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ORIGINAL ARTICLE

Impact of Exercise Heat Acclimation on Performance in Hot, Cool and Hypoxic Conditions

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Abstract

Purpose The aim of this study was to confirm the impact of heat acclimation on aerobic performance in hot conditions and elucidate the transfer of heat adaptations to cool and hypoxic environments.

Methods Ten males (VO_{2peak}: 4.50 ± 0.50 L/min) completed two three-week interventions consisting of heat acclimation (HA: 36°C and 59% RH) and temperate training (TEMP: 18°C and 60% RH) in a counter-balanced crossover design. Training weeks consisted of four work-matched controlled heart rate sessions interspersed with one intermittent sprint session, and two rest days. Before and after the interventions VO_{2peak} and 20-min time trial performance were evaluated in COOL (18°C), HOT (35°C) and hypoxic (HYP: 18°C and FiO_2: 15.4%) conditions.

Results Following HA, VO_{2peak} increased significantly in HOT (0.24 L/min [0.01, 0.47], $P = 0.040$) but not COOL ($P = 0.431$) or HYP ($P = 0.411$), whereas TEMP had no influence on VO_{2peak} ($P \geq 0.424$). Mean time trial power output increased significantly in HOT (20 W [11, 28], $P < 0.001$) and COOL (12 W [4, 21], $P = 0.004$), but not HYP (7 W [−1, 16], $P = 0.075$) after HA, whereas TEMP had no influence on mean power output ($P \geq 0.110$). Rectal (−0.13°C [−0.23, −0.03], $P = 0.009$) and skin (−0.7°C [−1.2, −0.3], $P < 0.001$) temperature were lower during the time trial in HOT after HA, whereas mean heart rate did not differ ($P = 0.339$).

Conclusions HA improved aerobic performance in HOT in conjunction with lower thermal strain and enhanced cardiovascular stability (similar heart rate for higher workload), whereas the mechanistic pathways improving performance in COOL and HYP remain unclear.

Keywords Altitude · Cross-acclimation · Cross-adaptation · Heat adaptation · Hot temperature · Temperate conditions

Introduction

Heat acclimation is known to improve peak oxygen uptake (VO_{2peak}) and endurance performance in hot conditions [20, 25, 49]. These improvements stem from integrative adaptations that enhance heat dissipation, fluid balance, cardiovascular stability, and skeletal muscle metabolism under heat stress [38, 48]. However, whether these adaptations improve exercise performance in cool conditions remains a topic of debate [30, 34]. Similarly, while some have shown heat acclimation to benefit endurance performance in hypoxic conditions [11, 23], others have not [55].

Following ~ 1–3 weeks of heat acclimation, significant improvements in VO_{2peak} [25, 49, 54, 57], power output at VO_{2peak} (W_{peak}) [31, 32, 44], lactate threshold [25, 31, 44] and endurance performance [25, 27, 44, 51] have been reported in cool conditions. The proposed pathways via which performance in cooler environments may be improved

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following heat acclimation include a lower metabolic rate during exercise at a given workload [48, 67, muscle glycogen sparing [5, 67], increased lactate threshold [25, 67], plasma volume (PV) expansion [52, 66], improved myocardial function (in rodent models) [15, 16], and increased skeletal muscle force generation [21, 42]. Despite the potential for these pathways to mediate performance improvements, some studies have reported that VO_{2peak} and time trial performance in cool conditions remain unchanged in well-trained male cyclists following 10–14 days of heat acclimation/acclimatization [19, 20, 55]. As such, the transfer of benefits stemming from training in the heat to performance in cooler environments remains contentious. To elucidate the transferability of any training effect, heat acclimation studies with a control group (i.e. training in temperate conditions) and performance tests conducted in both hot and cool conditions are required.

The transfer of heat adaptations to performance in hypoxic conditions (i.e. cross-adaptation) has gained interest in recent years [4, 7, 62, 63]. Heled et al [11] were the first to examine the potential for heat acclimation to improve performance in hypoxia. The authors reported that VO_{2peak} in hypoxia (FiO_2: 0.15) was unaffected after 12 days of heat acclimation, but that the onset of blood lactate accumulation occurred at a higher heart rate and oxyhemoglobin saturation (SpO_2) was elevated when walking at 7 km/h. Similarly, White et al [61] reported that VO_{2peak} was unaffected in hypoxia (FiO_2: 0.12) following 10 days of heat acclimation, but that training in the heat might confer an improvement (~1.6%; $P=0.07$) in time trial performance. A separate study found that time trial performance in hypoxia (FiO_2: 0.14) was improved by ~4.8% following 10 days of heat acclimation, whereas training in cool conditions provided no performance benefit (~0.8%) [23]. It has also been shown that 10 days of heat acclimation had no effect on VO_{2peak} in hypoxia (FiO_2: 0.13) [55], while others reported a lower heart rate and higher SpO_2 during exercise at 65% VO_{2peak} in hypoxic conditions (FiO_2: 0.12) after heat acclimation, but not after training in cool conditions [8]. The purported mechanisms associated with performance improvements in hypoxia following heat acclimation include an expansion of PV that facilitates the maintenance of cardiac output and SpO_2 (i.e. O_2 delivery), along with reduced body temperature and improved blood flow distribution [7, 62]. Exposure to heat stress also activates the expression of heat shock proteins (HSP), which leads to acquired thermotolerance and cellular cross-tolerance [7, 22]. The expression of HSP72 and HSP90 increases the molecular stability of hypoxia-inducible factor-1α (HIF-1α), which stimulates erythropoiesis [53] and angiogenesis [6]. Heat acclimation has thus been suggested to provide a pathway for improving performance in hypoxia via enhanced cytoprotection and improved O_2 transport and delivery [7, 23, 62]. Although heat acclimation has been shown to improve certain aspects of endurance performance (e.g. higher SpO_2 and lower submaximal heart rate) in hypoxic conditions, the benefits to self-paced (i.e. time trial) exercise are unclear and those to VO_{2peak} are absent.

Therefore, the aim of this study was to elucidate the transfer of heat adaptations to VO_{2peak} and time trial performance in cool and hypoxic conditions, and confirm the impact of heat acclimation on these parameters in hot conditions following whole-body pre-heating. A cross-over design with participants also training in temperate conditions was employed to isolate the transferability of heat acclimation adaptations from those of training, to performance in cool and hypoxic conditions. It was hypothesized that heat acclimation would improve both VO_{2peak} and time trial performance in the heat, but only time trial performance in cool and hypoxic conditions.

Methods

Participants

Ten endurance-trained (Tier 2–3) male cyclists and triathletes participated in and completed the study [28, 36]. Their characteristics on enrolment for age, height, body mass, VO_{2peak} and W_{peak} were: 34 ± 7 years, 177 ± 6 cm, 75.6 ± 7.5 kg, 4.50 ± 0.50 L/min, and 416 ± 39 W. All participants undertook personal training regimens and regularly competed in amateur endurance cycling races. Inclusion criteria for the study included cycling at least 250 km per week and undertaking cycling training for the previous 2 years at least. None of the participants had a prior history of exertional heat illness. The project was approved by the Anti-Doping Laboratory Qatar ethics committee (F2015000201) and conformed to the standards of the Declaration of Helsinki. Written informed consent was obtained from all participants before the beginning of testing.

Experimental Design

All participants completed two 3-week training interventions consisting of exercise heat acclimation (HA: 35.5 ± 1.8°C and 59.0% ± 7.7% relative humidity: RH) and work-matched exercise in temperate conditions (TEMP: 18.0 ± 0.5°C and 59.5% ± 9.9% RH) in a counter-balanced cross-over design (Fig. 1). The training interventions were separated by 21 ± 17 weeks and took place in Doha, Qatar. To minimise the influence of heat acclimatization, testing during the summer months was avoided. However, because of the high average daily peak temperatures of 23 to 42°C from the coldest to warmest months of the year in Qatar, respectively [56], participants were encouraged to minimise outdoor

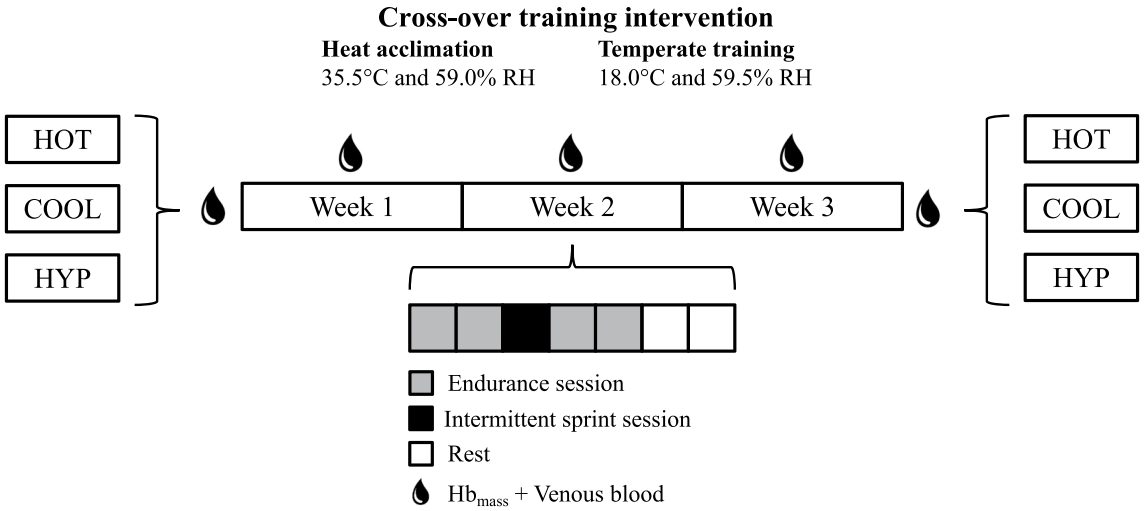

Fig. 1 Schematic representation of the three-week cross-over heat acclimation and work-matched temperate training intervention. Training consisted of four controlled heart rate endurance sessions and one intermittent sprint session per week. Prior to and following each intervention, performance tests (VO_{2peak} and 20-min time trial) were performed in hot (HOT: 35°C, 60% RH, 20.93% FiO_2, with pre-heating to a rectal temperature of 38.5°C), cool (COOL: 18°C, 60% RH, 20.93% FiO_2), and hypoxic (HYP: 18°C, 60% RH, 15.4% FiO_2) conditions. Hemoglobin mass (Hb_{mass}) measurements and venous blood samples were taken prior to, on days 3, 10 and 18 (Hb_{mass}) or 4, 11 and 19 (venous blood), and following the intervention

training to once per week for a minimum of 3 weeks before the first laboratory visit. Two participants had 4- and 5-week wash-outs due to time constraints, but these were following TEMP. Training consisted of five sessions per week including four controlled heart rate endurance sessions (days 1, 2, 4, 5, 8, 9, 11, 12, 15, 16, 18 and 19) and one intermittent sprint session (days 3, 10 and 17). Days 6, 7, 13, 14, 20 and 21 were rest days. Prior to and following HA and TEMP, participants completed three performance tests in hot (HOT: 35°C, 60% RH, 20.93% FiO_2, with pre-heating to a rectal temperature (T_{re}) of 38.5°C), cool (COOL: 18°C, 60% RH, 20.93% FiO_2), and hypoxic (HYP: 18°C, 60% RH, 15.4% FiO_2; ~2500 m simulated altitude) conditions with a convective airflow of 3 m/s in each environment. Each testing session was separated by 48 h and included an incremental cycling test to exhaustion to determine VO_{2peak} and 20-min time trial separated by 30 min of passive rest. Testing session order was randomized between participants but was maintained for each participant across interventions. The intervention was part of a larger project with physiological and hematological adaptations presented in a companion paper [40].

Training Intervention

Participants attended the laboratory at the same time of day to conduct the training sessions. The controlled heart rate training sessions were designed to produce a workload that corresponded to the total work (kJ) completed in 60 min if the power output associated with 65% VO_{2peak} was maintained. It was calculated as:

$$\text{Total work (kJ)} = \text{P65\%} \times 3600 \,/\, 1000$$

where, P65% is the power output corresponding to 65% VO_{2peak}, 3600 is time in seconds, and 1000 is the conversion from joules to kilojoules. Total work was increased to 105% in week two and 110% in week three of the initial workload to sustain the thermal stimulus and week-to-week training duration. The same progression was applied during both training interventions. Each training session involved an initial period (20% of total work) of cycling at a constant workload equivalent to 65% VO_{2peak} on a Lode ergometer (Corival, Groningen, Holland). Over the remainder of each session, computer software (Lode ergometry manager 9.0) adjusted resistance every 30 s so that an exercising heart rate associated with 65% VO_{2peak} was maintained. Participants were instructed to maintain a steady cadence > 80 rev/min. The initial constant workload period was designed to increase heart rate and core temperature and promote the onset of sweating. Heart rate during HA was clamped 7 beats/min higher than in TEMP to maintain a similar relative training intensity between conditions [38, 64], in response to the direct effect of temperature (i.e. hyperthermia) on the sinoatrial node [17] and to vagal withdrawal and sympathetic activation [9].

The intermittent sprint session consisted of a 20-min warm-up followed by five all-out sprints of 30 s on an SRM cycle ergometer (Schoberer Rad Meßtechnik, Jülich,

Germany) set to isokinetic mode at 105 rev/min. Participants were instructed to remain seated and were verbally encouraged throughout all sprints. The sprints were interspersed with 4 min 30 s of light resistance (~ 50 W) pedaling to reduce venous pooling in the lower extremities and minimize feelings of light-headedness or nausea. After the final sprint, participants rested for 5 min and then completed a 10-min cool-down at a light resistance (50–100 W) to ensure 60 min of heat exposure per day during HA and exercising duration was matched in TEMP training. During both the intermittent sprint and controlled heart rate training sessions participants consumed plain water ad libitum, participants were encouraged to drink an amount estimated from the previous days sweat rate to minimize post-training body mass losses to < 1%.

Performance Tests

Participants attended the laboratory at the same time of day and were instructed to ingest 7 mL/kg body mass of water 1 h prior to the start of the test. Upon arrival to the laboratory, participants were given a T_{re} probe to self-insert and asked to provide a urine sample for the measurement of urine specific gravity (USG: Pocket refractometer PAL-10S, Atago, Tokyo, Japan). A USG > 1.020 was considered a marker of hypohydration and participants were asked to consume additional water (300 mL) prior to commencing the trial. After instrumentation (heart rate and skin temperature) and baseline measures, participants rested in the environmental chamber for 15 min in the COOL and HYP trials prior to commencing the incremental cycling test. In the HOT trial, participants lay supine in a temperature-controlled bath (41.9 ± 0.9 °C) inside the environmental chamber set to HOT conditions until reaching a T_{re} of 38.5°C. Pre-heating was conducted to ensure participants undertook the performance tests in HOT in a hyperthermic state. Participants then towel dried, changed into cycling attire (cycling shorts, socks and shoes) and commenced the incremental cycling test 5 min after exiting the bath. The ramp protocol commenced with unloaded pedaling and increased by 1 W every 2 s (30 W/min) until volitional fatigue on the Lode ergometer. Oxygen uptake was measured continuously using an online breath-by-breath cardiopulmonary system (Oxycon Pro, CareFusion, Rolle, Switzerland). VO_{2peak} and W_{peak} were determined as the highest 30 s average of the second-by-second VO_2 and power output data, respectively.

Following the incremental test, participants recovered in the environmental chamber for 30 min, which included 20 min of passive rest and a 10-min self-selected warm-up, before performing the 20-min time trial. In the HOT trial, ambient temperature (37.4 ± 2.7 °C) during the 30-min recovery period was adjusted to maintain T_{re} at ~ 38.5°C prior to commencing the time trial, which was performed on the SRM ergometer. Participants were asked to maintain the highest sustainable effort for 20 min, which simulates the demands of a 16.1-km (10-mile) time trial. Feedback during the time trial was limited to the time remaining, with no other information provided. Participants were permitted to drink water ad libitum. A similar protocol with trained cyclists in our laboratory reported a coefficient of variation of 1.1% in performance [2].

Physiological and Perceptual Measurements

Body core temperature was continuously monitored with a T_{re} probe (MRB, Ellab A/S, Hillerød, Denmark) inserted 15 cm beyond the anal sphincter and attached to a precision digital thermometer allowing measurement to the nearest 0.1°C (DM 852, Ellab A/S, Hillerød, Denmark). Skin temperature was monitored at four sites with iButton™ temperature sensors/data loggers (Maxim Integrated Products, Sunnyvale, CA) used to calculate mean skin temperature (T_{sk}) [43]. Temperature measurements were taken during the resting baseline period prior to the VO_{2peak} test and at 2 min intervals during the test. SpO_2 was measured via pulse oximetry (8000SL; Nonin Medical Inc, Plymouth, MN) on the right middle finger at the same intervals. During the 30-min recovery period and the time trial T_{re} and T_{sk} measures were taken at 5 min intervals and SpO_2 upon completion. Heart rate was monitored continuously with a telemetric chest-strap transmitter (T-31 Polar Electro, Lake Success, NY) with resting heart rate determined as the average of 1 min after a 10-min seated period. Participants were asked to keep a 24-h food diary before testing and to replicate their diet before the second and third trials.

Hematological Measures

Hemoglobin mass (Hb_{mass}) was determined via the modified optimized CO rebreathing technique [50]. Briefly, participants rebreathed a 1.2 mL/kg bolus of CO for 2 min after a period of seated rest. Arterialized capillary fingertip blood samples were analyzed in quintuplicate (ABL 90 FLEX, Radiometer, Brønshøj, Denmark) prior to and 7 min following the start of the rebreathing period to determine the percentage of hemoglobin saturated with CO. Expired CO was determined using a Draeger Pac 7000 (Lubeck, Germany). Absolute Hb_{mass} (in g) was then calculated following corrections for remaining CO in the rebreathing apparatus, washout of CO following the procedure and estimates of residual lung volume. Between-day duplicate measurements were taken prior to (pre) and after (post) each intervention. In any case where Hb_{mass} differed by > 2% between duplicate measurements, the test was repeated. During the intervention, single Hb_{mass} measurements were taken on days 3, 10 and 18 (represented as days 4, 11 and 19 in Table 2). Hb_{mass}

was used to calculate blood volume (BV), red cell volume (RCV) and plasma volume (PV) using hemoglobin concentration (Hb) and hematocrit (Hct) values from venous blood samples (Beckman Coulter DxH 800, Beckman Coulter, Miami, FL) collected following 10 min of seated rest prior to training on days 4, 11 and 19 using the formulas:

$$\text{BV (mL)} = \left(\text{Hb}_{\text{mass}}(\text{g})/\text{Hb}(\text{g/dL})\right) \times 100$$

$$\text{RCV (mL)} = \text{BV(mL)} \times (\text{Hct}/100)$$

$$\text{PV (mL)} = \text{BV - RCV}$$

Statistical Analyses

All statistical analyses were performed in R statistical software (v 4.2.1) [39] and data are presented as means and 95% confidence intervals [CI], unless otherwise indicated. Linear mixed effect models were estimated using the 'lme4' package [1], utilizing restricted maximum likelihood and 'nloptwrap' optimizer, with each model including participant ID as a random intercept. Q-Q plots and histograms were used to assess data normality. For the analysis of VO_{2peak}, W_{peak}, peak heart rate and T_{sk} during the incremental test to exhaustion, linear mixed effect models were implemented with testing environment (COOL, HYP, HOT), training condition (TEMP, HA) and training status (pre, post) used as fixed effects. Training status (pre, post) was implemented as an ordered factor. Initial and final T_{re} and SpO_2 recorded during the incremental test were analyzed using the same random and fixed effects as the incremental exercise test outcome variables, except time was added as an ordered fixed effect with two levels (initial, final). For the analysis of power output, T_{re}, T_{sk} and heart rate during the time trial, the same random and fixed effects as the incremental exercise test outcome variables, except time was an ordered factor with four levels (4, 11, 19, Post). Hematological variables recorded at rest (i.e. PV and Hb_{mass}) were analyzed using training condition and day of training (Pre, 4, 11, 19, Post) as fixed effects. Pairwise comparisons were conducted using the 'emmeans' package [24] and the Kenward-Roger method was used to approximate degrees of freedom. Significance was accepted at $P < 0.05$.

Results

Incremental Exercise Test

VO_{2peak} was not significantly different between HA and TEMP (0.07 L/min [−0.03, 0.16], $P = 0.171$) and did not differ from pre- to post-intervention (0.06 L/min [−0.03, 0.16], $P = 0.186$; Fig. 2). However, VO_{2peak} in COOL was significantly higher than in HYP (0.61 L/min [0.49, 0.72], $P < 0.001$) and HOT (0.28 L/min [0.16, 0.39], $P < 0.001$), while VO_{2peak} in HYP was significantly lower than in HOT (−0.33 L/min [−0.44, −0.21], $P < 0.001$). Following HA, VO_{2peak} increased significantly in HOT (0.24 L/min [0.01, 0.47], $P = 0.040$), but not in COOL (0.09 L/min [−0.14, 0.32], $P = 0.431$) or HYP (0.10 L/min [−0.13, 0.32], $P = 0.411$). Following TEMP, VO_{2peak} did not significantly change in any condition (all $P \geq 0.424$).

W_{peak} during the incremental exercise test was not significantly different between HA and TEMP (1 W [−4,7], $P = 0.644$), but significantly increased from pre- to post-intervention (11 W [6, 17], $P < 0.001$; Fig. 2). W_{peak} in COOL was significantly higher than in HYP (40 W [33, 47], $P < 0.001$) and HOT (41 W [34, 48], $P < 0.001$), but there was no difference between HYP and HOT ($P = 0.744$). Following HA, W_{peak} increased in HOT (25 W [12, 39], $P < 0.001$) but not in COOL (10 W [−4, 23], $P = 0.159$) or HYP (10 W [−4, 24], $P = 0.147$). Following TEMP, W_{peak} was not significantly different in any condition (all $P \geq 0.170$).

Peak heart rate during the incremental exercise test was not significantly different between HA and TEMP (1 beat/min [−1, 2], $P = 0.477$) and did not differ from pre- to post-intervention (0 beats/min [−1, 2], $P = 0.667$; Fig. 2). Peak heart rate in HOT was significantly higher than in COOL (3 beats/min [1, 5], $P = 0.005$) and HYP (7 beats/min [5, 9], $P < 0.001$), while peak heart rate in HYP was significantly lower than COOL (−4 beats/min [−6, −2], $P < 0.001$). Peak heart rate did not significantly change following either HA or TEMP in any condition (all $P \geq 0.16$).

Initial T_{re} was similar between HA and TEMP (−0.00°C [−0.07, 0.07], $P = 0.971$), as well as from pre- to post-intervention (0.03°C [−0.04, 0.09], $P = 0.429$; Table 1). Final T_{re} was significantly higher in HOT compared to COOL (0.89°C [0.78, 1.01], $P < 0.001$) and HYP (1.02°C [0.90, 1.13], $P < 0.001$), while COOL was significantly higher than HYP (0.12°C [0.01, 0.24], $P = 0.04$). Final T_{re} was significantly higher than initial T_{re} during all incremental exercise tests (0.25°C [0.19, 0.32], $P < 0.001$). T_{sk} was not significantly different between HA and TEMP (0.1°C [−0.2, 0.3], $P = 0.640$), and did not differ from pre- to post-intervention (0.0°C [−0.2, 0.3], $P = 0.693$). Mean T_{sk} was significantly higher in HOT compared to COOL (4.7°C [4.4, 5.0], $P < 0.001$) and HYP (4.7°C [4.4, 5.0], $P < 0.001$), but there was no significant difference between COOL and HYP ($P = 0.921$).

SpO_2 was significantly higher during HA than TEMP (1.2% [0.5, 1.9], $P < 0.001$), but did not differ from pre- to post-intervention (0.4% [−0.2, 1.1], $P = 0.204$; Table 1). SpO_2 was significantly lower in HYP than HOT (−6.3% [−7.1, −5.5], $P < 0.001$) and COOL (−7.1% [−8.0, −6.3],

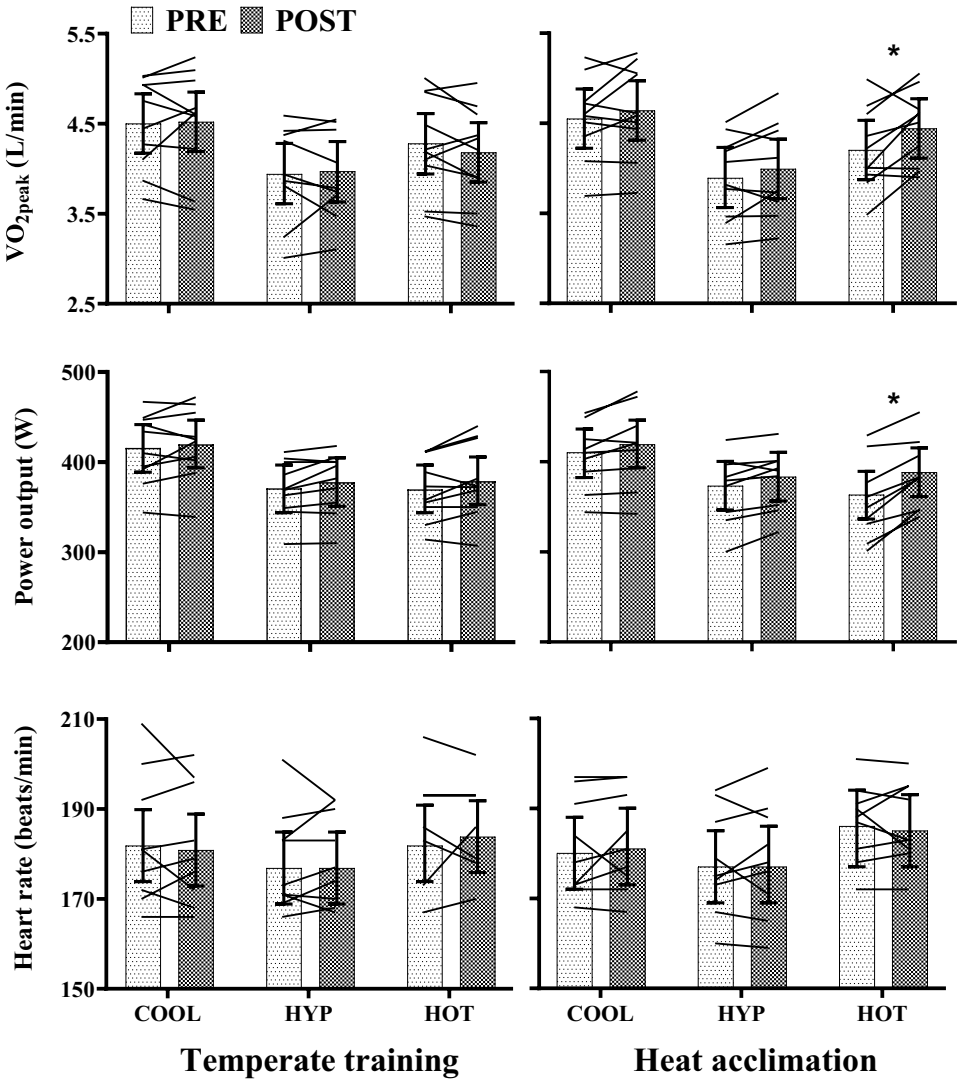

Fig. 2 Peak power output, peak oxygen uptake (VO_{2peak}) and peak heart rate during the incremental exercise test in cool (COOL), hypoxic (HYP) and hot (HOT) conditions, before and after temperate training (TEMP) and exercise heat acclimation (HA). $n = 10$ except for peak power output in HA ($n = 9$). Data presented as means (bars), 95% confidence intervals (error bars) and individual responses (black lines). * Significantly different from pre- to post-intervention ($P \leq 0.04$)

Table 1 Rectal temperature, skin temperature and oxyhaemoglobin saturation during the incremental exercise test in cool (COOL), hypoxic (HYP) and hot (HOT) conditions, before and after temperate training (TEMP) and exercise heat acclimation (HA)

Variable		COOL		HYP		HOT	
		Pre	Post	Pre	Post	Pre	Post
Initial T_{re} (°C)	TEMP	37.3 [37.1, 37.5]$^{\wedge}$	37.3 [37.1, 37.5]$^{\wedge}$	37.2 [37.0, 37.5]$^{\wedge}$	37.3 [37.1, 37.6]$^{\wedge}$	38.6 [38.4, 38.8]	38.7 [38.5, 38.9]
	HA	37.3 [37.1, 37.5]$^{\wedge}$	37.3 [37.1, 37.5]$^{\wedge}$	37.4 [37.1, 37.6]$^{\wedge}$	37.3 [37.1, 37.5]$^{\wedge}$	38.6 [38.4, 38.8]	38.5 [38.3, 38.7]
Final T_{re} (°C)	TEMP	37.6 [37.4, 37.9]$^{\wedge}$	37.8 [37.5, 38.0]$^{\wedge}$	37.5 [37.3, 37.8]$^{\wedge}$	37.6 [37.4, 37.9]$^{\wedge}$	38.6 [38.4, 38.8]	38.7 [38.5, 39.0]
	HA	37.8 [37.5, 38.0]$^{\wedge}$	37.8 [37.6, 38.0]$^{\wedge}$	37.6 [37.4, 37.8]$^{\wedge}$	37.7 [37.4, 37.9]$^{\wedge}$	38.6 [38.4, 38.8]	38.5 [38.3, 38.7]
T_{sk} (°C)	TEMP	30.1 [29.6, 30.6]$^{\wedge\#}$	30.6 [30.1, 31.1]$^{\wedge}$	30.7 [30.2, 31.2]$^{\wedge}$	30.7 [30.2, 31.2]$^{\wedge}$	35.2 [34.7, 35.7]	35.1 [34.6, 35.6]
	HA	30.7 [30.2, 31.2]$^{\$\wedge}$	30.7 [30.2, 31.2]$^{\wedge}$	30.1 [29.6, 30.6]$^{\$\wedge}$	30.5 [30.0, 31.0]$^{\wedge}$	35.6 [35.1, 36.1]	35.1 [34.6, 35.6]
Initial SpO_2 (%)	TEMP	99 [97, 100]$^{\#\wedge}$	99 [98, 100]$^{\#\wedge}$	94 [92, 95]$^{\wedge}$	93 [92, 94]$^{\wedge}$	97 [95, 98]	96 [94, 97]
	HA	99 [97, 100]$^{\#\wedge}$	99 [98, 100]$^{\#\wedge}$	94 [92, 95]$^{\wedge}$	94 [93, 96]$^{\wedge}$	96 [95, 98]	97 [96, 99]
Final SpO_2 (%)	TEMP	92 [90, 95]$^{\#\wedge}$	94 [92, 95]$^{\#}$	84 [82, 86]$^{\wedge}$	84 [82, 86]$^{\wedge}$	95 [94, 97]	95 [93, 96]
	HA	95 [93, 98]$^{\$\#}$	96 [93, 98]$^{\#}$	86 [85, 88]$^{\$\wedge}$	87 [85, 88]$^{\$\wedge}$	94 [91, 97]	96 [94, 99]

T_{re} rectal temperature, T_{sk} mean skin temperature, SpO_2 oxyhaemoglobin saturation. Data presented as means with [95% confidence intervals]. $^{\$}$Significantly different to TEMP training ($P \leq 0.049$); $^{\wedge}$ Significantly different to HOT ($P \leq 0.043$); $^{\#}$ Significantly different to HYP ($P \leq 0.042$)

$P < 0.001$), with a non-significant difference between COOL and HOT of 0.8% [0.2, 1.7] ($P = 0.057$). Final SpO_2 was significantly lower than initial SpO_2 during all incremental exercise tests (−4.8% [−5.6, −4.1], $P < 0.001$).

Time Trial Performance

Mean power output during the time trial was not significantly different between HA and TEMP (3 W [−0, 6], $P = 0.089$), but significantly increased from pre- to post-intervention (8 W [5, 12], $P < 0.001$); Fig. 3). Mean power output in COOL was significantly higher than in HYP (34 W [30, 38], $P < 0.001$) and HOT (42 W [38, 46], $P < 0.001$), while mean power output in HYP was significantly higher than HOT (8 W [4, 12], $P < 0.001$). Following HA mean power output significantly increased in COOL (12 W [4, 21], $P = 0.004$) and HOT (20 W [11, 28], $P < 0.001$), but not HYP (7 W [−1, 16], $P = 0.075$). Following TEMP mean power output did not significantly change in any condition (all $P \geq 0.110$).

Mean heart rate during the time trial was not significantly different between HA and TEMP (−1 beat/min [−1, 0], $P = 0.163$; Fig. 3). Mean heart rate in HOT was significantly higher than in COOL (3 beats/min [2, 4], $P < 0.001$) and HYP (6 beats/min [5, 7], $P < 0.001$), while mean heart rate in HYP was significantly lower than COOL (−3 beats/min [−3, −2], $P < 0.001$). Mean heart rate did not significantly change in any condition following either intervention (all $P \geq 0.090$).

Mean T_{re} during the time trial was significantly lower in HA than TEMP (−0.05°C [−0.09, −0.01], $P = 0.015$), but did not differ from pre- to post-intervention (−0.00°C [−0.04, 0.04], $P = 0.951$; Fig. 4). Mean T_{re} was significantly higher in HOT compared to COOL (0.44°C [0.39, 0.49], $P < 0.001$) and HYP (0.55°C [0.50, 0.60], $P < 0.001$), while mean T_{re} in HYP was significantly lower than in COOL (−0.11°C [−0.16, −0.06], $P < 0.001$). Following HA, mean T_{re} was significantly lower in HYP (−0.10°C [−0.20, −0.00], $P = 0.044$) and HOT (−0.13°C [−0.23, −0.03], $P = 0.009$), but not COOL ($P = 0.618$). Following TEMP, mean T_{re} was significantly higher in HYP (0.11°C [0.02, 0.21], $P = 0.024$) and HOT (0.16°C [0.06, 0.25], $P = 0.002$), but not COOL ($P = 0.184$).

Mean T_{sk} during the time trial was not significantly different between HA and TEMP (0.1°C [−0.1, 0.3], $P = 0.362$), but did significantly decrease from pre- to post-intervention (−0.3°C [−0.4, −0.1], $P = 0.003$; Fig. 4). Mean T_{sk} was significantly higher in HOT compared to COOL (4.9°C [4.7, 5.1], $P < 0.001$) and HYP (5.0°C [4.8, 5.2], $P < 0.001$), but there was no significant difference between COOL and HYP ($P = 0.339$). Following HA, mean T_{sk} was significantly decreased in HOT (−0.7°C [−1.2, −0.3], $P < 0.001$), but not COOL ($P = 0.085$) or HYP ($P = 0.145$). Following TEMP,

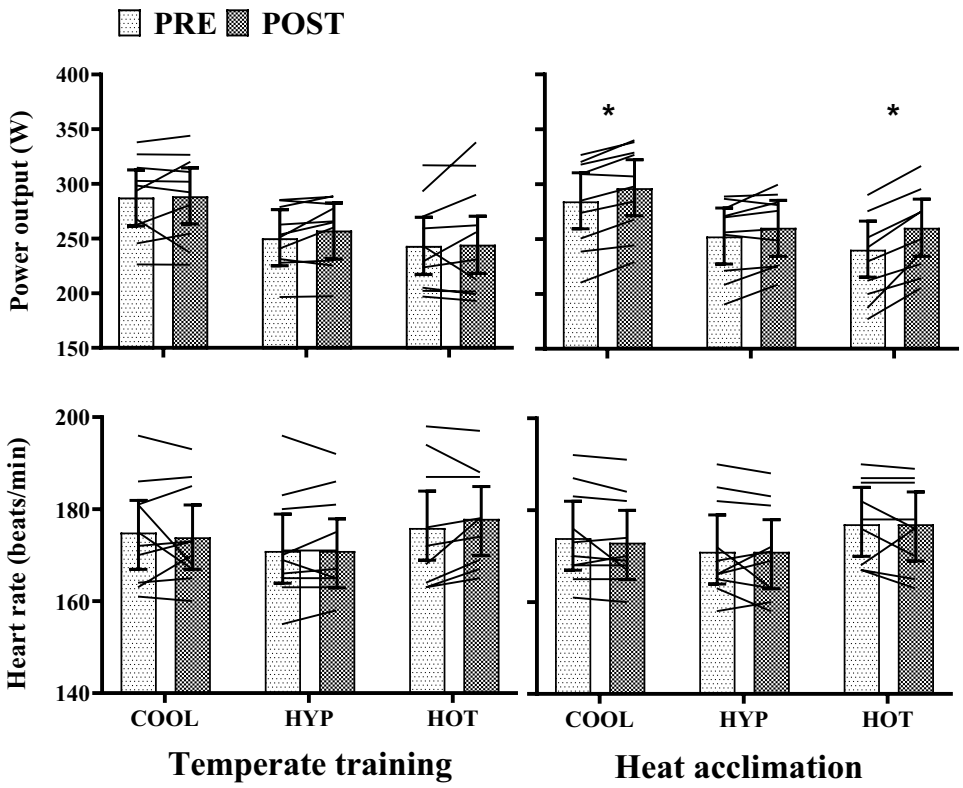

Fig. 3 Mean power output and heart rate during the time trial in cool (COOL), hypoxic (HYP) and hot (HOT) conditions, before and after temperate training (TEMP) and exercise heat acclimation (HA). $n = 10$ except for mean power output in HOT following HA ($n = 9$). Data presented as means (bars), 95% confidence intervals (error bars) and individual responses (black lines). * Significantly different from pre- to post-intervention ($P \leq 0.004$)

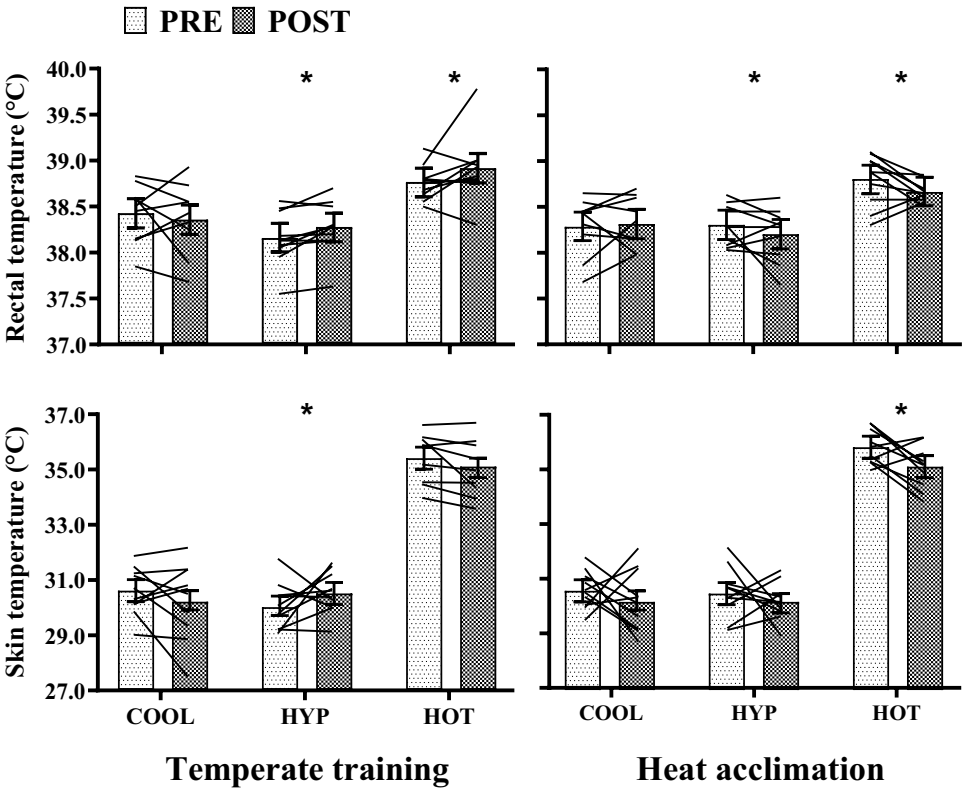

Fig. 4 Mean rectal temperature and skin temperature during the time trial in cool (COOL), hypoxic (HYP) and hot (HOT) conditions, before and after temperate training (TEMP) and exercise heat acclimation (HA). Data presented as means (bars), 95% confidence intervals (error bars) and individual responses (black lines). * Significantly different from pre- to post-intervention ($P \leq 0.044$)

mean T_{sk} significantly increased in HYP (0.5°C [0.1, 0.9], $P = 0.023$), but not COOL ($P = 0.112$) or HOT ($P = 0.127$).

Hematological Responses

PV was not significantly different between HA and TEMP (37 mL [−30, 103], $P = 0.277$; Table 2). During HA, PV increased significantly from pre-intervention to day 11 (168 mL [23, 314], $P = 0.024$) and 19 (150 mL [0, 300], $P = 0.050$), as well as during TEMP from pre-intervention to day 11 (166 mL [20, 311], $P = 0.027$) and 19 (157 mL [7, 307], $P = 0.041$). There was a decrease in PV following TEMP from day 11 (161 mL [−16, −307], $P = 0.030$) and day 19 (153 mL [−2, −303], $P = 0.046$) to post-intervention. Hb_{mass} was significantly lower in HA than TEMP (−18 g [−24, −11], $P < 0.001$; Table 2). During HA, Hb_{mass} decreased significantly from pre-intervention to day 4 (−22 g [−8, −37], $P = 0.003$), day 11 (−17 g [−2, −32], $P = 0.022$) and day 19 (−21 g [−6, −36], $P = 0.007$); whereas there was no change in TEMP ($P \geq 0.445$).

Heat Adaptations and Training

Resting heart rate decreased post-intervention in HA (−5 beats/min [−9, −2], $P = 0.007$)), but not TEMP ($P = 0.749$; Table 3). Resting T_{re} was significantly lower post-intervention in HA than TEMP (−0.26°C [−0.48, −0.04], $P = 0.200$). Mean T_{re} was similar during HA and TEMP (−0.02°C [−0.15, 0.10], $P = 0.683$) and remained unchanged from pre- to post-intervention ($P \geq 0.529$). However, mean power

Table 2 Plasma volume and hemoglobin mass during temperate training (TEMP) and exercise heat acclimation (HA)

Measure		Intervention day				
		Pre	4	11	19	Post
Plasma Volume (mL)	TEMP	3577 [3198, 3957]	3665 [3286, 4045]	3743 [3363, 4123]*	3734 [3353, 4115]*	3582 [3202, 3961]$^{£¥}$
	HA	3544 [3164, 3924]	3588 [3207, 3969]	3712 [3332, 4092]*	3694 [3313, 4075]*	3581 [3201, 3961]
Hemoglobin mass (g)	TEMP	951 [866, 1036]	953 [869, 1038]	953 [868, 1038]	954 [870, 1039]	949 [864, 1033]
	HA	948 [863, 1032]	926 [841, 1010]*$^{\$}$	931 [846, 1015]*$^{\$}$	927 [842, 1012]*$^{\$}$	941 [856, 1025]$^{€}$

Data presented as means with [95% confidence intervals]. * Significantly different to Pre ($P \leq 0.04$); $^{€}$ Significantly different to day 4 ($P \leq 0.02$); $^{£}$Significantly different to day 11 ($P \leq 0.03$); $^{¥}$ Significantly different to day 19 ($P \leq 0.05$); $^{\$}$ Significantly different to TEMP ($P \leq 0.001$)

Table 3 Resting heart rate and rectal temperature, mean rectal temperature, whole-body sweat rate, mean power output, training time and work completed on days 1 and 19 of temperate training (TEMP) and exercise heat acclimation (HA)

Variable		Intervention day	
		1	19
Resting heart rate (beats/min)	TEMP	57 [52, 63]	57 [52, 62]
	HA	58 [53, 63]	53 [48, 58]$*
Resting T_{re} (°C)	TEMP	37.2 [37.0, 37.4)	37.1 [36.9, 37.4)
	HA	37.0 [36.8, 37.2)	36.9 [36.6, 37.1)$
Mean T_{re} (°C)	TEMP	38.3 [38.1, 38.4]	38.2 [38.1, 38.4]
	HA	38.2 [38.0, 38.3]	38.2 [38.1, 38.4]
Whole-body sweat rate (L/h)	TEMP	0.9 [0.7, 1.2]	0.8 [0.6, 1.1]
	HA	1.5 [1.3, 1.8]$	1.9 [1.7, 2.2]$*
Mean power output (W)	TEMP	201 [176, 226]	215 [190, 240]*
	HA	169 [144, 194]$	193 [168, 218]$*
Time (min)	TEMP	58.5 [54.0, 63.0]	60.1 [55.5, 64.8]
	HA	70.2 [65.6, 74.7]$	67.8 [63.4, 72.2]$
Work (kJ)	TEMP	704 [643, 765]	772 [711, 833]*
	HA	703 [642, 765]	773 [712, 834]*

T_{re} rectal temperature. Data presented as means with [95% confidence intervals]. $ Significant difference to TEMP training ($P \leq 0.04$); * Significantly different to day 1 within same condition ($P \leq 0.04$)

output was significantly higher in TEMP than HA (27 W [19, 36], $P < 0.001$) and increased significantly from pre- to post-intervention in both TEMP (14 W [1, 27], $P = 0.04$) and HA (24 W [12, 36], $P < 0.001$). Training time was similar from pre- to post-intervention ($P = 0.529$), but took longer in HA (10.1 min [6.8, 13.6], $P < 0.001$), whereas work completed was similar between TEMP and HA (0 kJ [−3, 4], $P = 0.784$), but increased from pre- to post-intervention (69 kJ [66, 73], $P < 0.001$). Whole-body sweat rate was higher during HA than TEMP (0.86 L/h [0.71, 1.01], $P < 0.001$) and increased significantly from pre- to post-intervention in HA (0.38 L/h [0.17, 0.59], $P = 0.001$).

Discussion

To our knowledge, this is the first study to compare the impact of HA against TEMP training on VO_{2peak} and time trial performance in HOT, COOL and HYP conditions in a group of trained cyclists. Our data demonstrate that VO_{2peak} increased by 6% (0.24 L/min) in HOT following HA, but not in COOL or HYP. During the 20-min time trial, mean power output increased by 8% (20 W) in HOT and 4% (12 W) in COOL following HA, with a non-significant increase of 3% (7 W [−1, 16]) in HYP. The increased power output during the time trial in HOT was associated with a similar heart rate to pre-HA, lower mean T_{re} (0.13°C) and T_{sk} (0.7°C), and a higher whole-body sweat rate (during HA). T_{re} was also lower in HYP (0.10°C) after HA. The improvements in VO_{2peak} in HOT and time trial performance in HOT, COOL and HYP occurred in conjunction with an increase in PV (4.2%) and reduction Hb_{mass} (2.2%) on day 19 of HA, but a return towards baseline post-intervention (PV: 1.0%; Hb_{mass}: −0.7%). These data confirm the benefits of HA on aerobic capacity and time trial performance in HOT and provide evidence of improvements in time trial performance in COOL and HYP. These improvements are associated with greater cardiovascular stability (i.e. higher workload for similar heart rate) and lower thermal strain during exercise in HOT, whereas the mechanistic pathways improving performance in COOL and HYP remain less clear.

Exercise Performance in a Hot Environment

The increase in VO_{2peak} (6%) and W_{peak} (7%) during the incremental test (Fig. 2) and the improvement in time trial power output (8%; Fig. 3) in HOT when initiated in a hyperthermic state after HA support previous findings. Indeed, HA has been shown to increase VO_{2peak} by 4%–10% and W_{peak} by 2%–8% following whole-body pre-heating [20, 25, 49]. Time trial performance has also been shown to increase by 8%–10% [20, 25], with a meta-analysis indicating that HA generally enhances performance under heat stress by ~ 7% [59]. Of note, the increase in VO_{2peak} and W_{peak} in HOT following HA almost fully restored the decrement in these parameters relative to COOL prior to HA, as evidenced by VO_{2peak} increasing from 92% to 98% and W_{peak} from 89% to 95% of COOL. Concurrently, time trial performance increased from 85% to 92% of that achieved in COOL prior to HA. Had the time trial in HOT not been initiated in a hyperthermic state (T_{re}: ~ 38.5°C, T_{sk}: ~ 35°C) and a warm-up routine commensurate with outdoor cycling been conducted, perhaps the initial decrement in performance would not have been as pronounced. Notwithstanding, the

restoration of performance in HOT relative to COOL conditions has previously been observed in laboratory [20] and field settings [41]. In a study by Keiser et al. [20], a ~ 10% increase in VO_{2peak} under heat stress (35ºC) following HA fully compensated for the initial reduction when compared to values obtained in 18°C, whereas W_{peak} and 30-min time trial power output were almost completely restored (both ~ 98%). Racinais et al [41] reported that following two weeks of heat acclimatization, trained cyclists could produce a similar time (~ 66 min) over 43 km in hot (36°C) as in cold (~ 8°C) conditions. The 15% improvement in time was associated with a 15% improvement in mean power output (256 to 294 W) relative to an initial time trial conducted in the heat at the start of acclimatization, although still 3% lower than mean power output (304 W) in cold conditions. Our data therefore reaffirm the impact of HA on aerobic capacity and performance under heat stress.

The pathway via which improvements in aerobic capacity and performance under heat stress occur involves integrative adaptations that include a lowered resting body temperature, improved thermoregulatory responses, increased total body water and expanded PV, improved skeletal muscle metabolism, enhanced cardiovascular stability, and increased thermal tolerance [14, 38]. In the current study, T_{re} and T_{sk}, heart rate and SpO_2 during the incremental test were similar prior to and following HA (Table 1), but W_{peak} increased by 25 W and VO_{2peak} by 0.24 L/min. Similarly, time trial performance in HOT was improved by 20 W after HA, concomitant with a lower mean T_{re} (0.13°C) and T_{sk} (0.7°C), along with a similar heart rate to pre-HA (Figs. 2, 3). These improvements in VO_{2peak} and performance reflect an enhanced capacity to both thermoregulate and maintain O_2 delivery during exercise at a high workload. However, while we noted a 150 mL expansion of PV on the final day of HA (i.e. day 19), which has been suggested to increase vascular filling pressure to support cardiovascular stability (i.e. increase stroke volume and maintain cardiac output and arterial blood pressure) [33, 52, 65], post-HA measurements indicate that PV was only ~ 37 mL higher than pre-HA.

Previous studies have highlighted the potential for PV expansion following HA to increase VO_{2peak} in the heat by increasing cardiac output [25], but also that no association was found between changes in PV and time trial performance [20]. In cool conditions, acute (e.g. dextran, albumin infusion) PV expansion (200–400 mL) has been shown to improve cardiovascular stability and increase VO_{2peak} in untrained and moderately active individuals [3, 10, 12]. In contrast, cardiovascular stability was not affected in endurance trained individuals following an acute 400 mL expansion [13]. During exercise in the heat, a 13%–15% acute expansion of PV (500–600 mL) has been shown not to enhance VO_{2peak} and self-paced exercise performance, despite maximal cardiac output increasing [20, 60]. The lack of improvement in performance has been attributed to the concomitant hemodilution that accompanies a large acute PV expansion, which may offset any perfusion and O_2 delivery improvements to exercising muscles, especially in endurance trained individuals [3, 18]. In the current study, the ~ 4% expansion of PV on day 19 occurred in conjunction with a ~ 2% decrease in Hb_{mass} (i.e. ~ 3% decrease in Hb concentration) and a return towards baseline values post-HA (Table 2). As such, the combination of a lower thermal strain experienced during exercise in HOT following HA, in response to an improved whole-body sweat rate (Table 3), along with attenuated sinoatrial node and sympathetic activity [9, 17], appears to have contributed to improve VO_{2peak} and time trial performance.

Exercise Performance in a Cool Environment

This study used a counter-balanced cross-over design in which absolute workload and relative exercise intensity where matched between HA and COOL. The unique approach allowed for isolating the effects of training in the heat from those of training in cool conditions on the adaptive process and transferability of adaptations to performance in hot, cool and hypoxic conditions. Our data indicate that VO_{2peak} in COOL was not enhanced following HA (Fig. 2). This finding is in line with three previous reports of a lack of increase in VO_{2peak} in well-trained (VO_{2peak} > 60 mL/min/kg) individuals following 8–14 days of HA, with two studies noting a PV expansion of 6% and 15% [19, 20, 54]. The expansion of PV in these studies was larger than in the current study on day 19 of HA (4.2%) and following the intervention (1%). In contrast, others have demonstrated a 4% to 13% improvement in VO_{2peak} in less trained participants (VO_{2peak} 48–55 mL/min/kg), with two studies reporting a 3% and 8% PV expansion [49, 54, 57, 58]. However, in similarly trained participants (VO_{2peak}: 41–61 mL/min/kg), a PV expansion of 3.7% did not improve VO_{2peak} [55]. Of note, Lorenzo et al [25] observed a 5% increase in VO_{2peak} in trained athletes (VO_{2peak}: 67 mL/min/kg) following a 10-day HA regimen, which the authors attributed in part to a moderate (6.5%) expansion of PV and increase in maximal cardiac output (9%), concomitant to a small (3.5%) hemodilution. Across individuals of different aerobic fitness, it appears that the potential for PV expansion to increase VO_{2peak} may relate to the balance between an increase in stroke volume and cardiac output, and a decrease in hemoglobin concentration (i.e. hemodilution), and thus arterial O_2 content [3]. Despite the lack of change in VO_{2peak} in the current study, 20-min time trial performance was improved by 4% (12 W) in COOL following HA (Fig. 3), a finding supported by previous work with HA regimens of 10 to 21 days [25, 27, 44, 51].

Recently, Lundby et al [26] reported that VO_{2peak} in cool conditions increased by 6% and 4% in elite female (VO_{2peak}

59 mL/min/kg) and male (VO_{2peak} 75 mL/min/kg) cyclists after longer-term HA (i.e. 5 × 50 min session per week for 5 week), respectively. The authors also reported a 10% and 7% improvement in 15-min time trial power output in females and males, along with a 6% and 4% increase in power output at lactate threshold (i.e. 3 mmol/L), respectively. These improvements correlated with a 4% increase in Hb_{mass} in both sexes, whereas skeletal muscle properties (i.e. citrate synthase activity, fiber type distribution, and capillary density) were unaffected [26]. Although these data suggest that prolonged HA-induced increases in Hb_{mass} contribute to improve aerobic capacity and performance in cool conditions, similar long-term HA studies with elite male cyclists (VO_{2peak}: 77–78 mL/min/kg) from the same research group failed to show greater improvements in VO_{2peak}, 15-min time trial performance and power output at 4 mmol/L following HA compared to cool training, despite a 2%–5% increase in Hb_{mass} after HA and either a 5% increase or 3% decrease in PV [45, 46]. A lack of change in VO_{2peak} and W_{peak} in cool conditions following 5.5 weeks of HA was also reported in well-trained sub-elite male cyclists (VO_{2peak}: 60 mL/min/kg), along with a similar improvement (6%) in 15-km time trial performance to that of training in cool conditions [29]. In a companion paper, it was reported that Hb_{mass} and PV increased in response to HA and cool training, with larger but non-significant increases following HA than training in cool conditions (3% vs. 0.2%; 8% vs. 5%, respectively) [35]. The lack of consistent benefit from HA across studies may stem from low statistical power and the potential for marginal performance differences to be observed in elite athletes [26]. In the current 3-week HA study, Hb_{mass} decreased (~2%) during HA and returned to pre-intervention values after HA, whereas PV experienced the opposite pattern (Table 2). As such, the 4% improvement in time trial performance noted in COOL following HA may relate to the integrated adaptations highlighted to enhance performance in HOT, along with adaptations not measured in the current study and requiring further investigation, including improved efficiency, lactate threshold and cardiovascular function.

Exercise Performance in a Hypoxic Environment

VO_{2peak} in HYP (FiO_2: 15.4%) was not significantly improved following HA in the current study (Fig. 2). The lack of increase in VO_{2peak} at altitude (FiO_2: 0.12 to 0.15) following HA corroborates previous findings [11, 55, 61]. Similarly, 20-min time trial performance in HYP did not increase significantly following HA (Fig. 3). However, the 7 W ([−1, 16], $P = 0.075$) difference in self-paced exercise performance aligns with data from White et al [61] who reported a 'possible' HA benefit (~2%, $P = 0.07$) to 16-km time trial performance at altitude (FiO_2: 0.12), along with the significant improvement (~5%) noted by Lee et al [23] at an FiO_2 of 0.14 over the same distance.

Based on previous findings, improvements in time trial performance at altitude may stem from an elevated SpO_2 and/or lower heart rate during exercise at a given submaximal workload [8, 11]. Although SpO_2 was not measured during the time trials in the current study, mean heart rate was similar from pre- to post-intervention, including in HYP, which suggests greater cardiovascular stability following HA for a slightly, albeit not significantly, higher mean power output (7 W [−1, 16]). Moreover, while mean T_{re} was 0.10ºC lower during the time trial in HYP following HA, it is unlikely to have meaningfully influenced performance (Fig. 4). As such, the mechanistic pathways mediating the benefit of HA on performance in HYP remain to be elucidated. Potential pathways include acquired thermotolerance and cellular cross-tolerance [7, 22], as well as erythropoiesis [53] and angiogenesis [6] stemming from the expression of HSP increasing the molecular stability of HIF-1α. However, as we noted a decrease in Hb_{mass} after HA, enhanced O_2 transport and delivery are unlikely to have mediated any improvement in endurance performance in HYP. As such, the benefits of HA on endurance performance in HYP remain unclear and require further investigation, with longer (e.g. 5-week) HA regimens potentially providing insight into how increases in Hb_{mass} might enhance performance.

Conclusion

Heat acclimation significantly improved VO_{2peak} in HOT conditions and time trial performance in HOT and COOL, with a non-significant change in HYP of 7 W [−1, 16]. In contrast, training in temperate conditions, which was matched for both absolute and relative training workload with HA, did not affect aerobic capacity and time trial performance. While there were links between performance and greater cardiovascular stability and lower thermal strain in HOT following HA, the benefits of heat adaptations on endurance performance in COOL and HYP are less clear. This lack of clarity raises further questions regarding the mechanisms that support endurance performance improvements in cool and hypoxic conditions after heat acclimation.

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Data availability Data from the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interests The authors have no relevant financial or non-financial interests to disclose.

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