in H+H and CONT at
312 3wkP. There were no clear between or within group differences in RPE following each
313 respective 3-km TT.

314

315 **INSERT FIGURE 3**

316

317 **Pre Time-trial Questionnaires**

318 *Paragraph 19:* The perceived capacity of H+H to fulfil their 3-km TT performance potential
319 was likely reduced from Baseline to Post (ES = -0.48 (-1.02; 0.06)), resulting in a likely
320 greater reduction compared to HOT at Post (ES = -0.85 (-1.70; 0.00)), and CONT at 3wkP
321 (ES = -1.53 (-3.04; 0.01)). Motivation likely increased in HOT from Baseline to Post (ES =
322 0.43 (-0.06; 0.92)) and in CONT from Post to 3wkP (ES = 0.20 (-0.19; 0.60)), however was
323 likely reduced in H+H during the same period (ES = -1.12 (-2.12; -0.12)). This resulted in
324 very likely reduction in motivation from Post to 3wkP in CONT compared to H+H (ES =
325 1.12 (0.24; 1.99)).

326 *Paragraph 20:* Perceived importance of the 3-km TT likely increased both in HOT (ES =
327 0.45 (-0.17; 1.08)) and H+H ((ES = 0.46 (-0.09; 1.01)) from Baseline to Post, but was unclear
328 in CONT. While perceived importance remained likely elevated in HOT until 3wkP (ES =

0.49 (0.16; 0.82) vs. Baseline), it decreased from Post to 3wkP in H+H (ES = -0.38 (-0.72; 0.05)). General fatigue was likely reduced from Post to 3wkP in HOT (ES = -0.43 (-0.98; 0.11) and possibly reduced in CONT (ES = -0.16 (-0.46; 0.15)). However, H+H had likely greater increase in general fatigue both from Post to 3wkP (ES = 0.54 (0.09; 0.99)), as well as Baseline to 3wkP (ES = 0.60 (0.02; 1.18)). As a result, 3wkP fatigue was likely lower in both HOT and CONT when compared to H+H at both Baseline and Post (CONT vs. H+H: ES = -0.76 (-1.32; -0.20) vs. Post; ES = -0.83 (-1.50; -0.16) vs. Baseline; HOT v H+H: ES = -1.06 (-1.76; -0.35) vs. Post; ES = -0.69 (-1.43; 0.05) vs. Baseline). All other between and within group differences were unclear.

Running Economy

Paragraph 21: All RE between and within group differences were trivial, unlikely or unclear. ,HR was likely reduced in all groups when comparing Baseline to Post (expressed as a percentage of maximum HR), with no clear between group differences (HOT: 79.4 ±4.7% vs. 76.8 ±4.6% ES = -0.49 (-0.90; -0.07); H+H: 86.0 ±3.6% vs. 82.6 ±5.2% ES = -0.57 (-1.07; -0.07); CONT: 84.8 ±3.1% vs. 82.8 ±3.8% ES = -0.49 (-1.01; 0.03)). HR was possibly further reduced at 3wkP in H+H and CONT, and maintained in HOT. As a result, all groups had a reduced submaximal HR from Baseline to 3wkP (HOT: 79.4 ±4.7% vs. 76.6 ±5.2%, ES = -0.52 (-1.04; 0.00), likely; H+H: 86.0 ±3.6% vs. 81.0 ±6.2% ES = -0.85 (-1.46; -0.24), very likely; CONT: 84.8 ±3.1% vs. 81.8 ±3.8%, ES = -0.72 (-1.21; -0.24), very likely).

Haematology

Paragraph 22: PV increased by 3.8 ±6.0% in HOT during the exposure period (ES = 0.13 (-0.07; 0.34)), with this change possibly greater when compared to both H+H (ES = 0.20 (-0.12; 0.52) and CONT (ES = 0.17 (-0.13; 0.47), Figure 4). At 1wkP, PV remained likely elevated in HOT compared to H+H (ES = 0.68 (-0.09; 1.46)). All differences in HOT and H+H were deemed unclear by 3wkP, and all CONT time course differences throughout the study duration were unlikely or trivial. BV increased in HOT by 3.3 ±3.9% (ES = 0.11 (-0.02; 0.24)) during the exposure period, which was possibly greater when compared to H+H during the same period (ES = 0.15 (-0.05; 0.35)). However, all other within and between group differences were unclear or trivial.

Paragraph 23: Hb_{mass} was increased by 3.8 ±1.8% in H+H during the exposure period (784 ±197 vs. 813 ±203 g; ES = 0.14 (0.08; 0.21)), and remained elevated from Baseline by 3.3 ±1.9% at 3wkP (ES = 0.12 (0.05; 0.19)). This change was greater than the TE from Baseline. However, all within and between group differences were trivial, unlikely or unclear. There were no clear correlations in any group between 3-km TT performance and PV, BV, Hb_{mass}, HR, or RE.

INSERT FIGURE 4 HERE

DISCUSSION

Paragraph 24: This study investigated the effects of three weeks of independent heat interval training or LHTL hypoxic exposure combined with heat interval training in well-trained middle distance runners. The main finding was that 3-km TT performance was only

improved three weeks following HOT training, despite small but positive physiological adaptations (ie. PV) lasting up to one week post exposure. Despite H+H demonstrating positive haemotological adaptations (i.e.Hb_{mass}) above that of temperate training alone, there were no performance improvements. Accordingly, the initial hypothesis that LHTL combined with heat training would be of greatest performance benefit was not supported.

Paragraph 25: Three-km time-trial performance was improved in temperate conditions following heat interval training in all HOT participants at 3wkP. This adds further support to previous research indicating enhanced temperate performance following heat exposure INSERT SCOON (21, 26). A novel finding was that the performance peak in all participants occurred three weeks following heat exposure, but combining LHTL and heat training did not further enhance 3-km TT performance. Direct comparison to previous studies investigating combined LHTL and heat (5), or studies that did not find enhanced temperate performance following heat training (18, 19, 24) should be done so tentatively. This is due to a lack of control group (5, 26), the absence of training load data prior or during the study (21), the assessment of performance within two weeks of exposure (18, 19, 24) and/or the high number of fatiguing maximal tests in a short time frame, which could have reduced the athletes motivation to perform (18). The current protocol of intermittent heat exposure over a three week period, with several weeks of temperate training prior to competition is a practical protocol that can be used to enhance performance in well-trained endurance athletes.

Paragraph 26: It is apparent that heat interval training provides greater 3-km TT performance improvements than combining with LHTL, although physiological explanations for these observations remain elusive. Indeed, there was no clear relationship between any of the physiological measures and 3-km TT performance. As further exploration, heat acclimation can induce a number of cardiovascular periard – fix CITE (1) and thermoregulatory INSERT SAWKA 2011 (2) adaptations to tolerate heat stress, including

399 increased PV (21, 22), $VO_{2max,}$ running economy and power at lactate threshold (21, 24).
400 These adaptations have been suggested to be ergogenic in both hot (21, 26) and temperate
401 conditions (7). We suggest the 270 min/week heat exposure (i.e. 3 x 90 min sessions per
402 week) was sufficient to increase in PV in HOT (by 3.8 ±6.0%), though only until 1wkP, and
403 not at 3wkP when 3-km TT performance improved. In contrast, PV in both H+H and CONT
404 were not increased by more than 1.2% above baseline values at any time during the study,
405 despite H+H receiving the same heat dose as HOT. Such absence of PV expansion in H+H
406 contrasts with previous combined heat and hypoxic findings (5), and warrants further
407 exploration.

408 *Paragraph 27:* As athletes with lower training status have a greater adaptive potential than
409 highly trained athletes (39), it is possible the early season training status of athletes in
410 previous combined heat and LHTL research (5) contributed to the greater PV increases
411 compared more established training status of the current participants. The suggestion of an
412 optimal PV volume to enhance performance (CITE coyle) may provide background as to why
413 performance in HOT did not occur until PV values returned to normal at 3wkP. In addition
414 to training status, the PV response in the present study may also relate to the nature and dose
415 of the environmental stimuli. Hypoxia has been shown to induce hemoconcentration and
416 reduce PV (31). The heat dose in the present study was sufficient to prevent PV reduction in
417 H+H; however, it was unable to match the PV increase in HOT. Thus, heat stimuli appears
418 to prevent hypoxic induced hemoconcentration, however it may be that a greater dose of heat
419 stimuli is required to compensate PV beyond the losses from hypoxia. Further research is
420 required to assess if any other heat training benefits could be negated due to hypoxic
421 exposure. However based of the current data, we recommend that when combining heat and
422 hypoxia, a greater heat dose may elicit PV responses equivalent to heat exposure alone.

Paragraph 28: Running economy has been shown to be improved with endurance performance and has been reported to improve following simulated LHTL exposure in elite middle distance runners (30). In the present study there were only trivial improvements in RE in all training conditions. Moreover, similar to previous research (5), submaximal HR remained unchanged between groups. While RE has been reported to be increased immediately following LHTL alone (17), there does not appear to be any benefit of concurrent heat and altitude or heat alone on RE. Accordingly, the improvements observed in 3-km TT performance observed in the heat group cannot be explained by changes in RE.

Paragraph 29: A recent meta-analysis has shown that Hb_{mass} increases by ~1.1% per 100 h of altitude exposure, and remains elevated by 3.3% for up to 20 days following exposure (12). Similarly, the present study revealed H+H had a 3.8 ±1.8% increase in Hb_{mass} with ~290 h of hypoxic exposure, while no increases occurred in HOT and CONT. Despite H+H having an increase in Hb_{mass}, the lack of performance changes in H+H supports previous research showing that the changes in Hb_{mass} from the hypoxic exposure has minimal impact on 3km-TT performance (27).

Paragraph 30: Considering no associations were observed between the measured physiological adaptations and 3-km TT performance, other unmeasured physiological adaptations, not limited to enhanced thermoregulatory regulation, increased cardiac and skeletal muscle metabolic efficiency (CITE cross talk Minson/Cotter), or non-physiological factors may provide explanations for the observed performance responses. The uncoupling of performance and physiology changes is not uncommon in trained individuals (27), and factors such as perception of effort, motivation and fatigue can contribute to overall endurance performance outcomes (25). At the 3-km TT at 3wkP, fatigue was increased in H+H, despite TL being reduced during the non-exposure period. At the same time point, motivation and perceived time-trial importance was reduced in H+H, but increased in HOT

and CONT. It is likely that the combined psycho-physiological changes in the HOT underlie the observed performance changes. Whilst speculative, the combined perceptions of increased motivation and importance of the 3-km TT garnered HOT contributed positively to improved 3-km TT performance. Physiological adaptations to training were mostly trivial in CONT, while any beneficial effect of the physiological adaptations associated with the H+H may have been minimised by a negative psychological response. Potentially, the combined stress of heat and hypoxia prevented appropriate recovery from the hard training sessions in the heat, thus lingering to supress performance outcomes. While it could be argued the combined stress of heat and hypoxia may have been reduced if the treadmill sessions were matched for cardiovascular strain rather than absolute workload (%vVO_2), the absolute training load provides a more practical application of training prescription in trained individuals, particularly due to the intermittent nature of the sessions. Future investigations incorporating a staggered or reduced combination of heat and hypoxia are required (i.e. reduction in number of heat sessions or an incremental hypoxic dose). These findings illustrate the importance of considering both physiology and psychological aspects when aiming to elicit performance enhancements in well-trained athletes.

LIMITATIONS

Paragraph 31: Despite the above findings, some limitations should be acknowledged. Although participants were blinded to the specific temperature and oxygen concentrations during the study, they were unable to be blinded to their assigned environmental conditions. Furthermore, the heat and hypoxic environmental stimuli in the study were simulated and therefore may not be replicated in natural heat or hypoxic environments. Specifically, physiological adaptations resulting from hypobaric hypoxia or simulated normobaric hypoxia

are suggested to differ (CITE Millet 2012, saugy 2014), however recent evidence suggests no difference in VO_{2max} or 3-km run time-trial (CITE Saugy 2016). However, we recommend future research to investigate if similar results would occur in athletes living and training a natural environment. Another limitation is that we only investigated 3-km TT running performance benefits in a temperate environment. The physiological adaptations resulting from heat and LHTL exposure often enhance athlete's aerobic capacity. To assess this, future research could assess endurance performance over a longer duration in which there is a greater reliance on energy provision from aerobic sources.

CONCLUSIONS

Paragraph 32: In summary, three weeks of interval training in a hot environment may enhances 3-km TT performance in a temperate environment in the weeks following exposure. The present results showed that whilst adding LHTL to heat interval training can elicit a haematological response; these physiological changes do not result in improved 3-km TT performance. Collectively, these findings indicate that combining LHTL with heat exposure does not provide additional benefit over heat training alone and the incorporation of heat into a training camp maybe a simple approach to improving athletic performance. However, factors such as psychology of the athlete, dose of stimuli, environment and training status should be considered when including heat or hypoxia as part of an athlete's training program.

ACKNOWLEDGMENTS

Paragraph 33: The authors wish to thank the Australian Institute of Sport and Australian Sports Commissions Big Ideas Fund for their financial support, as well as Douglass Hanly Moir for assistance with blood analysis. A special thanks to all staff at New South Wales Institute of Sport, University of Canberra Research Institute for Sport and Exercise, University of Technology Sydney and the Department of Physiology at the Australian Institute of Sport for their support during the study and assistance during data collection. In particular, Chis Gore, Laura Garvican-Lewis, Kevin Thompson, Val Chan, Brad Clark, Leanne Meyers, Graeme Albon, Jamie Plowman and Sabrina Skorski. The authors have no conflicts of interests, and the results of the present study do not constitute endorsement by ACSM. The results of this study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

References

1. Bandura A. Guide for constructing self-efficacy scales. *Self-efficacy beliefs of adolescents*. 2006;5(307-337).
2. Barnett A, Maughan RJ. Response of unacclimatized males to repeated weekly bouts of exercise in the heat. *Br J Sports Med*. 1993;27(1):39-44.
3. Batterham AM, Hopkins WG. Making meaningful inferences about magnitudes. *Int J Sports Physiol Perf*. 2006;1(1):50-7.
4. Bonetti DL, Hopkins WG. Sea-level exercise performance following adaptation to hypoxia: a meta-analysis. *Sports Med*. 2009;39(2):107-27.
5. Buchheit M, Racinais S, Bilsborough J et al. Adding heat to the live-high train-low altitude model: a practical insight from professional football. *Br J Sports Med*. 2013;47(Suppl 1):i59-i69.
6. Cohen J. *Statistical Power Analysis for the Behavioral Sciences* 2nd ed. Hillsdale, (NJ): Lawrence Erlbaum Associates; 1988. 567 p.

519 7. Corbett J, Neal R, Lunt H, Tipton M. Adaptation to heat and exercise performance
520 under cooler conditions: a new hot topic. *Sports Med*. 2014;44(10):1323-31.
521 8. Ely BR, Lovering AT, Horowitz M, Minson CT. Heat acclimation and cross tolerance
522 to hypoxia. *Temperature*. 2014;1(2):107-14.
523 9. Foster C. Monitoring training in athletes with reference to overtraining syndrome.
524 *Med Sci Sports Exerc*. 1998;30(7):1164-8.
525 10. Foster C, Florhaug JA, Franklin J et al. A new approach to monitoring exercise
526 training. *J Strength Cond Res*. 2001;15(1):109-15.
527 11. Gore CJ, Bourdon PC, Woolford SM, Ostler LM, Eastwood A, Scroop GC. Time and
528 sample site dependency of the optimized co-rebreathing method. *Med Sci Sports*
529 *Exerc*. 2006;38(6):1187-93.
530 12. Gore CJ, Sharpe K, Garvican-Lewis LA et al. Altitude training and haemoglobin mass
531 from the optimised carbon monoxide rebreathing method determined by a meta-
532 analysis. *Br J Sports Med*. 2013;47 Suppl 1:131-9.
533 13. Hahn AG, Gore CJ, Martin DT, Ashenden MJ, Roberts AD, Logan PA. An evaluation
534 of the concept of living at moderate altitude and training at sea level. *Comp Biochem*
535 *Physiol A*. 2001;128(4):777-89.
536 14. Halson SL. Monitoring training load to understand fatigue in athletes. *Sports Med*.
537 2014;44 Suppl 2:S139-47.
538 15. Hopkins W. Spreadsheets for analysis of controlled trials, with adjustment for a
539 subject characteristic. *Sportscience*. 2006;10:46-50.
540 16. Hopkins WG, Hewson DJ. Variability of competitive performance of distance
541 runners. *Med Sci Sports Exerc*. 2001;33(9):1588-92.

17. Humberstone-Gough CE, Saunders PU, Bonetti DL et al. Comparison of live high: train low altitude and intermittent hypoxic exposure. *J Sports Sci Med.* 2013;12(3):394-401.

18. Karlsen A, Racinais S, Jensen MV, Nørgaard SJ, Bonne T, Nybo L. Heat acclimatization does not improve VO2max or cycling performance in a cool climate in trained cyclists. *Scand J Med Sci Sports*. 2015;25:269-76.

19. 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. *Am J Physiol Heart Circ Physiol*. 2015;309(5):H750-H61.

20. Levine BD, Stray-Gundersen J. "Living high-training low": effect of moderate-altitude acclimatization with low-altitude training on performance. *J Appl Physiol*. 1997;83(1):102-12.

21. Lorenzo S, Halliwill JR, Sawka MN, Minson CT. Heat acclimation improves exercise performance. *J Appl Physiol*. 2010;109(4):1140-7.

22. Luetkemeier MJ, Thomas EL. Hypervolemia and cycling time trial performance. *Med Sci Sports Exerc*. 1994;26(4):503-9.

23. Millet GP, Roels B, Schmitt L, Woorons X, Richalet JP. Combining hypoxic methods for peak performance. *Sports Med*. 2010;40(1):1-25.

24. Neal RA, Corbett J, Massey HC, Tipton MJ. Effect of short-term heat acclimation with permissive dehydration on thermoregulation and temperate exercise performance [Internet]. *Scand J Med Sci Sports*. 2015. Available from: http://dx.doi.org/10.1111/sms.12526. doi:10.1111/sms.12526.

25. Pageaux B. The psychobiological model of endurance performance: an effort-based decision-making theory to explain self-paced endurance performance. *Sports Med*. 2014;44(9):1319-20.

26. Racinais S, Buchheit M, Bilsborough J, Bourdon PC, Cordy J, Coutts AJ. Physiological and performance responses to a training-camp in the heat in professional Australian football players. *Int J Sports Physiol Perform*. 2014;9(4):598-603.

27. Robertson EY, Saunders PU, Pyne DB, Aughey RJ, Anson JM, Gore CJ. Reproducibility of performance changes to simulated live high/train low altitude. *Med Sci Sports Exerc*. 2010;42(2):394-401.

28. Saugy JJ, Schmitt L, Hauser A et al. Same performance changes after Live High-Train Low in normobaric versus hypobaric hypoxia. *Front Physiol* [Internet]. 2016. Available from: http://journal.frontiersin.org/article/10.3389/fphys.2016.00138. doi:10.3389/fphys.2016.00138.

29. Saunders PU, Pyne DB, Telford RD, Hawley JA. Factors affecting running economy in trained distance runners. *Sports Med*. 2004;34(7):465-85.

30. Saunders PU, Telford RD, Pyne DB, Hahn AG, Gore CJ. Improved running economy and increased hemoglobin mass in elite runners after extended moderate altitude exposure. *J Sci Med Sport*. 2009;12(1):67-72.

31. Sawka MN, Concertino VA, Eichner ER, Schnieder SM, Young AJ. Blood volume: importance and adaptations to exercise training, environmental stresses, and trauma/sickness. *Med Sci Sports Exerc*. 2000;32(2):332.

32. Sawka MN, Coyle EF. Influence of body water and blood volume on thermoregulation and exercise performance in the heat. *Exerc Sport Sci Rev*. 1999;27:167-218.

33. Sawka MN, Leon LR, Montain SJ, Sonna LA. Integrated physiological mechanisms of exercise performance, adaptation, and maladaptation to heat stress. *Compr Physiol*. 2011;1:1883 - 928.

34. Schmidt W, Prommer N. The optimised CO-rebreathing method: a new tool to determine total haemoglobin mass routinely. *Eur J Appl Physiol*. 2005;95(5-6):486-95.

35. Schmit C, Duffield R, Hausswirth C, Coutts AJ, Le Meur Y. Pacing adjustments associated with familiarisation: heat vs. temperate environments. *Int J Sports Physiol Perform* [Internet]. 2015. doi:10.1123/ijspp.2015-0572.

36. Schmit C, Le Meur Y, Duffield R et al. Heat-acclimatization and pre-cooling: a further boost for endurance performance? *Scand J Med Sci Sports* [Internet]. 2015. Available from: http://dx.doi.org/10.1111/sms.12629. doi: 10.1111/sms.12629.

37. Stray-Gundersen J, Chapman RF, Levine BD. "Living high-training low" altitude training improves sea level performance in male and female elite runners. *J Appl Physiol*. 2001;91(3):1113-20.

38. Tanner R, Gore C. *Physiological tests for elite athletes*. Human Kinetics; 2013. p. 397-410.

39. Taylor NAS, Cotter JD. Heat adaptation : guidelines for the optimisation of human performance : review article. *Int SportMed J*. 2006;7(1):p.33-57.

Figure 1: Outline of study design, illustrating the exposure and non-exposure training periods. Along with the incremental treadmill testing, haemoglobin mass (CO-rebreathing) and a performance 3-km time-trial (3-km TT) were conducted. Testing protocols were conducted following exposure (post), one week (1wkP) and three weeks following exposure (3wkP).

Figure 2: Mean (± SD) weekly internal training load (TL), expressed as session rating of perceived exertion (sRPE) (RPE x duration in minutes). Data is divided into the two weeks prior (baseline), three weeks of environmental stimuli (exposure), and the three weeks following exposure where all training was conducted in temperate, sea-level conditions (non-exposure). No difference between groups in TL across the study period. **Likely within-group reduction in TL in HOT and H+H from Exposure to Non-Exposure. ^Likely between-group reduction from Exposure to Non-Exposure in H+H compared to both HOT and CONT. AU- arbitrary units.

Figure 3. Change in 3-km running time-trial performance expressed as a percent change (%) from Baseline ±90% CL for H+H (A), HOT (B), and CONT (C). *Likely within group difference from Baseline.

676 **Figure 4.** Percent change (%) from Baseline in A) Hb_{mass}, B) PV and C) BV. Groups are
677 indicated by the symbols HOT (●), H+H (○) and CONT (□).

678

679 1. Périard JD, Travers GJS, Racinais S, Sawka MN. Cardiovascular adaptations supporting human
680 exercise-heat acclimation. *Auton Neurosci*. 2016;196:52-62.
681 2. Sawka MN, Wenger CB, Pandolf KB. Thermoregulatory responses to acute exercise-heat
682 stress and heat acclimation. In. *Comp Physiol*: John Wiley & Sons, Inc.; 2011, pp. 157–85.
683 3. Scoon GSM, Hopkins WG, Mayhew S, Cotter JD. Effect of post-exercise sauna bathing on the
684 endurance performance of competitive male runners. *Journal of Science and Medicine in*
685 *Sport*. 2007;10(4):259-62.

686