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Factors contributing to the change in thermoneutral maximal oxygen consumption after iso-intensity heat acclimation programmes

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ABSTRACT

The factors explaining variance in thermoneutral maximal oxygen uptake ($\dot{V}O_{2max}$) adaptation to heat acclimation (HA) were evaluated, with consideration of HA programme parameters, biophysical variables and thermo-physiological responses. Seventy-one participants consented to perform iso-intensity training (range: 45%–55% $\dot{V}O_{2max}$) in the heat (range: 30°C-38°C; 20%−60% relative humidity) on consecutive days (range: 5-days-14-days) for between 50-min and-90 min. The participants were evaluated for their thermoneutral $\dot{V}O_{2max}$ change pre-to-post HA. Participants' whole-body sweat rate, heart rate, core temperature, perceived exertion and thermal sensation and plasma volume were measured, and changes in these responses across the programme determined. Partial least squares regression was used to explain variance in the change in $\dot{V}O_{2max}$ across the programme using 24 variables. Sixty-three percent of the participants increased $\dot{V}O_{2max}$ more than the test error, with a mean ± SD improvement of 2.6 ± 7.9%. A two-component model minimised the root mean squared error and explained the greatest variance (R^2; 65%) in $\dot{V}O_{2max}$ change. Eight variables positively contributed ($P < 0.05$) to the model: exercise intensity (%$\dot{V}O_{2max}$), ambient temperature, HA training days, total exposure time, baseline body mass, thermal sensation, whole-body mass losses and the number of days between the final day of HA and the post-testing day. Within the ranges evaluated, iso-intensity HA improved $\dot{V}O_{2max}$ 63% of the time, with intensity – and volume-based parameters, alongside sufficient delays in post-testing being important considerations for $\dot{V}O_{2max}$ maximisation. Monitoring of thermal sensation and body mass losses during the programme offers an accessible way to gauge the degree of potential adaptation.

KEYWORDS
Hot training; maximal oxygen consumption; endurance

1. Introduction

Maximal oxygen uptake ($\dot{V}O_{2max}$) is the highest rate at which oxygen can be taken up and utilised during severe intensity exercise, setting the upper-limit for adenosine tri-phosphate production via oxidative phosphorylation (Bassett & Howley, 2000). Among other variables, $\dot{V}O_{2max}$ is a primary determinant of endurance performance (Coyle et al., 1988; Joyner et al., 2020), and is the most common method of prescribing exercise intensity in training studies (Milanović et al., 2015; Scribbans et al., 2016; Waldron et al., 2021). Improvements in $\dot{V}O_{2max}$ have been historically linked to physical training programmes (Midgley et al., 2007); however, the potential additive effect of training in hot environments has been more recently debated (Nybo & Lundby, 2016).

Serial exposure to the heat, combined with physical training, can be used for the purposes of heat acclimation (HA), thereby increasing tolerance to hot or humid environments. It has also been recognised that increases in plasma volume (PV), exercising cardiac output in cool and hot conditions ($\dot{Q}$) (Lorenzo et al., 2010; Nielsen et al., 1993) skin blood flow (Lorenzo & Minson, 2010) and sustained exercising limb blood flow, despite exercising in hot conditions (Nielsen et al., 1993) are a significant element of the phenotypic adaptation. Based upon features of the Fick principle, these changes may enhance systemic delivery of arterial blood and subsequent $\dot{V}O_{2max}$ adaptation (Poole et al., 2022; Wagner, 2011). Indeed, more recent evidence has emerged to demonstrate that haemoglobin mass

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can be increased following extended periods (~5-weeks) of HA (Oberholzer et al., 2019; Rønnestad et al., 2022, 2021), which despite the reported limited increases in $\dot{V}O_{2max}$, could theoretically increase oxygen carrying capacity and is consistent with the centrally-mediated improvements of $\dot{V}O_{2max}$ described among healthy, active participants (Lundby et al., 2017; Mortensen et al., 2005). In addition, increases in mitochondrial protein content have been reported after 3-weeks of HA, alongside modest increases in $\dot{V}O_{2max}$ (Maunder et al., 2021). Collectively, there is physiological reasoning to support the notion that selected approaches to HA could enhance thermoneutral $\dot{V}O_{2max}$, yet this topic remains controversial and, despite recent meta-analytical evidence to support thermoneutral $\dot{V}O_{2max}$ increases following HA, the results are not conclusive and the reasons for differences between study outcomes remain unclear (Waldron et al., 2021).

The protocols used to induce HA are wide-ranging and have been described in detail previously (Tyler et al., 2016). A common and accessible approach to HA is the so-called "iso-intensity" model, whereby the intensity of exercise is fixed to a pre-determined level, usually based on a fraction of baseline thermoneutral $\dot{V}O_{2max}$ or selected physiological threshold. It is noteworthy that this approach is often based on the $\dot{V}O_2$-work rate relationship in cooler conditions, meaning that the chosen work rate in the heat could represent a higher relative threshold of $\dot{V}O_{2max}$ during acclimation. Examples of this strategy are studies exercising participants at set mechanical intensities equivalent to 50% (Lorenzo et al., 2010; Waldron et al., 2019) and 60–65% $\dot{V}O_{2max}$ (Pivarnik et al., 1987; Travers et al., 2020), which have been reported to increase $\dot{V}O_{2max}$ to varying degrees. Other factors, such as the chosen ambient temperature (typically 30–40°C), relative humidity (typically 20–80%), number of induction days (typically 5–14-days) and daily session duration (typically 1–2-h) require consideration, as these will determine the total training and thermal load imposed across the programme (Waldron et al., 2021). However, meta-analyses of published HA studies have not clearly identified which combination of factors impart the greatest effect on $\dot{V}O_{2max}$ adaptation (Benjamin et al., 2019; Waldron et al., 2021)

Other inter-participant physical or physiological characteristics might also contribute to inter-individual adaptation capacity, such as the thermo-physiological response to the HA programme or baseline fitness levels that have been investigated in relation to the heat-acclimated phenotype (Corbett et al., 2018). Finally, there are strong theoretical grounds, alongside direct empirical (Waldron et al., 2019) and meta-analytical evidence (Waldron et al., 2021), to support that the number of days between the final day of HA and post-HA $\dot{V}O_{2max}$ testing is partly responsible for the reported outcome. For example, the fitness-fatigue paradigm refers to a phenomenon whereby a suitably dosed training stimulus (such as a physical training block) elicits an initial negative fatigue-like effect, which decays exponentially in the following days but overshadows the more sustained positive adaptations (i.e. fitness). Fitness subsequently manifests more slowly, once the fatiguing effect has sufficiently decayed (Calvert et al., 1976). With periods of intense training, across consecutive days, and in combination with other stressors (i.e. heat), it is likely that these fatigue-like effects accumulate and cause a delay in multi-system measures of cardio-respiratory adaptation, such as $\dot{V}O_{2max}$. As with other parameters of HA protocols in the literature, there has been little attention paid to this potentially important feature of programme design, and the inevitable inconsistency between individuals and HA studies makes it difficult to determine the optimal number of days required to maximise $\dot{V}O_{2max}$ adaptations following training cessation.

The parameters of a HA programme, such as ambient conditions, thermo-physiological responses, training or participant characteristics are also typically correlated (i.e. multicollinearity). For example, perceived exertion and heart rate have an underlying relationship, while training volume would naturally relate to total thermal load (defined as product of training time [min] and ambient temperatures [°C]). The combination of these factors prevents the application of some statistical processes (i.e. regression techniques) that might be used to evaluate the optimal design of a HA programme for enhancement of $\dot{V}O_{2max}$ and understand which physiological responses are associated with favourable adaptation. In addition, most HA studies are not conducted with the specific intention of improving $\dot{V}O_{2max}$ in a thermoneutral environment. For these reasons, wider inferences made in relation to HA design are restricted to results of meta-analyses, which are naturally limited by the inconsistency of study designs and are therefore less generalisable. However, with extensive recruitment, across longer study periods, larger samples can be recruited to address a common research question. This accommodates the use of more appropriate statistical procedures to explore the many potential factors associated with $\dot{V}O_{2max}$ adaptation. This information would be useful for individuals planning a heat training programme with the intention of improving $\dot{V}O_{2max}$ and determine which physiological responses during the programme should be monitored to signal a positive response. Therefore, the aim of the current study was to evaluate the factors explaining variance in thermoneutral $\dot{V}O_{2max}$ adaptation to training in a hot environment, with consideration of training programme parameters, individual biophysical variables and physiological responses to training.

2. Methods

2.1. Participants

A total of 71 active male adults (Table 1) provided written informed consent to take part in this research. Participants were 18–40 years of age, healthy, recreationally active, as defined by taking part in endurance training at least three times per week and some form of competitive sport on a weekly basis. None of the participants had taken part in outdoor hot weather training in the previous three-months and were at least three-months without exposure to hot environmental (~30°C) temperatures. None of the participants used hot bathing or other form of passive heating for the purpose of eliciting a training effect. All of the participants were deemed to be unacclimatised to the heat. All participants completed a food diary for 48-h prior to their first test, which was replicated with similar content and volume for subsequent tests. Participants were asked to refrain from alcohol and any supplementation during the study period and arrive at the laboratory having eaten a standardised meal and consumed 500 ml of fluid in the previous 2-h. Euhydration was verified via urine analysis using an osmometer (<600 $mOsmol \cdot kg^{-1}$ H_2O, Osmocheck, Vitech Scientific Ltd, UK). Ethical approval was provided by the institutional ethics committee for all studies, which were conducted in accordance with the 2013 Helsinki declaration, with the exception of registration in a trials database.

Table 1. Participant, training and testing variables (mean ± SD and range).

	Mean (SD)			Range		
Participants (n = 71)						
Age (years)	24	±	4	18	to	36
Body mass (kg)	76.7	±	7.9	58.0	to	113.0
Baseline $\dot{V}O_{2max}$ (mL/kg/min)	47.1	±	11.0	25.7	to	72.0
Training and testing variables						
Intensity (%$\dot{V}O_{2max}$)	50	±	4	45	to	55
Total exposure time (min)	543	±	376	250	to	1260
Ambient acclimation temperature (°C)	34	±	3	30	to	38
Ambient acclimation humidity (°C)	31	±	11	20	to	60
Daily session time (min)	66	±	13	50	to	90
Total HA duration (days)	7.7	±	3.6	5	to	14
Thermal load (AU)	2207	±	310	1650	to	2700
Additional training hours/ week (h)	5	±	4	1	to	19
Post-HA testing delay (days)	3	±	1	1	to	4

Note: $\dot{V}O_{2max}$ = maximal oxygen uptake; HA = heat acclimation or acclimatisation; all variables were entered as predictor variables in the subsequent analysis. Minor deviations in planned parameters, such as intensity, HA duration or thermal load are accounted for by adjustments during the protocol.

2.2. Design

This study was designed to perform an exploratory analysis using partial least squares regression (PLSR) to predict the observed change in $\dot{V}O_{2max}$ following a period of HA from a comprehensive set of predictor variables. This was achieved by pooling the appropriate individual participant data from previous heat acclimation studies, where participants had been allocated to HA groups for interventional research (Waldron et al., 2019; Waldron et al., 2020), alongside previously unpublished research. All data were collected by the same research team members, using the same protocols and equipment. The selection of participants was such that HA groups encompassed variation in the laboratory conditions during HA for subsequent modelling. For descriptive purposes, the mean, standard deviation and range of participant characteristics and training and testing variables are reported in Table 1. The $\dot{V}O_{2max}$ of participants was assessed before and after the HA programmes and expressed relative to body mass. There were no sustained changes in the body mass of participants pre-to-post HA using a paired *t*-test for *a-priori* analysis ($P < 0.05$). The parameters of the HA sessions were chosen to maintain the stimulus for heat adaptation based on available reviews (Tyler et al., 2016; Waldron et al., 2021), whilst manipulating key features of training to permit variation of the imposed stressors. There was also natural variation in the biophysical characteristics of participants and their training background, which were also considered as potential predictor variables. In addition, the number of days between post-testing and final HA day varied from 1 to 4-days. The groups all followed an iso-intensity approach to HA, with the mechanical load associated with selected thresholds of baseline $\dot{V}O_{2max}$ controlled. Temperatures and relative humidity systematically varied, thereby manipulating the thermal load of the participants. Given the nature of the subsequent statistical analysis, these variations offered valuable contribution to the dataset. In addition to data presented in Table 1, the distribution of ambient dry-bulb temperatures were: 30°C ($n = 20$; 28.2%), 33°C ($n = 16$; 22.5%), 36°C ($n = 23$; 32.4%) and 38°C ($n = 12$; 16.9%). The distribution of HA session times was: 50-min ($n = 6$; 8.5%), 60-min ($n = 47$; 66.2%), 70-min ($n = 4$; 5.6%) and 90-min ($n = 14$; 19.7%). The distribution of total HA days was: 5-days ($n = 41$; 57.7%), 6-days ($n = 4$; 5.6%), 10-days ($n = 12$;

16.9%) and 14-days ($n = 14$; 19.7%). Participants were monitored for their thermo-physiological responses to the HA on a daily basis using heart rate telemetry, rating of perceived exertion (RPE), thermal sensation, mean resting and exercising core temperature (Tc), mean change in Tc from rest to exercise, and plasma volume expansion (PV%Δ) across the HA programme. The mean of the physiological responses during the HA programme and their relative change across the programme (i.e. percentage change from day-1 to final day) were also used as predictor variables. These are denoted as "relative change" variables hereafter. For clarity, a relative change variable was determined simply by the value on final HA day (b), minus the value on day 1 (b), divided by b and multiplied by 100 (i.e. ((b-a)/a) x 100). In total, 24 variables were entered into the PLSR model as candidate predictors, with the percentage change in $\dot{V}O_{2max}$ entered as the reference variable.

2.3. Incremental ramp tests for $\dot{V}0_{2max}$

Across all laboratory visits, participants were familiarised with the same cycle ergometer (Monark Exercise AB, Ergomedic 874E, Varberg, Sweden). In a cool, air-conditioned room (~20°C), participants then completed a 5-min self-selected warm-up prior to completing an incremental ramp test. The test was conducted at approximately 60–85 rev/min, starting at ~70–80 W and increasing by approximately 18–24 W/min until volitional exhaustion. Each participants' individual increments were the same across their two trials. Pulmonary gas was measured continuously using a breath-by-breath gas analyser (Jaeger Vyntus CPX, Hoechberg, Germany). The gas analyser was calibrated before every trial with gases of known concentration (15.95% oxygen, 4.97% carbon dioxide, BAL. nitrogen) and the turbine volume transducer was calibrated automatically by the system at flow values of 2 and 0.2 L/s (Hans Rudolph, Kansas City, KS). Maximal oxygen consumption was determined as the mean value recorded over the final 30-s of the test. Criteria for achieving $\dot{V}O_{2max}$ was: [1] reaching volitional exhaustion, [2] respiratory exchange ratio > 1.15, [3] final heart rate within 10 bpm of age-predicted maximum and [4] RPE > 19 (6–20 Borg scale). All criteria were met during the studies. Heart rate was recorded throughout the tests (Polar FT1, Polar Electro Oy, Kempele, Finland). Based on an inter-day, test-re-test analysis of 55 separate participants with mixed training backgrounds, this incremental test has a coefficient of variation of 2.0% in our laboratory, using the calculations provided by Atkinson and Nevill (1998). Time between repeated tests has varied between two and seven days. Power output at selected fractions of the $\dot{V}O_{2max}$ was determined from the relationship between $\dot{V}O_2$ and mechanical power output. All tests were performed at the same time of day (± 2-h).

2.4. Heat acclimation protocols

The power output corresponding to between 45% and 55% of the participants' baseline $\dot{V}O_{2max}$ was set as the external work intensity for the intervention and was monitored using power output on the same cycle ergometers. Given the nature of the ramp test, the exercise thresholds were determined from the linear $\dot{V}O_2$-power relationship using regression analysis, with two thirds of the ramp rate deducted (Whipp et al., 1981). All participants cycled at the same intensity and cadence for all visits to the chamber as part of the HA programme and no fans were used during the exercise trials. Participants wore cycling shorts, socks and training shoes, and cycled between 50-min and 90-min per HA session during their programmes. The thermal chamber was controlled from 30°C to 38°C temperature and 20% to 60% relative humidity, which was fixed for the duration of the HA programme, within-participant. Thermal load was calculated as the product of ambient temperature (°C) and exposure time (min). The selection of HA programmes were conducted across a range of 5 and 14 consecutive days. These parameters were selected to replicate the typical range of HA programme variables reported (Waldron et al., 2021). Body mass was recorded pre – and post-session, wearing only underwear, on every day of the HA programme to estimate whole-body sweat rate (WBSR) by subtracting post-exercise body mass from pre-exercise values (MPMS-230, Marsden Weighing Group, Oxfordshire, UK). No fluid intake was permitted during the HA sessions, until after post-session measurements. Relative change in body mass was used as a candidate predictor variable, which was determined as the percentage change in body mass (i.e. the daily delta value) lost during a single HA session between day-1 and the final HA day. For example, a participant losing 1.5 L of fluid (1.5 kg of body mass) on day 1 and 2 L on their final day, would have a 33% relative change (((2 kg – 1.5 kg) / 1.5 kg) x 100). A rectal thermometer was self-inserted ~10 cm past the anal sphincter or a tympanic measurement was recorded, as an indication of Tc. The tympanic devices in our laboratory underestimate rectal temperature by 0.8 ± 0.3°C but correlate strongly ($R^2 = 0.92$) while cycling at exercise intensities between 30% and 70% $\dot{V}O_{2max}$ in environmental temperatures of between 30°C and 35°C at a fixed relative humidity of 40%. We therefore consider the tympanic measures to underestimate core temperature but provide a valid index of core temperature change. However, subsequent scaling of data for regression modelling accounted for the anticipated differences in Tc between devices. Core temperature data were recorded at rest on each day of the programme (between 10-min and 30-min pre-exercise, outside of the heat chamber in air-conditioned rooms of ~20°C) and for the duration of the session inside the heat-controlled chamber. The mean Tc at rest and the mean Tc during all exercising HA sessions were measured, with the latter used as a global indication of thermoregulatory response. The percentage change in mean resting Tc to mean exercising Tc was also recorded as further indication of the participants' individual daily dynamic temperature response to the programme. For clarity, this demonstrates the mean change in the Tc from rest-to-exercise, thus accounting for any daily variations in starting or final Tc values reached across the programme. During all exercising periods of the HA programme, heart rate was recorded telemetrically in beats/min, alongside thermal sensation and RPE (6–20) at 10-min intervals. Thermal sensation was recorded on either a 7-point or 9-point analogue scale (−3 to +3 or − 4 to +4, respectively). Subsequent statistical treatment during the regression modelling accounted for the differences between scales.

2.4. Plasma volume

Prior to exercise on HA day 1 and the final day of exercise, participants rested in an upright seated position in an air conditioned room (~20°C) for 15-min. Changes in capillary hematocrit (Hct) and hemoglobin concentration [Hb] (Hemocue Ltd, Viking Court, Derbyshire, UK) were recorded to determine the relative change in plasma volume (Dill & Costill, 1974). Capillary blood was taken from the same site for measurement of [Hb] using a Hemocue Hb 201 +. Plasma volume changes (%) were reported between the first and last measurement.

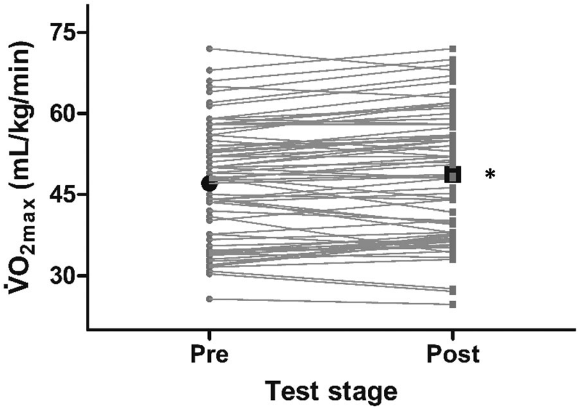

Figure 1. Pre – and post-heat acclimation testing of maximal oxygen consumption ($\dot{V}O_{2max}$). Darker and larger symbols are the mean of each group. Standard deviations are provided in the text. * = sig. different from pre-to-post ($p < 0.05$).

2.5. Statistical analysis

All data were collectively pooled to perform PLSR using RStudio (version 4.1.3, 2022, RStudio, Inc. software, Boston, MA), using the "*pls*' package. This statistical method was utilised to explain variation in the $\dot{V}O_{2max}$ change (response variable) based on the 24 candidate predictor variables. For clarity, all of the 24 candidate predictor variables are cited in Table 3. The mean responses refer to the average measurement of a given variable across the programme, whereas relative changes refer to percentage changes between the first and last day. The sample of 71 was deemed appropriate for analysis. Adopting the inverse square root approach for comparable PLS models, a sample estimate of $n = 69$ provided adequate sample for this model, based upon a conservative beta coefficient of ~ 0.2 between the predictor variables and the reference variable, with an alpha of 0.05 and 80% power (Hair et al., 2022). We chose to use PLSR because it is particularly suitable when predictor variables demonstrate collinearity. In PLSR, predictors are projected into latent variables to generate the minimal number of components that explain the most variance in the response variable. Data were scaled, such that each variable was divided by the standard deviation of the sample for that measurement. Scaling accounts for the different magnitude and scale of measurements in the x and y space, thus removing biased weighting in the PLSR. The PLSR model was cross-validated using $k =$ 10 folds, with the root mean squared error of the prediction (RMSEP) reported. The number of selected components was determined based upon the maximum fit of the overall model using the R^2 value and the minimum RMSEP. To objectively support this determination, the "*one-sigma*" function was applied in RStudio to return a model where the optimal RMSEP was within one standard error of the absolute optimum (Hastie et al., 2013). The one-sigma was the preferred option for selecting the number of components in the PLSR model, as it avoids subjective selection of components based on model fit and RMSEP. Finally, the *Jack-knife* function was used to determine the individual contribution of each predictor variable to the PLSR model (Martens & Martens, 2000). This function performs approximate *t*-tests of regression coefficients. For descriptive purposes, the pre-to-post change in $\dot{V}O_{2max}$ was evaluated using a paired *t*-test and a repeated measures effect size (dRM = Morris, 2008), which were also performed in RStudio.

3. Results

3.1. Pre-to post acclimation effects

Overall, 63% percent of the participants increased their $\dot{V}O_{2max}$ more than the coefficient of variation of the test with a mean ± SD change score of 2.6 ± 7.9% ($d_{RM} = 0.42$, $p < 0.001$). The mean ± SD of pre – and post-HA testing $\dot{V}O_{2max}$ values are presented in Figure 1, which were 47.1 ± 11.0 and 48.4 mL/kg/min. This translated to absolute value of 3.6 ± 0.8 L/min and 3.7 ± 0.9 L/min, respectively. Table 2 provides a descriptive overview of the physiological responses during training and change scores from pre-to-post testing.

3.2. Partial least squares regression model

Two components were selected in the final PLSR model based on the RMSEP minimisation from the *k*-fold cross-validation, as determined by the one-sigma analysis. The RMSEP value was 5.2% and the model explained 65% of the variance in the $\dot{V}O_{2max}$ change values. The contribution of each predictor variable is described below in Table 3.

4. Discussion

The aims of the current analysis were to establish: i) the optimal parameters for a HA programme when attempting to maximise the change in $\dot{V}O_{2max,}$ ii) evaluate the contribution of baseline biophysical variables, and iii) determine which physiological response variables are most important to monitor during the programme to identify a favourable adaptation. In total, 65% of the overall variance in $\dot{V}O_{2max}$ could be explained by the factors included in this two-component model. An increase in $\dot{V}O_{2max}$ was observed in 63% of cases, with a small-to-moderate effect size. A total of eight variables significantly contributed to this prediction model. Based on their positive associations, a total of four programme parameters are important to consider when designing a HA programme for this purpose, including (in order of contribution) the intensity of the exercise, the ambient temperature, the number of days and the total exposure time. The baseline body mass of the participants was also a positive contributor to the overall model, while mean thermal sensation and body mass losses (i.e. WBSR) were also strongly, positively associated with the change in $\dot{V}O_{2max}$. Finally, the highest contributor to the model was the number of days between the final day of HA and the post-testing day, with the

Table 2. Descriptive overview of the physiological responses during training and change scores from pre-to-post testing.

Training responses	Mean (SD)	Range
Mean HA core temperature (°C)	38.4 ± 0.3	37.6 to 39.4
Rest to exercise change in core temperature (%)	−3.8 ± 1.3	−6.4 to −0.6
Core temperature relative change (%)	−1.2 ± 0.7	−3.3 to 0.5
Mean RPE (6–20)	16.0 ± 0.9	13.5 to 18.0
Mean thermal sensation (−3 to +3 or − 4 to +4)	3.0 ± 0.5	2.0 to 4.0
Mean heart rate (% maximum)	79.7 ± 4.3	67.7 to 88.0
Mean RPE relative change (%)	−15.4 ± 3.4	−23.1 to −5.9
Mean thermal sensation relative change (%)	−33.0 ± 16.6	−50.0 to 0.0
Mean heart rate relative change (%)	−10.7 ± 4.3	−20.6 to −2.3
Mean exercising body mass loss (%)	1.2 ± 0.5	0.2 to 2.5
Body mass relative change (%)	77.8 ± 46.0	10.0 to 228.6
Plasma volume change (%)	9.5 ± 5.5	−6.6 to 18.9

Note: RPE = rating of perceived exertion; PV = plasma volume. "Relative changes' refer to percentage changes in those variables from the start to the end of the respective programme.

Table 3. A two-component partial least squares regression model predicting the change in maximal oxygen consumption after heat acclimation and the individual contribution of each predictor variable (n = 71).

Model	$\Delta\dot{V}O_{2max}$		
Components	2		
Adjusted RMSEP CV	5.2		
Adjusted R^2	0.65		
Predictor variables	***Estimate***	***t***	***P***
Biophysical variables			
Age (years)	0.751	1.496	0.169
Baseline body mass (kg)	0.875	2.341	0.044*
Baseline $\dot{V}O_{2max}$ (mL/kg/min)	0.104	0.314	0.761
Training and testing variables			
Intensity (%$\dot{V}O_{2max}$)	1.200	4.339	0.002*
Total exposure time (min)	0.504	2.873	0.018*
Ambient acclimation temperature (°C)	0.993	3.970	0.003*
Ambient acclimation humidity (°C)	0.579	1.180	0.268
Daily session time (min)	0.045	0.180	0.861
Total HA duration (days)	0.567	3.960	0.003*
Thermal load (AU)	0.563	1.573	0.150
Additional training hours/week (h)	0.092	0.305	0.767
Post-HA testing delay (days)	2.133	5.683	0.001*
Training responses			
Mean exercising HA core temperature (°C)	1.148	1.735	0.117
Rest to exercise change in core temperature (°C)	−0.690	−1.814	0.103
Core temperature relative change (%)	−0.185	−0.692	0.507
Mean RPE (6–20)	0.788	1.021	0.334
Mean thermal sensation	1.162	2.637	0.027*
Mean heart rate (% maximum)	0.861	1.914	0.088
Mean RPE relative change (%)	−0.024	−0.064	0.950
Mean thermal sensation relative change (%)	−0.658	−1.265	0.238
Mean heart rate relative change (%)	−0.172	−0.340	0.741
Mean exercising body mass loss (%)	1.514	2.807	0.020*
Body mass relative change (%)	0.226	0.493	0.634
Pre to post programme PV change (%)	1.196	2.024	0.074

Note: HA = heat acclimation or acclimatisation; RMSEP = root mean square error of prediction; CV = cross-validation; $\dot{V}O_{2max}$ = maximal oxygen uptake; PV = plasma volume.

greater number of days associated with the higher $\dot{V}O_{2max}$ increase.

It was anticipated that training programme parameters would be associated with the change in $\dot{V}O_{2max}$. It has been well established that training load (or impulse) is associated with the capacity to increase $\dot{V}O_{2max}$, which can be accrued using different modalities, volumes or intensities (Bacon et al., 2013; Milanović et al., 2015). Thus, enhancement of the endurance phenotype can be achieved in various ways, assuming that sufficient stimulus is provided. For example, fixed-intensity training at lower intensities (<70% $\dot{V}O_{2max}$) can take approximately 3-weeks to increase in $\dot{V}O_{2max}$ (Murias et al., 2010). Based on the current data, the addition of a thermal environmental stimulus to training can cause these adaptations in as few as 5-days, which is comparably faster than reported in the literature as a result of various endurance training programmes. Both volume (number of days and exposure time) and intensity (training intensity and ambient temperature) combined to explain variation in thermoneutral $\dot{V}O_{2max}$ change, such that much shorter timeframes (5–14 days) were sufficient in comparison to the aforementioned periods during thermoneutral training studies. The current data therefore characterise HA training approaches as more aggressive in nature, since the training and thermal loads are necessarily high to elicit the desired outcomes. In the HA literature, training "load" *per se* is often afforded less attention, yet the thermal impulse is commonly discussed in relation to thermoregulatory enhancement (Benjamin et al., 2019; Périard et al., 2015; Tyler et al., 2016). The inclusion of higher temperatures (irrespective of internal core temperature) and total HA exposure times reported here indicates that thermal impulse is also important for thermoneutral $\dot{V}O_{2max}$ adaptation. These higher temperatures would theoretically drive the skin temperature response, which is known to drive heat adaptation (Regan et al., 1996). Given that many training load-related variables were significant contributors to the current predictive model, it is recommended that "forcing" of the exercise stress signal during HA is important for athletes or practitioners wanting to maximise $\dot{V}O_{2max}$ changes. It should be noted that balance must be achieved between selected variables when using HA for development of $\dot{V}O_{2max}$. For example, training intensity and daily volume are reciprocally related, such that higher fractions of $\dot{V}O_{2max}$ would be unsustainable for prolonged periods. This naturally constrains the HA session intensity in the current model.

A key finding of the current analysis was the large association between $\dot{V}O_{2max}$ change and the number of days between the final HA day and post-testing. This has been studied previously in our laboratory, where the optimal number of days considered to permit super-compensation of the $\dot{V}O_{2max}$ change was approximately 96-h (Waldron et al., 2019). This is supported by prior research, where the greatest reductions, or lowest improvements, in $\dot{V}O_{2max}$ are reported when a post-HA programme period of 24-h is provided (Aoyagi et al., 1998; Chen et al., 2013; Febbraio et al., 1994). A demanding episode of physical training, particularly combined with HA, will intentionally induce transient overreaching or acute fatigue among participants. Consistent with historical models of the training dose–response relationship, such as the fitness-fatigue paradigm (Calvert et al., 1976) or general adaptation syndrome (Selye, 1950), when coupled with a short period of recovery post-training, it is likely that insufficient time is permitted to realise the training effect, yet longer periods offer greater adaptation time and decay of residual fatigue. Since the range of post-HA testing days was limited to 1–4 in the current analysis, we maintain our previous recommendation that 96-h is optimal for post-testing or planned tapering if $\dot{V}O_{2max}$ maximisation is desired.

It was noteworthy that all "relative change" response variables were negatively, yet non-significantly, associated with the $\dot{V}O_{2max}$ change outcome. These relative change scores were included in the current model because they reflect the apparent reduction in the training or thermal impulse, which is a consistently reported hallmark of iso-intensity acclimation (Périard et al., 2015). This is logically regarded as a potential limitation of the iso-intensity model, since as fitness increases during any training programme, the daily stimulus is decreased from baseline (Tyler et al., 2016). The implication is that progressively insufficient stimulus will lead to lower adaptation across time. This has been highlighted using iso-thermal HA models to enhance PV expansion (Patterson et al., 2004). However, the consistent trend in negative coefficients (albeit non-significant) reported herein indicate, at least in relation to $\dot{V}O_{2max}$ adaptation, that the greater the reduction in the physiological (i.e. heart rate) or perceptual (i.e. RPE) response variable across the programme, the greater the $\dot{V}O_{2max}$ increase. Owing to the decay in training or thermal impulse, this finding was unexpected but could be explained via programme engagement. That is, those with larger reductions in heart rate or RPE across the programme provides an apparent indication of adaptation to the training. It should also be considered that the $\dot{V}O_{2max}$ values and associated power outputs were measured in thermoneutral conditions and will also transiently decrease during the programme, which has been reported to reflect a cumulative fatiguing effect within the HA period (Waldron et al., 2019). This means that the combined effect of an in-programme temporary reduction in fitness, mixed with the reduced absolute ceiling value of $\dot{V}O_{2max}$ when exercising in hotter conditions for the HA programme, is likely to set the relative intensity of $\dot{V}O_{2max}$ to a higher threshold than stated among participants. Therefore, the loss in impulse (in absolute terms) is probably less than considered and would have a smaller impact across shorter HA programmes. In summary, while relative changes in the responses to the programme were not contributing factors, the appearance of negative changes in impulse might not necessarily mean that the training or thermal stimulus is insufficient for $\dot{V}O_{2max}$ changes and could be considered as a way of monitoring engagement with the HA programme when using an iso-intensity model.

Monitoring participants' responses to HA programmes via thermal sensation and daily body mass lost (WBSR) during exercise, significantly contributed to the $\dot{V}O_{2max}$ outcome. Body mass losses are easily measured in the laboratory or field and indicate the approximate sweating response to the heat-exercise combination. This is largely driven by increased heat production, which could have been a factor among those of larger body mass, who typically demonstrate greater absolute $\dot{V}O_2$ (i.e. metabolic heat production), despite a fixed relative intensity (Havenith et al., 1998). This could partly explain the contribution of baseline body mass to the current model, whereby heavier individuals produce greater absolute metabolic heat, thus providing a greater adaptation stimulus. Whilst those of smaller body mass typically demonstrate greater increases in core temperature compared to heavier individuals, this is achieved only when exercising at fixed absolute heat production (Cramer & Jay, 2015). This level of control was not provided in the current study. However, there was no contribution of mean core temperature responses to the current prediction model, perhaps owing to the homogenous values between participants; therefore, the role of body mass in $\dot{V}O_{2max}$ adaption is currently uncertain.

Surprisingly, PV expansion was not a significant contributor to the current model, despite positively associating with the change in $\dot{V}O_{2max}$, in both previous studies and non-significantly herein. The hypervolemic effect of HA and its relationship to $\dot{V}O_{2max}$ have been reported previously (Lorenzo et al., 2010), but the current data indicate that its capacity to predict the change in $\dot{V}O_{2max}$ was relatively less important amongst the accompanying variables. The thermal load imposed during the HA programme appears to partly explain the degree to which PV expands (Aoyagi et al., 1997) and this could lead to transient (rather than sustained) PV inflations when using iso-intensity models. This could have affected the relative predictive capacity of PV expansion here, but it remains clear that this is an important haematological response to HA, which might provide an initial stimulus for subsequent compensatory responses in Hb mass, as reported after ~5-weeks of iso-intensity HA in cyclists (Oberholzer et al., 2019; Rønnestad et al., 2022, 2021). Of course, a number of peripheral skeletal muscle adaptations might also support complete adaptation in $\dot{V}O_{2max}$ and are worthy of investigation in future studies.

5. Conclusion

Iso-intensity HA programmes improved thermoneutral VO_{2max} 63% of the time, with the analysis in the current study revealing four programme-based parameters – exercise intensity (% VO_{2max}), ambient temperature, number of HA training days and total exposure time – as important considerations when designing HA programmes to maximise this outcome. The most important consideration for optimising VO_{2max} adaptation following HA was a delay in post-testing, with a greater number of days associated with higher VO_{2max} increase, supporting the notion that HA should finish ~96-h prior to competition if this outcome is deemed to be a determinant of performance. The analysis also revealed that monitoring of thermal sensation and whole-body mass loss can provide an indication of adaptation for practitioners to potentially predict the degree to which VO_{2max} improves post-HA and increase the likelihood of optimal benefits from the programme.

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