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ORIGINAL ARTICLE

No ergogenic effects of a 10-day combined heat and hypoxic acclimation on aerobic performance in normoxic thermoneutral or hot conditions

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Abstract

Purpose Hypoxic acclimation enhances convective oxygen delivery to the muscles. Heat acclimation-elicited thermoregulatory benefits have been suggested not to be negated by adding daily exposure to hypoxia. Whether concomitant acclimation to both heat and hypoxia offers a synergistic enhancement of aerobic performance in thermoneutral or hot conditions remains unresolved.

Methods Eight young males ($\dot{V}O_{2\,max}$: 51.6 ± 4.6 mL min^{-1} kg^{-1}) underwent a 10-day normobaric hypoxic confinement ($F_iO_2 = 0.14$) interspersed with daily 90-min normoxic controlled hyperthermia (target rectal temperature: 38.5 °C) exercise sessions. Prior to, and following the confinement, the participants conducted a 30-min steady-state exercise followed by incremental exercise to exhaustion on a cycle ergometer in thermoneutral normoxic (NOR), thermoneutral hypoxic ($F_iO_2 = 0.14$; HYP) and hot (35 °C, 50% relative humidity; HE) conditions in a randomized and counterbalanced order. The steady-state exercise was performed at 40% NOR peak power output (W_{peak}) to evaluate thermoregulatory function. Blood samples were obtained from an antecubital vein before, on days 1 and 10, and the first day post-acclimation.

Results $\dot{V}O_{2\,max}$ and ventilatory thresholds were not modified in any environment following acclimation. W_{peak} increased by 6.3 ± 3.4% in NOR and 4.0 ± 4.9% in HE, respectively. The magnitude and gain of the forehead sweating response were augmented in HE post-acclimation. EPO increased from baseline (17.8 ± 7.0 mIU mL^{-1}) by 10.7 ± 8.8 mIU mL^{-1} on day 1 but returned to baseline levels by day 10 (15.7 ± 5.9 mIU mL^{-1}).

Discussion A 10-day combined heat and hypoxic acclimation conferred only minor benefits in aerobic performance and thermoregulation in thermoneutral or hot conditions. Thus, adoption of such a protocol does not seem warranted.

Keywords Heat acclimation · Hypoxic acclimation · Thermoregulation · Aerobic performance

Abbreviations

ANOVA	Analysis of variance
CO	Cardiac output
ELISA	Enzyme-linked immunosorbent assay
EPO	Erythropoietin
F_iO_2	Fraction of inspired oxygen
GME	Gross mechanical efficiency
HE	Heat
HR	Heart rate
HSP	Heat shock protein
HYP	Hypoxia
NOR	Normoxia
P_iO_2	Partial pressure of inspired oxygen
PV	Plasma volume
RH	Relative humidity
RPE	Rating of perceived exertion
S_pO_2	Capillary oxyhaemoglobin saturation
SV	Stroke volume
T_a	Ambient temperature
T_c	Core temperature
T_{re}	Rectal temperature

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T_{sk}	Weighted mean skin temperature
$\dot{V}O_2$	Submaximal oxygen uptake
$\dot{V}O_{2\,max}$	Maximal aerobic power
VT	Ventilatory threshold
W_{peak}	Peak power output
ΔT_{f-f}	Forearm–fingertip skin temperature difference

Introduction

Hypoxic acclimation has been shown to enhance maximal aerobic power ($\dot{V}O_{2\,max}$) in hypoxic (Schuler et al. 2007), normoxic thermoneutral (Levine and Stray-Gundersen 1997) and hot (Sotiridis et al. 2018a) conditions. These improvements are possibly mediated by an augmented red cell volume (Levine and Stray-Gundersen 2005) that in cooperation with the plasma volume (PV) contraction serves to increase arterial oxygen content (Siebenmann et al. 2015a). Similarly, heat acclimation has also gained popularity among athletes as a potential ergogenic aid in aerobic performance in hot conditions. There is a disagreement however, over whether such a protocol increases aerobic performance in normoxic thermoneutral conditions (Lorenzo et al. 2010; Rendell et al. 2017) or not (Keiser et al. 2015; Karlsen et al. 2015). Potential heat acclimation-induced ergogenic mechanisms include PV expansion (Lorenzo et al. 2010), an increase in exercise economy (Sotiridis et al. 2019; Sawka et al. 1983) and/or power output at the lactate threshold (Lorenzo et al. 2010; Rendell et al. 2017). Thus, the separate physiological mechanisms associated with heat and hypoxic acclimation might act synergistically to influence aerobic performance in thermoneutral or hot conditions following heat and hypoxic acclimation. Attempts have been made to address this hypothesis by several previous studies detailed below, and is the principal aim of the current study.

The potential improvements that can be derived from such a combined hypoxic and heat acclimation are associated with either, predominantly, haematological and cardiorespiratory modifications in the case of the former, or thermoregulatory modifications in the case of the latter. With regard to aerobic performance, several recent studies have employed live high-train low concomitant with heat protocols to explore the effects of this promising synergistic interaction. Buchheit et al. (2013) recruited professional football players who completed sport-specific training sessions in the heat and slept either at normobaric hypoxia [fraction of oxygen in the inspired air (F_iO_2) between 0.143 and 0.152] or normoxia over the course of 12 days. PV and performance on the Yo–Yo intermittent recovery test were substantially higher in both groups whereas the between-groups difference in haemoglobin mass (+ 3.2%) was higher than the typical error of measurement post-acclimation. The main limitation of the study was that no $\dot{V}O_{2\,max}$ measurements were obtained and the session rating of perceived exertion was used as an index of the training load. Using a counterbalanced, cross-over design, Rendell et al. (2017) had trained cyclists undergo a controlled hyperthermia protocol while sleeping overnight in normoxia and moderate hypoxia ($F_iO_2 = 0.156$) for 11 days. Haemoglobin mass remained unchanged as PV expanded following heat acclimation. Work done in a time trial (+ 4%), peak power output (W_{peak}) (+ 4%) and lactate threshold was higher following heat acclimation with no further differences noted when individuals had been sleeping in hypoxia. Finally, McCleave et al. (2017) hypothesised that the superposition of heat interval training into a 3-week live-high train-low hypoxic protocol ($F_iO_2 = 0.144$) would cause an additive effect on aerobic performance in well-trained runners. Paradoxically, the average observed increase in haemoglobin mass (+ 3.8%) did not translate into enhanced 3-km time trial performance. This lack of improvement was attributed to a negative psychological response and the inability of the selected performance test to reflect an improved aerobic capacity. Surprisingly, only Rendell et al. (2017) measured $\dot{V}O_{2\,max}$, the main determinant of aerobic capacity, in normoxic thermoneutral conditions. Examination of the thermoregulatory responses following a combined heat and hypoxia acclimation protocol by McCleave et al. (2018) revealed no change in the core temperature (T_c) response, PV and sweat rate. Given that participants of that study were exercising in ambient temperatures ranging between 30 °C and 35 °C thrice per week, the results might have been limited by virtue of the low heat dose applied. Buchheit et al. (2013) reported unchanged sweat rate, but a lower heart rate (HR) in the group that was sleeping in hypoxia. Unfortunately, no data on T_c were presented. In contrast, Rendell et al. (2017) stated that adaptation to the heat stressor was not negated by adding nocturnal hypoxia as reflected in lower rectal temperature (T_{re}) and skin temperature, lower HR and an expanded PV. The discrepancy between studies could be explained by differences in the hypoxic dose (Sotiridis et al. 2018b) or the ratio of the heat to hypoxic stimuli (McCleave et al. 2018) applied. Alternatively, the participants of the former studies that employed traditional fixed-work rate heat acclimation protocols may have experienced an ever-diminishing thermal adaptation impulse (Taylor 2014). The novelty of the present study mainly lies on the application of a continuous normobaric hypoxic confinement interspersed only by daily normoxic controlled hyperthermia heat-training sessions. Specifically, we applied heat (ten daily 90-min controlled hyperthermia sessions) and hypoxic (230 h living at $F_iO_2 \sim 14\%$) stimuli that according to the existing literature are potent enough to stimulate the desired thermoregulatory (Sotiridis et al.

2019; Rendell et al. 2017) and haematological (Siebenmann et al. 2015a) adaptations, respectively. Such an acclimation protocol could also mitigate the decrement in exercise capacity in hot hypoxic conditions as reflected in a lower time to exhaustion at 66% W_{peak} (Girard and Racinais 2014).

This study investigated aerobic performance and thermoregulatory adaptations in normoxic, hot and hypoxic environments following a 10-day combined heat and hypoxic acclimation protocol in young males. We tested the hypothesis that the combined stressor acclimation protocol would enhance aerobic performance and facilitate thermoregulatory function in normoxic and hot conditions.

Methods

Participants

Eight healthy recreationally active to trained males [average (range) $\dot{V}O_{2\,max}$: 51.6 (44.7–59.8) mL kg^{-1} min^{-1}, W_{peak}: 343 (304–390) W] not involved in high-level sport, participated in the present study. They refrained from travelling to altitudes > 2500 m and were not exposed to temperatures > 30 °C during the 4 weeks prior to the study. None of them had a history of any cardiorespiratory or haematological disease. They were requested not to consume any caffeine- and/or alcohol-containing beverages throughout the period of the study. All applicants performed a preliminary normoxic maximal test on a cycle ergometer to familiarise themselves with the test protocol. W_{peak}, as established from the preliminary test, served to determine the submaximal workload for: 1. the subsequent pre- and post-acclimation tests (40% of the preliminary W_{peak}), 2. the time to exhaustion test in hot hypoxic conditions (80% of the preliminary W_{peak}) and 3. the initial workload for the controlled-hyperthermia exercise-sessions (see "Heat acclimation").

Study design

A single-group repeated-measures study design was employed; participants performed a battery of criterion exercise performance tests prior to commencing the acclimation protocol, and immediately upon completion of the acclimation (Fig. 1). These tests comprised a modified $\dot{V}O_{2\,max}$ test conducted in three different environmental conditions on consecutive days. To assess exercise performance, they also performed a time to exhaustion test at a standardized workload in hot hypoxic conditions 6 h after the first maximal test. Following the 3 days of testing, participants rested for 24 h before commencing the acclimation protocol. During the acclimation protocol, they were confined to a hypoxic environment for 10 days (a total of 230 h). During this period, they exercised daily in a hot normoxic (the facility is situated at an altitude of 940 m) environment for 90 min. The participants rested for the first day after exiting the hypoxic environment and then the criterion exercise tests were repeated. For a given participant, the order of the exercise tests was the same as in the pre-acclimation period. The maximal exercise tests for each participant were held at the same time of the day (± 1 h) in a laboratory situated at 300 m above sea level (Ljubljana, Slovenia). The daily training sessions were also performed at the same time of the day (± 2 h).

Hypoxic acclimation

The hypoxic acclimation protocol was performed in a hypoxic facility situated at an altitude of 940 m. Each participant was confined to the hypoxic facility for more than 10 days (entering hypoxia in the morning of the first day and exiting in the evening of the eleventh day). They lived (22 h per day) at a simulated altitude of approximately 4000 m achieved by maintaining F_iO_2 at 0.141 ± 0.005 in the facility situated at 940 m, resulting in a partial pressure of oxygen in the inspired air (P_iO_2) of 90.4 ± 2.3 mmHg. Hypoxia within the facility was achieved using an oxygen

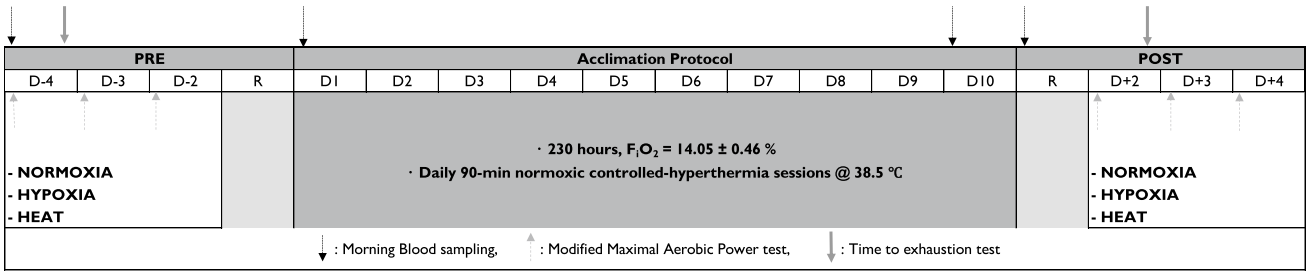

Fig. 1 Schematic illustration of the study design. Participants performed a battery of exercise performance tests prior to commencing the combined heat and hypoxic acclimation protocol and upon completion of the protocol, in a single-group within-subject repeated-measures study design

dilution system (B-cat, Tiel, The Netherlands), based on the vacuum pressure swing adsorption principle. The oxygen levels in each room were monitored and recorded continuously using oxygen sensors (PGM-1100, Rae Systems, San Jose, California, USA). The atmospheric pressure in the hypoxic facility was 690 ± 4 mmHg. Participants had ad libitum access to food and water and had the same daily menu throughout their sojourn at the Olympic Sports Centre. Participants were accommodated in double bedrooms for the duration of the study. During their 10-day sojourn at the Olympic Sports Centre, participants followed a daily schedule of lights out at 2300 h, and waking up at 0700 h. Body composition was measured on day 1 and day 10 of the hypoxic acclimation using Dual X-ray absorptiometry (DEXA, Hologic Discovery QDR, Hologic Inc., Bedford, Massachusetts, USA).

Heat acclimation

Heat acclimation was achieved with the controlled hyperthermia technique (isothermal clamping) (Fox et al. 1963). During all heat acclimation exposures, a combination of exercise (metabolic) and heat stress was used to elevate and maintain high T_{re} and skin temperature. The heat acclimation protocol consisted of ten 90-min daily sessions of cycle ergometry starting at a workload of 172 ± 15 W—corresponding to 50% of participants' normoxic W_{peak}—so that the target T_{re} of 38.5 °C was reached within 30–45 min of exercise (Gibson et al. 2017). Once the target T_{re} was reached, power was adjusted every 5 min to maintain T_{re} at the desired level. For reasons unrelated to the study, participants were passively heated for an additional 2 h after the training sessions 1 and 10. Participants were provided with 1.5 L of water during each exercise session in six doses of 250 mL at 15-min intervals. During the heat acclimation trials, the ambient temperature (T_a) was monitored at 5-min intervals using a temperature sensor (MSR 145, 175 Henggart, Switzerland). The average T_a and relative humidity (RH) in the laboratory during the training sessions were 37.4 ± 0.5 °C and 38.5 ± 1.7%, respectively. The wind speed was less than 0.5 m s^{-1}. T_{re} was measured continuously during the training sessions using a rectal thermistor probe (MSR 145, 175 Henggart, Switzerland) inserted 10 cm beyond the anal sphincter. Exercise HR was assessed using finger pulse oximeters (Wristox 3100 Nonin, Plymouth, Minnesota, USA) at 10-min intervals. Ratings of perceived exertion [RPE; 6-20 (Borg 1970)] were also recorded at 10-min intervals. During the training sessions, participants exercised on mountain bikes mounted on resistance trainers (Elite Cycling, Italy). Participants were trained in groups of 2 or 3 under strict supervision of the researchers.

Exercise testing

The format of the exercise trials has been previously described (Sotiridis et al. 2018a, 2019). All the pre- and post-training criterion exercise trials were conducted in a laboratory situated at 300 m above sea level (Ljubljana, Slovenia). The trials were conducted on a cycle ergometer (Daum, Electronic, Furth, Germany) and comprised two stages; a 30-min steady-state exercise immediately followed by an incremental exercise to exhaustion. During exercise, each participant pedalled at a preferred cadence (between 60 and 90 rpm), which they maintained via visual and verbal feedback throughout the trial. Before (Pre) and after (post) the 10-day training protocol, participants conducted three trials on three consecutive days. In the normal temperature and normoxic condition (NOR), participants breathed room air (pre: $P_iO_2 = 144 \pm 2$ mmHg, post: $P_iO_2 = 143 \pm 1$ mmHg) and exercised in thermoneutral conditions (pre: $T_a = 23.67 \pm 0.19$ °C and RH = 46.9 ± 1.2%, post: $T_a = 23.41 \pm 0.23$ °C and RH = 49.1 ± 0.8%). In the hypoxic condition (HYP), they inspired a hypoxic gas mixture (pre: $P_iO_2 = 93 \pm 2$ mmHg, post: $P_iO_2 = 92 \pm 3$ mmHg) and exercised in thermoneutral conditions (pre: $T_a = 23.32 \pm 0.19$ °C and RH = 45.8 ± 5.6%, post: $T_a = 23.16 \pm 0.26$ °C and RH = 50.2 ± 0.8%). In the hot condition (HE), the participants inspired room air (pre: $P_iO_2 = 144 \pm 1$ mmHg, post: $P_iO_2 = 139 \pm 2$ mmHg), but exercised in a hot environment (pre: $T_a = 35.08 \pm 0.89$ °C, RH = 42.0 ± 2.8%, post: $T_a = 35.34 \pm 0.25$ °C and RH = 46.8 ± 1.5%). The exercise trial sequence was randomized across participants and determined in a counterbalanced manner, using an incomplete Latin square design. A resting day was scheduled immediately before and after the training protocol to minimize the occurrence of fatigue during the exercise tests.

A metabolic cart (Quark CPET, Cosmed, Rome, Italy) was used to obtain the breath-by-breath respiratory responses during the exercise trials. Metabolic energy expenditure was calculated during submaximal exercise using the following equation:

$$M(\mathrm{W}) = \dot{V}\mathrm{O}_2 \frac{\left(\frac{\mathrm{RER}-0.7}{0.3}\right)e_c + \left(\frac{1-\mathrm{RER}}{0.3}\right)e_f}{60},$$

where RER is the respiratory exchange ratio, $\dot{V}O_2$ the oxygen uptake, and e_c and e_f are the caloric equivalents per litre of oxygen for the oxidation of carbohydrates (21.13 kJ) and fats (19.62 kJ), respectively. Metabolic heat production (H_{prod}) was estimated by subtracting the rate of mechanical work from metabolic energy expenditure. Before each trial, the O_2 and CO_2 analysers were calibrated using two different gas mixtures, and the pneumotachograph was calibrated

with a 3-L syringe, in accordance with the manufacturer's recommendation.

HR was derived from electrocardiographic recordings with electrodes in a precordial position. Cardiac output (CO) was determined using electrical impedance cardiography (Physioflow Q-Link, Manatec Biomedical, Paris, France), whereby changes in thoracic impedance during cardiac ejection are used to estimate stroke volume (SV). Six electrodes were placed at the base of the neck and on the xiphoid process. Electrical impedance cardiography has been validated against the direct Fick method during maximal incremental exercise in healthy participants (Siebenmann et al. 2015b). Capillary oxyhaemoglobin saturation (S_pO_2) was recorded using a finger pulse oximeter (Wristox 3100 Nonin, Plymouth, Massachusetts, USA). Participants were instructed to relax their hands during measurement of S_pO_2. Ratings of perceived exertion were obtained using a Borg scale (6–20). During all the trials, the participants wore only shorts and athletic shoes. The body mass was measured before each exercise testing on a weighing scale (± 0.05 kg) (TPT 5N, Libela ELSI, Celje, Slovenia).

Rectal temperature (T_{re}) was measured with a thermistor probe (MSR 145, Henggart, Switzerland) inserted 10 cm beyond the anal sphincter. Skin temperatures were measured at four sites (chest, arm, thigh and calf) using thermistors (MSR 147WD, Henggart, Switzerland) attached to the skin with a single piece of adhesive tape. The assessment of calf, thigh, chest and arm skin temperature enabled the calculation of weighted mean skin temperature (T_{sk}) (Ramanathan 1964). The ventral fingertip and forearm temperatures were also measured. The forearm–fingertip temperature difference (ΔT_{f-f}) has been validated as an index of vasomotor tone during steady-state exercise (Keramidas et al. 2013). Minute mass flow of secreted sweat was measured with a ventilated capsule placed on the forehead. Forced evaporation of sweat under the capsule (surface area 4.8 cm^2) was achieved by a constant flow of air (1.0 L min^{-1}) through the capsule. Minute mass flow of secreted sweat was estimated each minute from the difference between the temperature and the humidity of inflowing and outflowing air. Air temperature was measured with thermistors (LM35, National Semiconductor Corp., Santa Clara, CA, USA) and RH with capacitance hygrometers (Valvo air humidity sensor, Valvo-Philips GmbH, Hamburg, Germany).

Steady state and incremental exercise

Following a 2-min rest period, participants commenced pedalling on the cycle ergometer. During the first 2 min, the load was set at 90 W (warm-up). Thereafter, they cycled for 30 min at a steady-state work rate equivalent to 40% of their NOR W_{peak} attained during the preliminary testing. The initial work rate was set at 100 W (2 min), thereafter increasing by 20 W every minute until volitional exhaustion. During each trial, S_pO_2 and sweat rate data were recorded at minute intervals whereas HR, CO and SV were measured every 10 s. RPE values were recorded at 5-min and 2-min intervals during the steady state and the incremental load exercise, respectively. Attainment of $\dot{V}O_{2\,max}$, defined as the highest $\dot{V}O_2$ averaged over 30 s, was confirmed when participants met at least three of the following criteria, listed in order of priority: (a) severe fatigue or exhaustion leading to inability to maintain exercise at a given work rate (cycling cadence lower than 60 rpm), (b) a plateau in $\dot{V}O_2$, as indicated by the breath-by-breath values, despite an increase in power output (c) a subjective rating of perception of effort near maximal (Borg scale rating greater than 17) and (d) respiratory quotient greater than 1.10. W_{peak} was calculated according to the following equation:

$$W_{peak} = \text{work rate of last stage completed} + \left[(\text{work rate increment}) * (\text{time into current stage}/60)\right]$$

Time to exhaustion

Time to exhaustion was determined using a constant load test at 80% of the W_{peak} (274 ± 24 W). The test was held in hot (pre: $T_a = 34.4 \pm 0.5$ °C, 47 ± 4% RH, post: $T_a = 33.6 \pm 1.1$ °C, 54 ± 3% RH) hypoxic conditions on the same ergometer and chamber as the rest of the exercise tests. A metabolic cart (Quark CPET, Cosmed, Rome, Italy) was used to acquire the breath-by-breath respiratory responses during the time-to-exhaustion tests. After entering the climatic chamber, participants commenced breathing via a two-way respiratory valve (Model 2700, T-Shape, Hans Rudolph, Shawnee, Kansas, USA). The inspiratory side of the valve was connected via corrugated tubing to a meteorological balloon containing a calibrated hypoxic gas mixture (pre: $F_iO_2 = 0.135 \pm 0.007$, post: $F_iO_2 = 0.131 \pm 0.004$). This gas mixture was decompressed from a high pressure cylinder, humidified by passing through a water bath at room temperature and collected in the meteorological balloon. The subjects rested for 120 s while breathing the hypoxic gas mixture. The warm-up period included 165 s at 90 W followed by 15 s of rest. The test ended when participants were unable to maintain a cycling cadence above 60 revolutions per minute for more than 5 s. Constant verbal feedback was given to the participants by the researchers during the tests.

Haematology

Blood samples were obtained from an antecubital vein before, on days 1 (after 24 h in hypoxia) and 10, and the first day post-acclimation (24 h after exiting the hypoxic environment). The participants rested in a seated position for ≥ 10 min before blood samples were obtained. Blood was

taken using standard venipuncture technique at the hypoxic facility or at the laboratory where the criterion exercise tests were performed. Blood samples were allowed to clot for 1 h at 2–8 °C and were then centrifuged at 2000*g* for 15 min so that serum was completely separated from the cells. Serum was then stored in BD Vacutainers at − 20 °C for further analysis. With regard to the haemogram analysis, an additional 3 mL of whole blood was collected into standard EDTA tubes (Vacutainer, Greiner BIO one, Austria) and the blood sample was mixed to prevent clotting. Samples were analysed for haematocrit, haemoglobin, ferritin and reticulocyte concentration and erythrocyte count on an automated haematology analyser (ABX Micros ES 60, Horiba medical, Japan) twice within 3 h after the sample was obtained. Since every sample was analysed twice, the average value was computed and CV was less than 2%. Additional erythropoietin (EPO) was determined from 200 µL samples of serum using a two-site enzyme-linked immunosorbent assay (ELISA) (IVD, Erythropoietin, IBL, Germany). The quantification of the optical density was performed on a microplate reader BioTek (BioTek instruments, Elx808TM, Winooski, Vermont, USA) set at 405 nm and 450 nm. Renin (active) was determined from 50 µL samples of serum using an ELISA based on the sandwich principle (IVD, Renin (active), IBL, Hamburg, Germany). The quantification of the optical density was performed on a microplate reader BioTek (BioTek instruments, Elx808TM, Winooski, Vermont, USA) set at 450 nm. Heat shock protein (HSP) 70, HSP90 and VEGF concentrations were determined using ELISA (Enzo Life Sciences Inc., Farmingdale, NY, USA) in 50, 4 and 20 µL of plasma, respectively. Optical density was quantified on a microplate reader (Biotek Elx808TM; Biotek Instruments, Winooski, Vermont, USA) set at 450 nm and corrected at 630 nm for HSP70, HSP90 and VEGF. Two microplates were used for each participant's samples to eliminate the possible variability between the plates. All techniques were performed in accordance with the protocol provided by the manufacturer. Samples and standards were assayed in duplicate. The sensitivity of the measurements was 0.09 ng mL^{-1}, 8 pg mL^{-1}, 0.05 ng mL^{-1}, 0.81 pg mL^{-1}, 1.1 mIU mL^{-1} for HSP70, VEGF, HSP90, renin, EPO, respectively. Initial resting vascular volumes (pre-acclimation) were calculated from lean body mass by the equations of Sawka et al. (1992) and post-acclimation volumes were calculated by correcting that initial values for the percent change in PV (Strauss et al. 1951) and blood volume (Dill and Costill 1974). The specific heat capacity of the blood was estimated by the equation of Blake et al. (2000).

Data analysis

Statistical analyses were performed using Statistica 5.0 (StatSoft, Tulsa, OK). Cardiorespiratory and temperature data collected during steady-state exercise were analysed for the last 20 min of the steady-state exercise, unless stated otherwise. ΔT_{f-f} was analysed for the last 10 min of the steady-state exercise. Cardiorespiratory and thermoregulatory data were analysed using a two-way repeated-measures ANOVA (Environmental condition: NOR-HYP-HE; acclimation status: pre-post). Haematological variables were analysed using one-way repeated-measures ANOVA (time: pre-D1-D10-post). Heat training data were also analysed using one-way repeated-measures ANOVA (time: D1-D2-…-D10). When ANOVA revealed a significant *F* ratio for interaction and/or main effect, pairwise comparisons were performed with Tukey honestly significant difference post hoc tests. A *t* test for paired samples was performed to compare pre- to post-acclimation time to exhaustion in hot hypoxic conditions. RPE values, gains of the sweating response (relative change in sweating as a function of the relative change in T_{re}) and HSP70 plasma concentrations were analysed using the non-parametric Friedman test. The sweat rate and temperature data were analysed only for the steady-state exercise. Least square linear regression was used to determine the mean T_{re} threshold for initiation of sweating, as well as the gain of the sweating response. The reported values are averages of the individual thresholds and slopes derived from linear regression parameters. The ventilatory threshold (VT) was defined as $\dot{V}O_2$ that corresponded to the deflection point of the end-tidal $P_{ET}CO_2$ confirmed by the nadir in the $V_E/\dot{V}CO_2$ when plotted as functions of the workload during the incremental test (Binder et al. 2008). Data are presented as mean ± SD unless indicated otherwise. The alpha level of significance was set a priori at 0.05.

Results

Heat acclimation

With the exception of one participant who did not train owing to gastrointestinal problems and a concomitant fever (resting T_{re} of 38.1 °C) during day 4 of the acclimation protocol, participants completed all ten heat acclimation sessions. Table 1 demonstrates the progression of the adaptive response to heat acclimation sessions.

Participants' average T_{re} ranged between 38.4 ± 0.1 °C or 38.4 ± 0.2 °C (D1 and D8, respectively) and 38.6 ± 0.1 °C (D4 and D9) during the last 60 min of each heat acclimation session ($p = 0.51$). A higher HR was observed only at D9 compared to D1 (140 ± 9 vs. 152 ± 8 bpm, $p = 0.007$). Resting T_{re} ($p = 0.43$), ΔT_{re} ($p = 0.39$) as well as the time to reach the target T_{re} ($p = 0.23$) remained unchanged. Pre-exercise body mass decreased by 1.9 ± 1.4 kg over the course of the acclimation protocol with the body mass loss reaching significance

Table 1 Average daily exercise responses ($n = 8$) during heat acclimation

	D1	*D2*	*D3*	*D4*	*D5*	*D6*	*D7*	*D8*	*D9*	*D10*
T_{re} (°C) after 30th min	38.4 ± 0.2	38.5 ± 0.1	38.5 ± 0.1	38.6 ± 0.1	38.5 ± 0.1	38.5 ± 0.1	38.5 ± 0.1	38.4 ± 0.2	38.6 ± 0.1	38.5 ± 0.1
HR (bpm)	140 ± 9	142 ± 9	144 ± 9	148 ± 8	145 ± 11	148 ± 8	146 ± 8	145 ± 9	152 ± 8	147 ± 9
Resting T_{re} (°C)	37.1 ± 0.3	37.3 ± 0.2	37.2 ± 0.3	37.3 ± 0.3	37.3 ± 0.4	37.3 ± 0.3	37.4 ± 0.3	37.2 ± 0.2	37.3 ± 0.3	37.3 ± 0.2
ΔT_{re} (°C)	1.3 ± 0.3	1.2 ± 0.2	1.3 ± 0.3	1.4 ± 0.3	1.3 ± 0.3	1.2 ± 0.3	1.2 ± 0.2	1.3 ± 0.2	1.3 ± 0.3	1.3 ± 0.3
Time to reach 38.5 °C (min)	51 ± 14	45 ± 13	46 ± 10	40 ± 9	37 ± 10	37 ± 6	39 ± 9	47 ± 14	42 ± 8	41 ± 14
Pre-exercise mass (kg)	76.7 ± 7.5	76.1 ± 7.4	75.8 ± 7.1	75.4 ± 7.2	75.2 ± 7.3	75.2 ± 7.4	75.1 ± 7.2	75.0 ± 7.2	76.0 ± 6.8	74.8 ± 6.8
Whole-body sweat rate (L h^{-1})	–	–	1.7 ± 0.4	1.6 ± 0.3	1.5 ± 0.2	1.7 ± 0.3	1.8 ± 0.4	1.8 ± 0.4	1.9 ± 0.3	1.9 ± 0.4

on D7 ($p = 0.041$). Due to technical reasons, we were unable to measure body weight for the first 2 days of the heat acclimation protocol. However, participants were sweating more on D7–10 ($p < 0.01$ for days 7, 9, 10 and $p = 0.06$ for day 8) compared to D4.

In contrast to the pre-exercise body mass, similar pre- to post- acclimation values were observed for the body composition variables as assessed using DEXA. More specifically, there was no change in either body fat percentage (17.3 ± 2.1 vs. 17.5 ± 1.9%, $p = 0.11$), fat mass (12.8 ± 2.0 vs. 13.0 ± 1.7 kg, $p = 0.24$) or fat-free mass (61.6 ± 6.2 vs. 61.2 ± 5.6 kg, $p = 0.18$).

Haematology

Table 2 presents the haematological responses as measured over the course of the acclimation protocol. There was a main effect of time ($p < 0.001$) on haematocrit and haemoglobin concentration. Haematocrit was higher ($p = 0.021$) on D1 compared to baseline levels with mean values being 47.8 ± 2.3 and 45.3 ± 2.5%, respectively. Haematocrit tended to be even higher during D10 ($p = 0.10$) compared to D1. A similar pattern was detected for haemoglobin concentration with a profound increase on D10 compared to baseline values (149 ± 9 vs. 162 ± 9 g dL^{-1}, $p < 0.001$). Haemoglobin concentration on D1 remained unaltered compared to baseline ($p = 0.15$) but was lower than D10 ($p = 0.016$). Regarding the individual values, both indices were higher across all participants on D10 compared to pre-acclimation values.

The estimated PV was already contracted by D1 (− 7.7 ± 6.5%, $p = 0.023$) and was even lower by D10 (− 15.1 ± 8.5%, $p = 0.043$). The decrease in blood volume only reached statistical significance by D10 (− 7.8 ± 4.8%, $p < 0.001$), but not on D1 ($p = 0.09$). No individual variation was noted since the fall in plasma and total blood volumes was consistent across all participants. The four different sampling time points also enabled us to investigate the EPO kinetics (Fig. 2). Plasma EPO increased from baseline (17.8 ± 7.0 mIU mL^{-1}) on D1 by 10.7 ± 8.8 mIU·mL^{-1} ($p = 0.012$). By D10, plasma EPO had returned to baseline levels ($p = 0.90$) and tended to undershoot when participants exited the hypoxic environment ($p = 0.12$) (Fig. 2). Extracellular HSP90 concentration tended to increase when participants exited the hypoxic environment compared to D10 (40.0 ± 10.1 vs. 66.7 ± 27.9 ng mL^{-1}, $p = 0.076$). The renin concentration was lower on D1 compared to the baseline value (48.5 ± 24.7 vs. 34.0 ± 24.9 pg·mL^{-1}, $p = 0.048$). By D10, it had returned to baseline values ($p = 0.29$), and it remained at this level on the first day post-acclimation ($p = 0.61$). On the contrary, HSP70 and VEGF remained unaffected by the acclimation protocol ($p = 0.55$ and $p = 0.25$, respectively).

Table 2 Resting haematological variables pre-, on days 1 and 10 and the first day post-acclimation ($n=8$)

	Pre-acclimation	Day 1	Day 10	Post-acclimation
Haemoglobin (g L^{-1})	149 ± 9	154 ± 8[b]	162 ± 9[a]	156 ± 6[a]
Haematocrit (%)	45.3 ± 2.5	47.8 ± 2.3[a]	49.8 ± 2.7[a]	48.3 ± 1.8[a]
Plasma EPO (mIU·mL^{-1})	17.8 ± 7.0	28.5 ± 10.8[ab]	15.7 ± 5.9	10.6 ± 5.9
HSP70 (ng·mL^{-1})	0.15 (0.01–1.47)	0.22 (0.01–1.77)	0.15 (0.01–1.34)	0.09 (0.06–1.19)
HSP90 (ng·mL^{-1})	56.4 ± 25.7	41.8 ± 19.1	40 ± 10.1	66.7 ± 27.9[b]
Plasma volume (%)	–	− 7.7 ± 6.5[ab]	− 15.1 ± 8.5[a]	− 9.5 ± 5.7[a]
Renin (pg·mL^{-1})	48.5 ± 24.7	34.0 ± 24.9[a]	39.0 ± 22.7	42.1 ± 22.6
VEGF (ng·mL^{-1})	2.56 ± 2.40	2.95 ± 3.03	2.76 ± 2.80	2.72 ± 2.65

Values are mean ± SD except for HSP70 data which are presented as median (range)

EPO erythropoietin, *HSP70* extracellular heat shock protein 70, *HSP90* extracellular heat shock protein 90, *VEGF* vascular endothelium growth factor

[a]Significant difference to pre-acclimation

[b]Significant difference to day 10, $p<0.05$

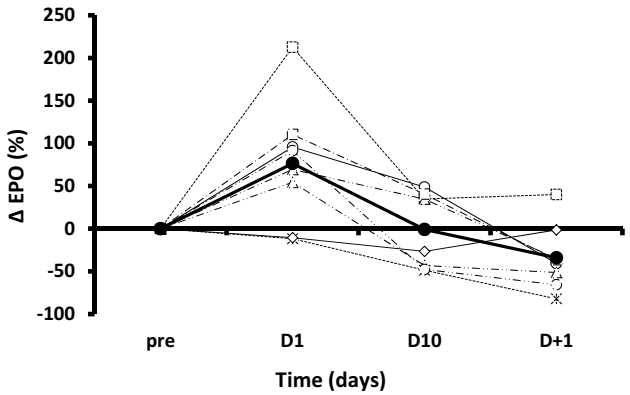

Fig. 2 Individual and average (bold) percentage changes in plasma erythropoietin (EPO) concentration before (Pre), during (days 1 and 10), and after (D + 1) the 10-day combined acclimation protocol

Exercise testing

Steady-state exercise

Table 3 provides cardiorespiratory responses during the 30-min steady-state exercise in all environmental conditions. An interaction effect between environmental condition and acclimation ($p=0.043$) is reflected on the post-acclimation reductions in HR observed in NOR (− 6 ± 13 bpm, $p=0.035$) and HYP (− 9 ± 12 bpm, $p=0.004$) which were not persistent in HE (− 2 ± 10 bpm, $p=0.96$). HR in NOR was lower than in HE and HYP across acclimation (main effect of environmental condition: $p<0.001$). Submaximal CO tended to be higher in HYP (18.4 ± 2.5 L·min^{-1}) than in NOR (16.5 ± 3.1 L·min^{-1}, $p=0.055$) but not HE (16.8 ± 2.2 L·min^{-1}, $p=0.12$). Submaximal SV was lower ($p=0.024$) post-acclimation in HE compared to NOR (130 ± 22 vs. 111 ± 12 mL, $p=0.014$). $\dot{V}O_2$ at 40% W_{peak} was lower in NOR post-acclimation ($p=0.010$). Even though a main effect of acclimation on gross mechanical efficiency (GME) ($p=0.013$) was subsequently observed, post hoc tests were unable to locate specific differences ($p>0.05$). S_pO_2 was higher post-acclimation in HYP (+ 3.5 ± 2.3%, $p=0.010$) but remained lower than NOR and HE (main effect of environmental condition: $p<0.001$). Minute ventilation (V_E) increased by ~ 5 L·min^{-1} post-acclimation across environmental conditions with the hypoxic values being persistently higher compared to NOR and HE (main effects of acclimation and environmental condition: $p<0.001$).

Incremental exercise and time to exhaustion

Figure 3 represents the individual data for $\dot{V}O_{2\,max}$ and W_{peak} in the three environmental conditions pre- and post-acclimation. The acclimation protocol did not increase $\dot{V}O_{2\,max}$ in any environment (main effect for acclimation: $p=0.52$) with mean percentage changes from pre-acclimation being − 2.6 ± 1.8%, + 0.3 ± 10.5% and − 1.8 ± 7.2% in NOR, HYP and HE, respectively. On the contrary, there was a main effect of both acclimation ($p<0.001$) and environment ($p<0.01$) for W_{peak}. Indeed, W_{peak} was, or tended to be, higher post-acclimation in NOR ($p<0.01$) and HE ($p=0.068$), respectively. It is notable that in HYP, five participants increased and three participants maintained their W_{peak} rendering the increase not statistically significant ($p=0.21$). Exercise performance in hot hypoxic conditions, as assessed by the time to exhaustion at 80% of the NOR W_{peak}, was improved post-acclimation by 12.9 ± 10.6% (249 ± 58 vs. 286 ± 89 s, $p=0.010$) with seven participants improving and one maintaining their pre-acclimation time to exhaustion.

An overview of aerobic performance measures is given in Table 4. VT was lower in HYP compared to NOR pre-acclimation ($p=0.022$). Furthermore, maximal CO was lower post-acclimation in HYP (22.3 ± 3.9 L·min^{-1}, $p=0.019$)

Table 3 Cardiorespiratory responses in the last 20 of the 30 min of constant work rate cycling before and after acclimation for the normoxic, the hypoxic and the trial in the heat

	Pre-acclimation			Post-acclimation		
	NOR	HYP	HE	NOR	HYP	HE
HR (bpm)	129 ± 17^{bc}	148 ± 12^{b}	145 ± 16	123 ± 11^{abc}	140 ± 10^{ab}	144 ± 13
CO (L min^{-1})	16.46 ± 3.12	18.42 ± 2.53	16.75 ± 2.19	16.08 ± 3.59	16.88 ± 2.88	15.94 ± 2.74
SV (mL)	125 ± 20	123 ± 15	116 ± 12	130 ± 22^{b}	121 ± 17	111 ± 12
$\dot{V}O_2$ (mL·min^{-1})	2212 ± 113	2190 ± 100	2150 ± 196	2070 ± 150^{a}	2122 ± 192	2048 ± 220
GME (%)	18.2 ± 1.4	18 ± 1.0	18.5 ± 0.8	19.3 ± 0.5	18.6 ± 1.4	19.4 ± 1.0
$\dot{V}_E$ (L min^{-1})	49.8 ± 4.5^{c}	61.6 ± 5.5^{b}	52.8 ± 5.0	54.6 ± 5.5^{ac}	66.6 ± 8.6^{ab}	57.3 ± 6.6^{a}
S_pO_2 (%)	95.9 ± 1.3^{c}	79.1 ± 5.1^{b}	95.7 ± 00.6	96.1 ± 1.1^{c}	82.6 ± 4.1^{ab}	95.4 ± 1.0

Heart rate, stroke volume and cardiac output values reflect the last minute of submaximal exercise (n = 8)

Values are mean ± SD

HR heart rate, *CO* cardiac output, *SV* stroke volume, $\dot{V}O_2$ submaximal oxygen uptake, *GME* gross mechanical efficiency, $\dot{V}_E$ minute ventilation, S_pO_2 capillary oxyhaemoglobin saturation

[a]Significant difference to values before HA

[b]Significant difference to heat values

[c]Significant difference to hypoxic values, $p < 0.05$

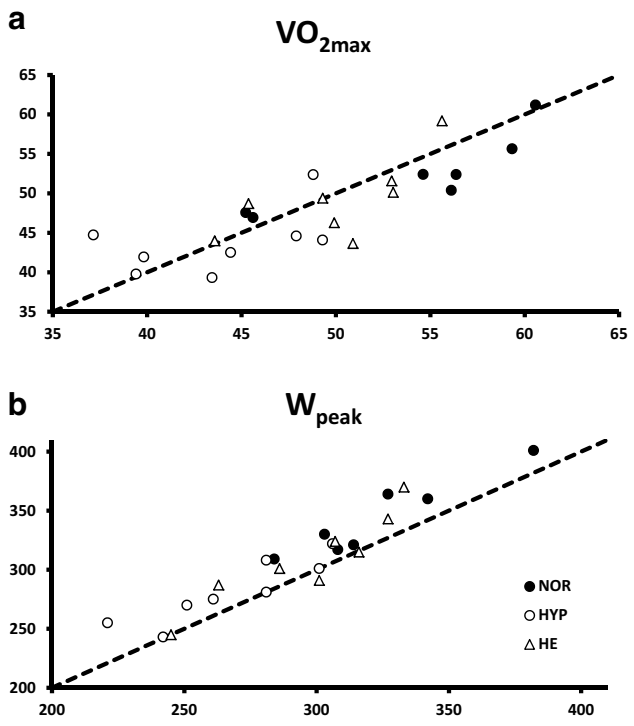

Fig. 3 Pre- (horizontal axis) and post- (vertical axis) acclimation individual data for **a** maximal aerobic power and **b** peak power output in normoxia (closed circles), hypoxia (open circles) and heat (triangles)

and HE (22.0 ± 2.5 L·min^{-1}, $p < 0.01$) compared to NOR (25.1 ± 3.4 L·min^{-1}). The substantial decreases in maximal CO in HE (compared to NOR) were also mirrored in a lower corresponding SV across acclimation status (141 ± 25 vs. 123 ± 15 mL pre, $p = 0.034$; 138 ± 19 vs. 118 ± 11 mL post, $p = 0.001$). On the contrary, there was no main effect for acclimation on either maximal CO ($p = 0.80$) or the corresponding SV ($p = 0.79$). Maximal V_E increased by ~ 15 L across environmental conditions (main effect of acclimation: $p = 0.006$). In contrast to the submaximal exercise, S_pO_2 did not increase post-acclimation in HYP (78.5 ± 3.0 vs. $81.3 \pm 4.6\%$, $p = 0.20$) and it remained lower than NOR and HE ($p < 0.001$).

Thermoregulatory data

Thermoregulatory responses during submaximal exercise are shown in Table 5. Resting T_{re} remained unchanged by the acclimation protocol ($p = 0.39$) or the environmental condition ($p = 0.54$). ΔT_{re} during submaximal exercise was lower in NOR compared to HYP and HE across acclimation ($p = 0.023$). The acclimation protocol did not affect ΔT_{re} ($p = 0.42$). T_{sk} was consistently higher in HE ($p < 0.001$). Furthermore, T_{sk} was higher post-acclimation in NOR compared to HYP (33.8 ± 0.5 vs. 33.4 ± 0.6, $p = 0.025$).

Main effects of acclimation were also observed for H_{prod} expressed in absolute values ($p = 0.013$) or adjusted relative to body surface ($p = 0.034$), implying lower values post-acclimation across environmental conditions. However, even the highest mean decrement observed in NOR did not reach statistical significance ($-6.59 \pm 7.61\%$, $p = 0.20$). H_{prod} normalized to body weight tended to be lower post-acclimation ($p = 0.091$). Figure 4 shows the individual data for absolute sweating thresholds and peak sweat rate. Similar values for the absolute sweating threshold were reported across acclimation status

Table 4 Maximal cardiorespiratory responses and peak power output during the incremental exercise to exhaustion pre- and post-acclimation in normoxic, hypoxic and the heat trials ($n=8$)

	Pre-acclimation			Post-acclimation		
	NOR	HYP	HE	NOR	HYP	HE
$\dot{V}O_{2\,max}$ (mL·kg^{-1}·min^{-1})	54.3 ± 5.8^{c}	43.8 ± 4.7^{b}	50.1 ± 4.0	52.4 ± 4.9^{c}	43.7 ± 4.1^{b}	49.1 ± 5.0
$\dot{V}O_{2\,max}$ (L·min^{-1})	4.12 ± 0.3^{c}	3.26 ± 0.36^{b}	3.83 ± 0.44	3.96 ± 0.40^{c}	3.29 ± 0.36^{b}	3.72 ± 0.53
W_{peak} (W)	321 ± 20^{bc}	268 ± 30^{b}	297 ± 31	343 ± 33^{abc}	282 ± 27^{b}	310 ± 38
CO_{max} (L·min^{-1})	25.0 ± 4.2	24.1 ± 3.9	22.1 ± 2.9	25.1 ± 3.4^{bc}	22.3 ± 3.9	22.0 ± 2.5
HR_{peak} (bpm)	177 ± 11	177 ± 7	180 ± 12	181 ± 13	172 ± 5^{b}	186 ± 8
SV_{peak} (mL)	141 ± 25^{b}	136 ± 23	123 ± 15	138 ± 19^{b}	130 ± 20	118 ± 11
S_pO_2 (%)	93.9 ± 1.7^{c}	78.5 ± 3.0^{b}	93.6 ± 2.8	94.6 ± 1.6^{c}	81.3 ± 4.6^{b}	94.6 ± 1.7
Anaerobic threshold (mL·kg^{-1}·min^{-1})	42.0 ± 4.5^{c}	35.2 ± 3.8^{b}	40.3 ± 3.9	38.6 ± 3.2	33.7 ± 4.1	38.3 ± 2.3
V_{Epeak} (L·min^{-1})	147 ± 33	136 ± 24	139 ± 23	162 ± 31	152 ± 19	152 ± 37

Values are means ± SD

$\dot{V}O_{2\,max}$ maximal aerobic power, W_{peak} peak power output, CO_{max} maximal cardiac output, HR_{peak} heart rate corresponding to maximal cardiac output, SV_{peak} stroke volume corresponding to maximal cardiac output, $V_{E\,peak}$ peak minute ventilation

[a]Significant difference to pre-acclimation values

[b]Significant difference to heat values

[c]Significant difference to hypoxic values, $p<0.05$

Table 5 Thermoregulatory responses at rest and during the constant-work rate cycling before and after acclimation for the normoxic, the hypoxic and the heat trials. ($n=8$)

	Pre-HA			Post-HA		
	NOR	HYP	HE	NOR	HYP	HE
$T_{re\,rest}$ (°C)	37.36 ± 0.26	37.29 ± 0.21	37.34 ± 0.24	37.28 ± 0.42	37.24 ± 0.40	37.36 ± 0.26
ΔT_{re} (°C)	0.42 ± 0.11	0.57 ± 0.12	0.60 ± 0.26	0.37 ± 0.21	0.50 ± 0.22	0.56 ± 0.29
T_{sk} (°C)	33.6 ± 0.7^{b}	33.7 ± 0.4^{b}	36.7 ± 0.3	33.8 ± 0.5^{bc}	33.4 ± 0.6^{b}	36.6 ± 0.4
ΔT_{f-f} (°C)	− 0.57 ± 1.49	− 0.37 ± 1.60	− 0.22 ± 0.25	0.22 ± 1.04	− 0.71 ± 1.52	− 0.27 ± 0.37
H_{prod} (W)	617 ± 38	621 ± 30	601 ± 58	575 ± 44	597 ± 58	566 ± 58
H_{prod} (W·kg^{-1})	8.2 ± 1.0	8.2 ± 0.9	7.9 ± 0.8	7.6 ± 0.6	7.9 ± 0.9	7.5 ± 0.7
H_{prod} (W·m^{-2})	316 ± 31	319 ± 26	307 ± 27	294 ± 20	307 ± 31	290 ± 24
$\dot{m}_{sw\,gain}$ (mg·cm^{-2} min^{-1})	1.17 ± 0.25	1.18 ± 0.27	1.13 ± 0.22	1.07 ± 0.17^{b}	0.97 ± 0.13^{b}	1.54 ± 0.35^{a}
$\dot{m}_{sw\,gain}$ (mg·cm^{-2}·min^{-1} °C^{-1})	1.96 ± 0.73	1.66 ± 0.76	1.06 ± 0.85	1.98 ± 0.74	1.99 ± 1.43	2.93 ± 1.49
Threshold T_{re} for sweating (°C)	37.45 ± 0.24	37.30 ± 0.20	37.39 ± 0.29	37.37 ± 0.31	37.23 ± 0.40	37.39 ± 0.26

Values are mean ± SD

T_{re} resting rectal temperature, ΔT_{re} difference from resting value in rectal temperature, T_{sk} weighted mean skin temperature, ΔT_{f-f} difference between forearm and fingertip skin temperatures, H_{prod} metabolic heat production, $\dot{m}_{sw}$ sweating rate

[a]Significant difference to values before HA

[b]Significant difference to heat values

[c]Significant difference to hypoxic values, $p<0.05$

and environmental conditions ($p>0.05$). On the contrary, there was a main effect of environmental condition ($p=0.001$) accompanied by an interaction effect between environmental condition and acclimation ($p=0.004$) on the peak sweat rate. Indeed, peak sweat rate was higher in HE (1.54 ± 0.35 mg·cm^{-2}·min^{-1}) compared to NOR (1.07 ± 0.17 mg·cm^{-2}·min^{-1}, $p=0.004$) and HYP (0.97 ± 0.13 mg·cm^{-2}·min^{-1}, $p=0.001$), but only post-acclimation. Peak sweat rate increased post-acclimation by 37 ± 33% in HE ($p=0.018$). A similar tendency for an interaction effect between acclimation and environmental condition ($p=0.067$) was noted for the gain of the sweating response which increased post-acclimation by 1.8 ± 1.6 mg·cm^{-2}·min^{-1} °C^{-1} in HE ($p=0.094$). ΔT_{f-f}

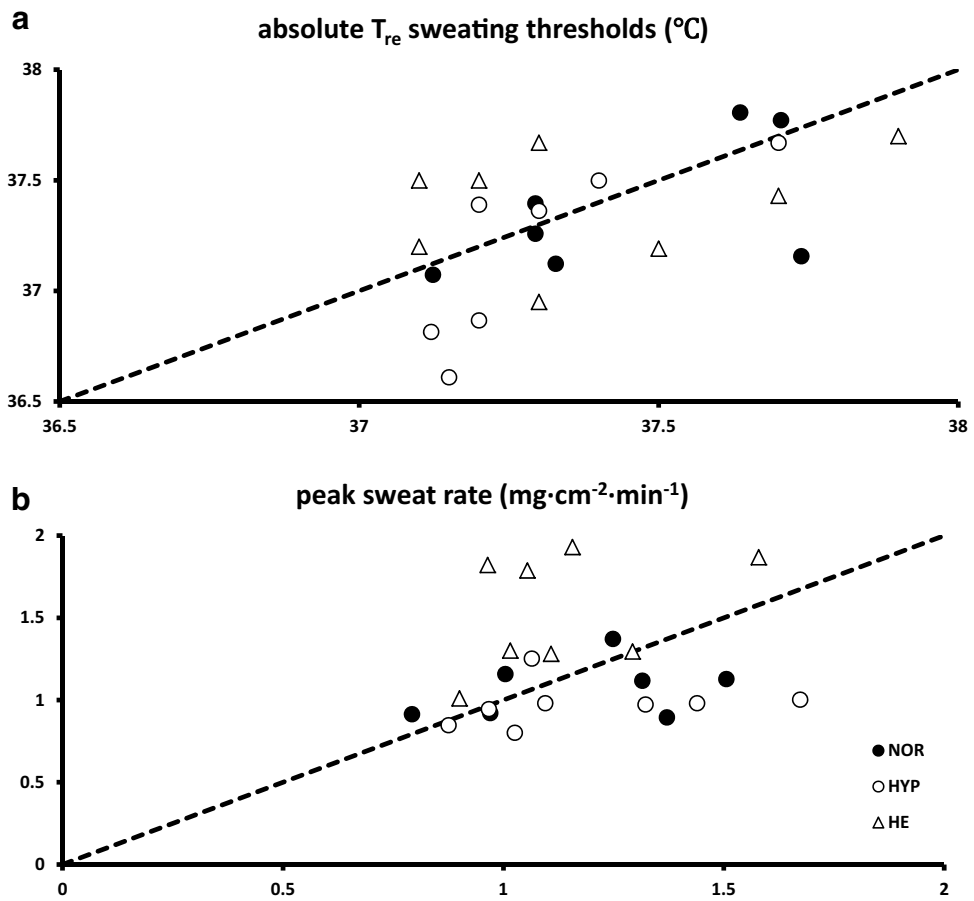

Fig. 4 Pre- (horizontal axis) and post- (vertical axis) acclimation individual data for **a** absolute sweating thresholds and **b** peak sweat rate in normoxia (closed circles), hypoxia (open circles) and heat (triangles)

remained unaltered by the acclimation protocol or environmental condition ($p = 0.70$ and $p = 0.61$, respectively). As a result of the PV contraction, the estimated specific heat capacity of the blood was lower even by day 1 (3553 ± 33 vs. 3520 ± 31 J·kg^{-1} °C^{-1}, $p = 0.021$) and remained in such levels by day 10 ($-1.6 \pm 1.0\%$, $p < 0.001$) but also the first day after participants exited the hypoxic environment ($-1.1 \pm 0.5\%$, $p = 0.006$).

Discussion

Aerobic exercise performance has been shown to be enhanced by the independent application of heat or hypoxic acclimation. The current study examined the interactive effects of heat and hypoxic acclimation protocols on aerobic performance in normoxic thermoneutral or hot conditions. The main findings of the study were that (1) the combined acclimation protocol did not elicit substantial benefits in aerobic performance, and (2) thermoregulatory adaptations, long associated with heat acclimation protocols, were blunted following the application of a combined heat and hypoxic acclimation protocol.

Exercise performance

The first study to employ a combined stressor (heat & hypoxia) acclimation protocol was promising a "conditioning cocktail for team-sport athletes exercising at sea level in temperate conditions" (Buchheit et al. 2013). The fitness level of the participants in the present study could be deemed similar to that study. Considering the lack of effect on $\dot{V}O_{2\,max}$, we cannot confirm that live-high train-low in the heat provides an ergogenic tool for aerobic performance in thermoneutral normoxic conditions. Rendell et al. (2017) have also reported unaltered $\dot{V}O_{2\,max}$ values, whereas Buchheit et al. (2013) and McCleave et al. (2017) did not measure $\dot{V}O_{2\,max}$ following a combined stressor acclimation protocol. In the former study, the hypoxic stimulus (240 km h) was probably not sufficient to elicit a substantial erythropoietic effect (Sotiridis et al. 2018b) as verified by no evident changes in haemoglobin mass. In contrast, higher hypoxic doses have potentiated increments of + 3.8% and 2.6% in haemoglobin mass in the latter studies, respectively. The differential results might be additionally explained by a baseline effect. Given that the individual haemoglobin mass level is training status-dependent (Heinicke et al. 2001), hypoxic acclimation protocols could increase haemoglobin mass,

especially in athletes of a lower fitness level (Robach and Lundby 2012) such as the team-sport athletes recruited by Buchheit et al. (2013). The possibility that the interaction of the heat with the hypoxic stimulus negated erythropoietic adaptations can be safely excluded on the basis that a separate controlled hyperthermia heat acclimation has been shown not to suppress haemoglobin mass (Rendell et al. 2017).

Even though we were unable to measure haemoglobin mass, we are quite confident that the applied hypoxic dose initiated the desired haematological adaptations. First, in our previous study, we observed an increase in $\dot{V}O_{2\,max}$ following a similar hypoxic acclimation protocol (Sotiridis et al. 2018a). The training stimulus was not of sufficient intensity to account for this increase in $\dot{V}O_{2\,max}$, especially since training HR was set to 50% HYP W_{peak}. Second, according to a recent meta-analysis, an approximate increase in haemoglobin mass of 4% is expected after the application of such a hypoxic dose (Garvican-Lewis et al. 2016). Finally, in a recent study that confined participants of a similar fitness level at a similar simulated altitude compared to the present study, an increase in haemoglobin mass was noted in all, but one, participants by day 12 (Siebenmann et al. 2015a). Regarding the inability of the hypoxia-induced haematological changes to evoke a $\dot{V}O_{2\,max}$ increase, it is notable that EPO concentration undershot baseline levels on the first day post-acclimation presumably permitting a selective destruction of young red blood cells (Alfrey et al. 1997). Alternatively, the estimated PV contraction might have been accompanied by a reduction in maximal SV and CO mediated by the decreased venous return. Nevertheless, considering our participants performed the maximal tests between the second and fourth day post-acclimation, a restoration of blood volumes towards baseline values may have accounted for the observed unchanged values of maximal CO and the corresponding SV. In HE, as our participants enhanced their sweating response (as reflected on peak sweat rate and the gain of the sweating response in HE), an even lower amount of blood would return to the heart post-acclimation indicated by lower maximal CO and corresponding peak SV values compared to NOR. In HYP, the failure of the CO to normalize towards baseline levels after hypoxic acclimatization has been long considered as the limiting factor for $\dot{V}O_{2\,max}$ (Saltin et al. 1968; Calbet et al. 2003).

With $\dot{V}O_{2\,max}$ unaffected, an independent increase in GME could account for improvements in aerobic performance as reflected on W_{peak}. In the absence of a control group or a sham treatment, a training/placebo effect could be considered likely. However, our own data (unpublished data, Sotiridis A, Debevec T and Mekjavic IB) suggest that gains in W_{peak} after a 10-day moderate-intensity training period are accompanied by improvements in $\dot{V}O_{2\,max}$ but not GME in participants of a lower training level. Exercise performance has been shown to improve after a combined stressor acclimation protocol when assessed using a Yo–Yo intermittent recovery test in professional football players (Buchheit et al. 2013), but not a 3 km time trial in trained runners (McCleave et al. 2017). In both studies, the respective improvement was not superior to the gains reported in exercise performance after an independent heat acclimation protocol. These data are in agreement with our recent findings that higher increments in W_{peak} are observed after a controlled-hyperthermia heat-acclimation protocol (Sotiridis et al. 2019), but not a continuous hypoxic confinement (Sotiridis et al. 2018a). Thus, the possibility that the hypoxic stressor adds significantly to the gains obtained by (heat) training is considered unlikely when exercise is conducted in normoxia. Regarding aerobic performance in hot hypoxic conditions, the employed acclimation protocol succeeded in alleviating the previously reported 51% decrement compared to thermoneutral normoxic conditions (Girard and Racinais 2014). Provided that W_{peak} was higher post-acclimation in HE, such improvement in time to exhaustion in hot hypoxic conditions might be attributed to the lower relative exercise intensity that individuals had to sustain during the trial. It is of note that all, but one, participants increased their time to exhaustion. Future research is needed to elucidate whether such a protocol might be recommended for professionals expected to perform long submaximal work, markedly elevating their T_c as a consequence, at high altitudes.

Thermoregulatory data

Previous controlled hyperthermia heat acclimation protocols (Rendell et al. 2017; Gibson et al. 2015; Fox et al. 1963; Sotiridis et al. 2019) have confirmed the development of positive phenotypic adaptations when humans are acclimatized to heat with the main ones being: reduced T_{sk} and T_{re}, augmented sweating response, lower HR and an expansion of PV. The present combined stressor approach that included a medium-term heat acclimation protocol only partially potentiated these adaptive responses. In HE, HR was not reduced probably serving as a compensatory mechanism to the reduced SV compared to NOR during submaximal exercise. The pronounced PV contraction may have stimulated this reflex. Resting T_{re} and T_{sk} remained unaffected. Our participants demonstrated a higher magnitude and gain of the forehead sweating response in HE post-acclimation despite an estimated lower specific heat capacity of the blood. This observation together with the fact that they did not start sweating at a lower T_{re} might reinforce the idea of distinguishing central from peripheral heat adaptations. Specifically, the augmented sweat rate at any given T_c may be attributed to an effect of training the sweat glands, which is unrelated to aerobic fitness. Decreases in absolute sweating thresholds have been repeatedly related to

decreases in resting T_{re} (Buono et al. 1998; Patterson et al. 2004). Conversely, as resting T_{re} remained unaltered in the present study, we did not expect any change in the sweating thresholds. For that type of analysis though, a more responsive index of T_c such as oesophageal temperature (Taylor et al. 2014) might be warranted.

This is not the first study to demonstrate that adding normobaric hypoxic confinement to a heat acclimation protocol will blunt the expected thermoregulatory adaptations. Recently, McCleave et al. (2018) reported "no changes in T_c, sweat rate or PV" and reasoned that a low ratio of heat to hypoxic exposure, the intermittent nature of the heat acclimation together with the fact that training sessions were conducted using the traditional fixed-workload protocol rather than the controlled hyperthermia technique could explain their findings. We do not agree with the authors that the stimuli should be quantified in a ratio of heat to hypoxic exposure. Both environmental stimuli should be applied as suggested by previous research if they are designed to mediate aerobic gains. Medium-term heat acclimation protocols that have repeatedly induced cardiovascular as well as thermoregulatory adaptations rarely propose more than 15 h of total heat exposure. On the contrary, a hypoxic exposure should exceed 200 h to elicit haematological adaptations (i.e. 225 h in the study by Hauser et al. (2017)). This is why the participants in the present study were confined for 230 h at a simulated altitude of ~ 4000 m and were training every day in the heat for 90 min. Furthermore, the intermittent nature of the heat acclimation elicited a certain degree of adaptation in the study by McCleave et al. (2018) given that the group that followed the same heat acclimation protocol (training thrice per week for a time period of 3 weeks adding up to 13.5 h of heat exposure), but did not live in hypoxic conditions, displayed lower core and skin temperatures, a reduced HR and an augmented sweat rate. Despite the fact that a controlled hyperthermia protocol exposes individuals to a constant forcing function that sustains the heat adaptation impulse (Taylor 2014), Gibson et al. (2015) elegantly showed that isothermal heat acclimation is as potent as a fixed-work rate protocol in inducing heat adaptation. Consequently, it comes as no surprise that the phenotypic response of that group was identical to the results of our recent study that involved a 10-day controlled hyperthermia heat acclimation protocol (Sotiridis et al. 2019). Finally, the actual heat acclimation-induced endogenous thermal load is usually underestimated as T_c remains high for the first 10–15 min after the completion of each acclimation session.

Haematological data

Our haematological data support the idea that the observed changes were predominantly due to the hypoxic acclimation; increased concentrations of haemoglobin and haematocrit suggesting a pronounced PV contraction, EPO kinetics reaching peak concentration on day 1 of the protocol and returning towards baseline levels on day 10 and renin concentration following the decline in PV. The estimated PV contraction was similar to the one we reported after a 10-day continuous hypoxic acclimation protocol using the same simulated altitude at the same facility (Sotiridis et al. 2018a). This persistent PV contraction is traditionally attributed to diuretic fluid loss over the course of the 10-day acclimation (Jain et al. 1980), a hypothesis that is further confirmed by virtue of the ever-decreasing daily pre-exercise body mass values. The combination of the acclimation protocols seems to have also impacted on the renin kinetics. In the Siebenmann et al. (2015a) study, renin activity decreased by day 3 and remained below baseline values for the remainder of the 28-day hypoxic confinement whereas Keramidas et al. (2016) reported a 25% decrease in renin concentration on day 2 of their acclimation protocol, which was thereafter restored to baseline. In both studies, care was taken to ensure that individuals maintained their normal daily activity levels. In the present study, a heat training-mediated stimulation of the renin–aldosterone axis might have counteracted the hypoxia-induced renin suppression. The low sensitivity of the ELISA kits accompanied by the excessive inter-individual variability did not enable us to observe changes in HSP70 and VEGF, respectively, in agreement with McCleave et al. (2018).

Limitations

The present study was not without limitations. The potential for type II error remains substantial due to the small sample size. Since our participants were aware of the environmental conditions they were living and training in, a placebo effect cannot be excluded in view of the increases in W_{peak}. However, the fact that minor gains were observed in the determinants of aerobic performance ($\dot{V}O_{2\,max}$, VT or GME) (Bassett and Howley 2000) clearly suggests that the acclimation-elicited ergogenic effect was trivial. The recruitment of the same individuals living in the same facility in normoxic conditions and training in the heat in a cross-over design would add valuable insight into the interactive effects of a combined heat and hypoxic acclimation on thermoregulatory function. Longer submaximal tests that would potentiate increases in T_c should, instead, be used for the evaluation of thermoregulation in professionals or endurance-type athletes (e.g. mountaineers) to ensure external validity. In such groups, T_c could escalate after long bouts of physical activity. Even though we are confident that the applied hypoxic dose was sufficient to initiate ventilatory as well as haematological adaptations, measures of haemoglobin mass are necessary to establish the erythropoietic effect in future studies. By employing the carbon monoxide rebreathing

method, the magnitude of the PV contraction is not confounded by hypoxia-elicited erythrocytosis. In the present study, baseline vascular volumes were calculated from lean body mass using the equations given by Sawka et al. (1992).

Conclusions

In conclusion, the findings of this study suggest that a 10-day combined heat and hypoxic acclimation protocol does not improve aerobic performance in normoxic thermoneutral or hot conditions. Thermoregulatory gains—repeatedly reported in previous heat acclimation studies—were comparatively absent following the application of a combined heat and normobaric hypoxic acclimation protocol in young males. Combining heat and hypoxic acclimation does not seem to be superior to traditional acclimation/training protocols in enhancing aerobic fitness and thermoregulation in normoxic thermoneutral or hot conditions. The selection of such a combined stressor acclimation protocol might be warranted when exercise is undertaken in hot hypoxic conditions.

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Author contributions AS, PM, MK and IBM conceived and designed the research. AS, PM, UC, MK and IBM performed experiments. AS and UC analysed the data. AS interpreted results. AS drafted the manuscript. AS, PM, UC, MK and IBM edited/revised the manuscript critically for important intellectual content.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the national research committee (National Committee for Medical Ethics, Ministry of Health, Republic of Slovenia, no. 0120-494/2018/9) and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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