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Inter-individual variation in the adaptive response to heat acclimation**Running Head:** variability in heat acclimation response**Original Investigation**

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ABSTRACT

Aim: To investigate inter-individual variance in adaptive responses to heat acclimation (HA).

Methods: 17 males ($\text{VO}_{2\text{max}}=58.8(8.4) \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) undertook 10-days (exercise + heat-stress [40°C, 50%RH]) HA. Adaptation was assessed by heat stress tests (HST; 60-minutes cycling, 35% peak power output) pre- and post-HA. **Results:** Inter-individual variability was

evident in adaptive responses e.g. mean(range) reduction in end-exercise $T_{\text{re}}=-0.70(-0.20 \text{ to } -1.32)^{\circ}\text{C}$, but, in the main, the variance in adaptation was unrelated across indices (thermal, sudomotor, cardiovascular, haematological), indicating independence between adaptation indices. Variance in adaptive responses was not correlated with aerobic capacity, history of previous HA, or the accrued thermal-dose. Some responses to the initial HST were related to the subsequent adaptations e.g. $\Delta\bar{T}_{\text{sk}}$ during the initial HST and the reduction in the within HST ΔT_{re} after HA ($r=-0.676$), but responses to the initial HST may also have been influenced by HST design e.g. ΔT_{re} correlated with metabolic heat production ($r=0.609$). Metabolic heat production also correlated with the reduction in the within HST ΔT_{re} after HA ($r=-0.514$). **Summary:** HA indices are mainly independent; ‘low’, or ‘high’, responders on one index do not necessarily demonstrate similar response across other indices. Variance in HA responses was not related to aerobic capacity, previous HA, or thermal-dose. Thermo-physiological responses to a HST might identify individuals who will benefit from HA. However, some initial responses are influenced by HST design, which may also affect the scope for demonstrating adaption. **Conclusion:** Variance in the HA response remains largely unaccounted for and future studies should identify factors contributing to this variance.

KEYWORDS: Acclimatization; adaptation; thermal; responders; non-responders

HIGHLIGHTS

- Although we demonstrated pronounced inter-participant variance in the adaptive response to heat, this was not explained by factors that have putatively been suggested to influence this response, such as maximal aerobic capacity, previous heat acclimation, or the thermal ‘dose’ accrued during the heat intervention.
- Classification of individuals as either ‘low’ or ‘high’ responders to heat may not be appropriate. Acclimation indices appear to be largely independent and individuals demonstrating a pronounced, or blunted, adaptive response on one index of acclimation do not necessarily demonstrate a similar response across other indices.
- Some of the thermo-physiological responses to an initial heat stress test undertaken before a programme of heat acclimation were related to the magnitude of subsequent adaptation, suggesting that this type of test may have utility in assessing baseline ‘heat-readiness’, as well as in identifying individuals who will most benefit from heat acclimation.
- Some of the initial responses may have been influenced by the heat stress test design, which could also affect the scope for demonstrating adaption, although most of the variance in the adaptive response remained unaccounted for.

1.0 INTRODUCTION

At the cohort level, the typical heat acclimation (HA) response is well characterised (for recent reviews see: Daanen *et al.*, 2018; Périard *et al.*, 2015; Tyler *et al.*, 2016). Broadly speaking, the heat-adapted phenotype is characterised by hypervolemia (Senay *et al.*, 1976), an increased sudomotor response (Nadel *et al.*, 1974), and reduced heart rate, rectal temperature (T_{re}), and mean body temperature ($\bar{T}_b$) during exercise at a given external work rate in the heat (Neal *et al.*, 2016b; Rendell *et al.*, 2017). However, whilst there is consistency between studies when the adaptive response to heat is viewed at the cohort level, where individual data are presented considerable heterogeneity is evident. For instance, Senay *et al.* (1976) demonstrated a typical group response for the plasma volume increase to a 10-day HA programme, yet the individual data show the final plasma volume expansion ranged from ~8 to 33%. In a related paper large variations in the reduction in exercise heart rate (~-2 to -32 beats·minute⁻¹) and T_{re} (~-0.3 to -1.2°C) were evident following the same 10-day HA programme (Wyndham *et al.*, 1976). Heterogeneity has also been demonstrated in the sudomotor adaptation (sweating rate) following HA (Mitchell *et al.*, 1976). These observations are consistent with later work by Racinais *et al.* (2012) who also noted high inter-individual variation in the adaptive response to a 6-day heat acclimatization programme (e.g. change in (Δ) plasma volume of -10 to + 20%) with apparent ‘responders’ and ‘non-responders’; similar findings were also reported by Racinais *et al.* (2014) following a 2-week acclimatization intervention. Although the variability reported by Racinais *et al.*, (2012 and 2014) might be attributable to the greater complexity of natural acclimatization compared to laboratory protocols (Edholm, 1966), recent research using a standard 10-day laboratory HA intervention also demonstrated a broad spectrum of adaptive responses to HA (Neal *et al.*, 2016b; Rendell *et al.*, 2017). Interestingly, it is unknown whether the response profile is consistent across HA indices, that is, whether individuals who have a pronounced, or

conversely low, adaptive response for a given index of HA, demonstrate the same response across the range of HA indices. However, cardiovascular changes can occur in the absence of significant alterations in plasma volume (Garrett *et al.*, 2009; Neal *et al.*, 2016a) and reductions in T_{re} and $\bar{T}_b$ post-HA have also been reported without plasma volume changes (Neal *et al.*, 2016a), whereas the plateau in the T_{re} adaptation during HA may precede pronounced sudomotor adaptation (Périard *et al.*, 2015). Given the apparent independence between some aspects of the HA response, it might be anticipated that the magnitude of response is specific to the HA index.

Understanding the basis for heterogeneity in the HA response has important practical utility for the screening and identification of individuals who will most benefit from undertaking HA, in optimising the HA process, and in identifying those individuals best suited to performing in a hot environment or those at increased risk of an adverse response e.g. poor heat tolerance (Epstein, 1990). The need to increase understanding of the factors underpinning the inter-individual variability in the HA response was highlighted as a priority in a 2012 International Olympic Committee consensus statement (Bergeron *et al.*, 2012), yet little subsequent progress has been made. Historic work suggests that a dose-response relationship between heat exposure and the magnitude of the adaptive response underpins some of the variability in the HA response (Fox *et al.*, 1963; Lind & Bass, 1963) although there may be a ceiling-effect for thermal ‘dose’ given that elevating T_{re} beyond 38.5°C during a HA intervention does not confer any additional benefit (Gibson *et al.*, 2015). Moreover, there is some evidence to suggest that individuals with a high maximal aerobic capacity (VO_{2max}) may be partially heat acclimated (Ravanelli *et al.*, 2018; Shvartz *et al.*, 1977), probably by virtue of some of their training adaption (e.g. hypervolemia) as well as through the high thermal-strain that can be elicited through their habitual exercise at high absolute

exercise intensities under temperate conditions (Ely *et al.*, 2009). Similarly, individuals with a high $\text{VO}_{2\text{max}}$ may acclimate more rapidly than individual with a lower $\text{VO}_{2\text{max}}$ (Pandolf *et al.*, 1977), whereas meta-analytic data suggests that the process of re-acclimation is more rapid than initial HA (Daanen *et al.*, 2018) and animal models have provided evidence of a HA memory, at least in terms of cytoprotection (Horowitz, 2016). Finally, whilst the roles of $\text{VO}_{2\text{max}}$ and anthropometric factors have historically been emphasised in explaining the thermophysiological responses to exercise in the heat, recent work has demonstrated that the metabolic heat production (H_{prod}) explained the largest amount of the inter-individual variance in the ΔT_{re} whereas the evaporative requirement for heat balance (E_{req}) explained the largest amount of variance in sweating rate (Cramer and Jay, 2015). However, there has, historically, been no attempt to standardise these parameters in protocols for assessing HA (e.g. Garrett *et al.*, 2009; Gibson *et al.*, 2015; Neal *et al.*, 2016ab; Pandolf *et al.*, 1977; Rendell *et al.*, 2017; Senay *et al.*, 1976; Shvartz *et al.*, 1977) raising the possibility that the design of the heat stress test (HST) might contribute to the variance observed in the response to the initial baseline assessment of acclimation state. Likewise, it might be anticipated that a large increase in T_{re} during the HST prior to HA, which might be influenced by H_{prod} rather than acclimation state *per se*, could provide the greatest scope for demonstrating an adaptive response thereafter.

Accordingly, the primary aim of the present study was to examine the putative factors underpinning the observed variance in the adaptive response to a standard HA intervention. We hypothesised that $\text{VO}_{2\text{max}}$, a history of previous HA, the thermal dosage experience during the HA intervention, and the baseline response to a standard HST, would be significant contributors to the variance in the HA response. We also investigated the extent to which the inter-individual variability in the magnitude of adaptive response to heat was

consistent across adaption indices, that is, whether individuals who have a pronounced, or conversely low, adaptive response on one index of HA demonstrate a similar response across other indices of HA, or whether the magnitude of adaptive response is specific to the index of adaption. Based upon the apparent independence between some indices of HA we hypothesised that the response profile would be non-uniform. Finally, we investigated the factors influencing the thermo-physiological responses to the initial HST, and whether this influenced the subsequent adaptive response to a HA intervention. Our hypothesis was that the highest T_{re} and whole-body sweating rates (WBSR) in the initial HST would be observed in those individuals with the highest H_{prod} and E_{req} , respectively, and that these high baseline responses would provide greater scope for evidencing adaptation.

2.0 METHODS

2.1 Participants

Seventeen trained males participated (Mean(SD) age: 22(5) years; height: 1.81(0.05) m; mass: 74.4(6.3) kg; body surface area (BSA, Dubois and Dubois, 1916) 1.94(0.10) m²; VO_{2max} : 58.8(8.4) mL·kg⁻¹·min⁻¹). These data were pooled from previously published studies (Neal *et al.*, 2016b; Rendell *et al.*, 2017). The studies received ethical approval from the Universities Science Faculty ethics committee and were conducted in accordance with the Declaration of Helsinki (2013). All participants completed a health history questionnaire and provided written informed consent.

2.2 Experimental design

A within-participant repeated-measures design was employed. All participants undertook a preliminary graded exercise test (GXT) under temperate ambient conditions (target ambient conditions: 22°C; 50%RH) in the seven day period prior to commencing the HA intervention.

Thereafter, participants undertook 11 consecutive days of exercise-heat exposures (target ambient conditions: 40°C; 50%RH). The first, sixth and eleventh day consisted of a standardised exercise HST for assessing the HA responses; the other days consisted of exercise-heat exposures using the controlled hyperthermia (CH) approach. Nine of the participants had previously undertaken a heat acclimation programme (3 to 18 months washout).

2.3 Experimental procedures

2.3.1 Graded Exercise Test

Participants initially cycled (Excalibur, Lode, The Netherlands) at 85-110 W, dependent upon the estimated fitness of the participant. After 20 minutes work-rate was incremented by 25 W every 3 minutes until fingertip capillary blood lactate concentration [Lac] was $\geq 4 \text{ mmol}\cdot\text{L}^{-1}$ (Biosen C-line, EKF Diagnostic, Cardiff, UK). Thereafter, following a five-minute recovery period, the participant cycled at 100 W for five minutes, before work-rate was increased by $25 \text{ W}\cdot\text{min}^{-1}$ until volitional exhaustion. $\dot{\text{V}}\text{O}_{2\text{max}}$ was defined as the highest 15 s $\dot{\text{V}}\text{O}_2$.

2.3.2 Exercise-Heat Stress Test

Participants cycled on a calibrated CompuTrainer cycle ergometer (RacerMate Inc., Seattle, Washington, USA) for 60 minutes at 35% of the GXT peak power. All HSTs were completed at the same time of day, within-participant.

2.3.3 Controlled Hyperthermia

Participants self-selected their initial work rate on the Computrainer cycle ergometer in order to elicit a target rating of perceived exertion (RPE [Borg, 1982]) of 15. This was maintained until $T_{\text{re}}=38.3^\circ\text{C}$, at which point external power output and convective cooling ($\sim 2\text{-}3 \text{ m}\cdot\text{s}^{-1}$)

were adjusted as appropriate to maintain the target T_{re} (38.5-38.7°C). Convective cooling was manipulated to facilitate the exercise component and provide some perceptual benefit, whilst maintaining a high mean skin temperature. The total exercise-heat exposure was 90-minutes per session. The time each individual spent with a $T_{re} > 38.5^{\circ}\text{C}$ during the CH sessions was used as an index of the thermal ‘dose’ accrued during the HA intervention as used previously (Zurawlew *et al.*, 2016).

2.4 General procedures

Participants wore the same clothes (shorts, undergarments, shoes) each day, abstained from alcohol throughout the experimental period and caffeine for 12 hours before exercise, and were instructed to consume a similar diet before each test and drink 500 mL of water 2 hours before every attendance. Participants were instructed to maintain their normal high-intensity training (except 24 hours before HSTs or GXTs) and replace an equivalent duration of low/moderate training with that completed in the laboratory to maintain usual training volume. To estimate WBSR, nude body mass was measured immediately before and after every exercise session (Industrial Electronic Weight Indicator, Model I10, Ohaus Corporation, Parsippany, New Jersey, USA), having adjusted for fluid consumption. During HST and controlled hyperthermia sessions 250 mL boluses of 3.6% carbohydrate solution (drink temperature 20°C) were ingested, immediately prior to commencing exercise and every 15 minutes thereafter. After every exercise session, participants were encouraged to drink *ad libitum* to ensure similar hydration for each of the following days.

Ambient conditions were measured by a wet-bulb globe temperature (WBGT) logger (Squirrel 1000, Grant Instruments, Cambridge, UK), T_{re} by a thermistor (Grant Instruments, Cambridge, UK) self-inserted approximately 15 cm beyond the anal sphincter and cardiac

frequency (f_c) by short range telemetry (Polar RS800, Polar Elector, Kempele, Finland). Participants were withdrawn from a session if $T_{re} > 40^\circ\text{C}$. During HSTs and GXTs, skin temperature (T_{sk}) was measured using thermistors on the chest, biceps, thigh and calf (Grant Instruments, Cambridge, UK). During HSTs expired gases (Douglas bag method) were measured at 15 minute intervals. VO_2 was measured breath-by-breath throughout the GXTs (Quark B2, COSMED, Rome, Italy).

Before and after HSTs 10 mL venous blood samples were obtained from the antecubital vein for the triplicate measurement of haemoglobin concentration [Hb] (201⁺ HemoCue, Sweden) and haematocrit (Hct) (Hawksley, England). Blood volume changes were determined according to Dill and Costill (1974).

2.5 Data analysis

Mean skin ($\bar{T}_{sk}$) calculated according to Ramanathan (1964) with $\bar{T}_b$ calculated using a two-compartment model (Jay *et al.*, 2007). H_{prod} was calculated according to ISO 8896 (Malchaire, 2004). The rate of dry heat exchange (H_{dry}) was calculated as:

$$H_{dry} = C + R \text{ (W/m}^2\text{)}$$

$$C = h_c (\bar{T}_{sk} - T_a) \text{ (W/m}^2\text{)}$$

$$R = h_r (\bar{T}_{sk} - T_r) \text{ (W/m}^2\text{)}$$

C and R represent convective and radiant heat exchange, respectively, T_a and $\bar{T}_{sk}$ denote ambient and mean skin temperatures ($^\circ\text{C}$), respectively, T_r is the mean radiant temperature ($^\circ\text{C}$), assumed to be equivalent to ambient temperature in the laboratory setting, h_c is the convective heat transfer coefficient, and h_r is the radiant heat transfer coefficient:

$$h_c = 8.3 v^{0.6} \text{ (W}\cdot\text{m}^{-2}\cdot\text{K}^{-1}\text{)}$$

$$h_r = 4\epsilon\sigma (BSA_r/BSA) ((\bar{T}_{sk} + T_r) / 2 + 273.15)^3 \text{ (W}\cdot\text{m}^{-2}\cdot\text{K}^{-1}\text{)}$$

Where: v is air velocity ($\text{m}\cdot\text{s}^{-1}$), ϵ is skin emissivity (0.95), σ is the Stefan-Boltzmann constant ($5.67\cdot 10^{-8} \text{ W}\cdot\text{m}^{-2}\cdot\text{K}^{-4}$), and BSA_r/BSA is the non-dimensional effective radiant surface area for a seated individual valued at 0.70.

Respiratory heat exchange (H_{resp}) was calculated as:

$$E_{\text{res}} + C_{\text{res}} = 0.0173(H_{\text{prod}})(5.87 - P_a) + 0.0014(H_{\text{prod}})(34 - T_a) \text{ (W/m}^2\text{)}$$

Where: E_{res} and C_{res} are evaporative and convective heat loss from the respiratory tract, respectively, and P_a is the ambient vapor pressure (kPa).

The rate of evaporation required for heat balance (E_{req}) was expressed as:

$$E_{\text{req}} = H_{\text{prod}} - H_{\text{dry}} - H_{\text{resp}} \text{ (W/m}^2\text{)}$$

The maximum rate of evaporation to the environment (E_{max}) was determined by:

$$E_{\text{max}} = h_e (P_{\text{sk},s} - P_a) \text{ (W/m}^2\text{)}$$

where h_e is the evaporative heat transfer coefficient, calculated as the product of h_c and the Lewis relation coefficient (16.5 K/kPa), and $P_{\text{sk},s} - P_a$ is the skin-air vapor pressure gradient

The value of $P_{\text{sk},s}$ was calculated based on $\bar{T}_{\text{sk}}$ using Antoine's equation:

$$P_{\text{sk},s} = 10 \cdot \exp [18.956 - 4.030.18/(\bar{T}_{\text{sk}} + 235)] \text{ (kPa)}$$

As per convention, heat balance parameters were calculated in W/m^2 ; however, these values are expressed in W or W/kg^{-1} where appropriate.

2.6 Statistical Analysis

Heat acclimation was assessed using the data obtained from the pre vs. post HA HSTs conducted on day 1 and day 11 of the HA intervention. A range of indices were used to assess HA including: thermal (end-exercise T_{re} and $\bar{T}_b$, the within HST ΔT_{re} and $\Delta \bar{T}_b$), cardiovascular (average exercise heart rate), sudomotor (WBSR), and haematological (Δ blood volume). Data are expressed as mean(SD) unless otherwise stated. To identify the factors influencing the thermo-physiological responses to the initial HST we used an approach similar to that described by Cramer and Jay (2015). Statistical analyses were undertaken using SPSS (IBM Version. 22, IBM, New York, New York, USA) with alpha set *a priori* as ≤ 0.05 . Strength of relationship between variables was assessed using Pearson's product-moment correlation. Correlation coefficients were considered as strong (≥ 0.60), moderate (0.40 to 0.59), and weak (0.20 to 0.39) (Cohen, 1998). Within-individuals differences were assessed by paired samples t-test. Between-individuals differences were assessed by independent samples t-tests.

3.0 RESULTS

At the cohort level, a clear adaptive response was evident following the HA programme, as evidenced by significant reductions in the mean(SD) end-exercise T_{re} and $\bar{T}_b$, a reduced within HST ΔT_{re} and $\Delta \bar{T}_b$, a lower average exercise heart rate, increased WBSR and pronounced hypervolemia. However, inspection of the individual responses revealed notable inter-participant variation in the range of adaptive responses (Table 1).

	Pre HA Mean(SD) min:max	Post HA Mean(SD) min:max	Pre-post HA change Mean(SD) min:max
End exercise T_{re} ($^{\circ}\text{C}$)	38.79(0.43) 38.18:39.74	38.09(0.40)*** 36.98:38.71	-0.70 (0.34) -0.20:-1.32
ΔT_{re} ($^{\circ}\text{C}$)	1.53(0.53) 0.78:2.34	1.23(0.44)** 0.37:2.17	-0.30(0.32) 0.47:-0.71
End Exercise $\bar{T}_b$ ($^{\circ}\text{C}$)	38.65(0.46) 38.02:39.65	37.86(0.35)*** 37.15:38.62	-0.79(0.29) -0.35:-1.25
$\Delta \bar{T}_b$ ($^{\circ}\text{C}$)^a	1.51(0.45) 0.82:2.16	1.13(0.33)*** 0.50:1.71	-0.38(0.27) 0.27:-0.79
Whole body sweat rate ($\text{L}\cdot\text{hr}^{-1}$)	1.45(0.33) 1.09:2.22	1.79(0.49)*** 1.15:2.89	0.34(0.29) 0.02:1.03
Blood volume (%)	100.0(0.0) 100.0:100.0	106.5(2.8)*** 102.0:112.9	6.5(2.8) 2.0:12.9
Average heart rate (beats$\cdot$minute^{-1})^a	150(11) 135:174	129(8)*** 120:144	-21(5) -12:-29

Table 1: Effect of heat acclimation (HA) on thermophysiological indices measured during a standard heat stress test undertaken before and after heat acclimation (n=17, except ^a where n=16).

Significant difference from pre HA is denoted by: ** = $P < 0.01$; *** = $P < 0.001$.

The inter-participant range for $\text{VO}_{2\text{max}}$, expressed in absolute terms, was 3.49 to 5.05 $\text{L}\cdot\text{min}^{-1}$. Absolute $\text{VO}_{2\text{max}}$ was not related to the magnitude of reduction in end-exercise T_{re} ($P = 0.930$) or $\bar{T}_b$, ($P = 0.785$), the reduction in the within HST ΔT_{re} ($P = 0.722$) or $\Delta \bar{T}_b$ ($P = 0.714$), the

increase in WBSR ($P = 0.405$) or blood volume ($P = 0.410$) or the reduction in average heart rate ($P = 0.086$) following the HA intervention. The inter-participant range for $\text{VO}_{2\text{max}}$, expressed in relative terms was 45.2 to 74.6 $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$. The individual relative $\text{VO}_{2\text{max}}$ was not related to the magnitude of reduction in end-exercise T_{re} ($P = 0.947$) or $\bar{T}_{\text{b}}$ ($P = 0.686$) the reduction in the within HST ΔT_{re} ($P = 0.852$) or $\Delta \bar{T}_{\text{b}}$ ($P = 0.868$), the increase in WBSR ($P = 0.252$) or blood volume ($P = 0.381$), or the reduction in average heart rate ($P = 0.089$) following the HA intervention.

Nine of the participants had undergone a prior HA intervention before participating in the present study. Independent samples t-test indicated that prior experience of heat acclimation did not affect the reduction in end-exercise T_{re} (prior heat exposure = $-0.32(0.34)^{\circ}\text{C}$ vs. no prior exposure = $-0.40(0.19)^{\circ}\text{C}$, $P = 0.555$) or $\bar{T}_{\text{b}}$ (prior heat exposure = $-0.83(0.25)^{\circ}\text{C}$ vs. no prior exposure = $-0.74(0.33)^{\circ}\text{C}$, $P = 0.566$), the reduction in the within HST ΔT_{re} (prior heat exposure = $-0.39(0.22)^{\circ}\text{C}$ vs. no prior exposure = $-0.19(0.39)^{\circ}\text{C}$, $P = 0.194$) or $\Delta \bar{T}_{\text{b}}$ (prior heat exposure = $-0.39(0.19)^{\circ}\text{C}$ vs. no prior exposure = $-0.38(0.36)^{\circ}\text{C}$, $P = 0.980$), increase in WBSR (prior heat exposure = $0.25(0.18) \text{ L}\cdot\text{hr}^{-1}$ vs. no prior exposure = $0.45(0.36) \text{ L}\cdot\text{hr}^{-1}$, $P = 0.158$) and blood volume (prior heat exposure = $6.9(3.1)\%$ vs. no prior exposure = $6.1(2.6)\%$, $P = 0.581$) or the reduction in average exercise heart rate (prior heat exposure = $-15(8) \text{ beats}\cdot\text{min}^{-1}$ vs. no prior exposure = $-11(6) \text{ beats}\cdot\text{min}^{-1}$, $P = 0.264$).

The total time individual spent in CH sessions with a $T_{\text{re}} > 38.5^{\circ}\text{C}$ was 456(64) minutes (range 326:552 minutes). Total time spent with a $T_{\text{re}} > 38.5^{\circ}\text{C}$ was not significantly correlated with any of the adaption indices. Likewise, the average external work rate sustained during each CH session, expressed either in absolute ($101(16) \text{ W}$, range 69:130 W) or relative terms ($1.37(0.28) \text{ W}\cdot\text{kg}^{-1}$, range 0.86:1.99 $\text{W}\cdot\text{kg}^{-1}$) was not correlated with the reduction in the end-

exercise T_{re} ($P = 0.986$, $P = 0.939$, respectively), end-exercise $\bar{T}_b$ ($P = 0.489$, $P = 0.888$, respectively), the within session ΔT_{re} ($P = 0.614$, $P = 0.981$, respectively), $\Delta \bar{T}_b$ ($P = 0.718$, $P = 0.620$, respectively), Δ blood volume ($P = 0.726$, $P = 0.344$, respectively) and the reduction in average exercise heart rate ($P = 0.077$, $P = 0.068$, respectively). However, there was a significant moderate negative relationship between the average absolute power sustained in each CH session and the increase in WBSR ($r = -0.530$, $P = 0.029$), but the relative power sustained in each CH session was not significantly related to WBSR ($P = 0.054$).

The baseline responses to the pre-HA HST were correlated with a number of the adaption indices (Figure 1 a-f). The reduction in end-exercise T_{re} following HA was correlated with the pre-HA HST end-exercise T_{re} ($r = -0.490$, $P = 0.046$) and the baseline [Hb] ($r = 0.550$, $P = 0.022$). The reduction in the within session ΔT_{re} following HA was correlated with the end exercise $\bar{T}_{sk}$ ($r = -0.529$, $P = 0.029$), $\Delta \bar{T}_{sk}$ ($r = -0.676$, $P = 0.004$) and $\Delta \bar{T}_b$ ($r = -0.526$, $P = 0.036$) in the pre-HA HST. The reduction in end-exercise $\bar{T}_b$ following HA was correlated with end-exercise T_{re} ($r = -0.638$, $p = 0.006$), $\bar{T}_{sk}$ ($r = -0.527$, $P = 0.030$), $\bar{T}_b$ ($r = -0.646$, $P = 0.005$) and the within session ΔT_{re} ($r = -0.660$, $P = 0.004$), $\Delta \bar{T}_{sk}$ ($r = -0.573$, $P = 0.020$) and $\Delta \bar{T}_b$ ($r = -0.706$, $P = 0.002$) in the pre-HA HST. The reduction in the within session $\Delta \bar{T}_b$ following HA was correlated with the end-exercise $\bar{T}_{sk}$ ($r = -0.679$, $P = 0.004$) and $\bar{T}_b$ ($r = -0.600$, $P = 0.014$) and the ΔT_{re} ($r = -0.514$, $P = 0.042$), $\Delta \bar{T}_{sk}$ ($r = -0.827$, $P < 0.001$), $\Delta \bar{T}_b$ ($r = -0.697$, $P = 0.003$), in the pre-HA HST. The increase in WBSR following HA was correlated with the average exercise $\bar{T}_{sk}$ in the pre-HA HST ($r = -0.565$, $P = 0.018$), whereas the decrease in average exercise heart rate following HA was correlated with the average ($r = -0.713$, $P = 0.002$) and end-exercise ($r = -0.757$, $P = 0.001$) heart rate in the pre-HA HST. The increase in blood volume following HA was not related to any of the variables measured in the pre-HA HST.

To examine the specificity of the adaptive response *i.e.* whether those having a pronounced, or more limited, response for one adaption index also demonstrated a similar response for other indices of HA, correlation analysis were performed between the thermal indices of adaption (end-exercise T_{re} and $\bar{T}_b$, within HST ΔT_{re} and $\Delta \bar{T}_b$) and the thermoregulatory (WBSR), haematological (Δ blood volume) and cardiovascular (average exercise heart rate) indices of adaption. This analysis indicated that the magnitude of increase in WBSR following HA was moderately related to the magnitude of the reduction in the within HST ΔT_{re} ($r = 0.487$, $P = 0.048$), but there were no other significant relationships between the indices of adaption.

Our analysis of the factors influencing the thermo-physiological responses to the initial HST (figure 2 a-d) demonstrated that the absolute H_{prod} (596(56) W, range 509:738 W) was strongly correlated with the within HST ΔT_{re} ($r = 0.609$, $P = 0.009$) and moderately correlated with the within HST session $\Delta \bar{T}_b$ ($r = 0.523$, $P = 0.038$) and WBSR ($r = 0.525$, $P = 0.030$). The relative H_{prod} ($8.1(0.9)$ W·kg⁻¹, range 6.7:10.2 W·kg⁻¹) was moderately correlated with the end exercise T_{re} ($r = 0.508$, $P = 0.037$) and the within HST ΔT_{re} ($r = 0.584$, $P = 0.014$). E_{req} ($r = 0.685$, $P = 0.002$) and E_{req}/E_{max} ($r = 0.669$, $P = 0.003$) were strongly correlated with WBSR. Thereafter, we investigated whether those variables identified as being significantly related to our indices of acclimation in the initial HST were also related to the subsequent magnitude of adaptive response for that parameter. This analysis demonstrated a moderate negative correlation between the reduction in the within session ΔT_{re} following the HA intervention and the absolute H_{prod} in the initial HST ($r = -0.514$, $P = 0.035$), but there were no further significant correlations.

4.0 DISCUSSION

Our findings demonstrate that the individual-variation in the adaptive responses to the 10-day HA intervention was not related to baseline $\text{VO}_{2\text{max}}$, previous exposure to a HA intervention, or the thermal ‘dose’ accrued during the HA intervention. In addition, there was limited evidence for strong relationships between the various indices of acclimation, indicating that the characterising of individuals as ‘high’, or ‘low’, responders to HA should be done so with reference to specific indices of HA, rather than as a ‘global’ classification. Importantly, some of the thermo-physiological responses during the initial HST were related to the magnitude of subsequent adaptive responses to the HA intervention, which suggests that some of these baseline responses may be useful in estimating the potential benefits that an individual may obtain from HA. However, we urge some caution, because the T_{re} and WBSR responses during the initial HST were also related to the inter-participant differences in H_{prod} and E_{req} , indicating that the design of the HST may also influence some of these initial thermo-physiological responses. Moreover, the inter-participant differences in H_{prod} during the initial HST were also related to the reduction in the within session ΔT_{re} following HA, indicating that the design of the HST may have influenced the scope for demonstrating adaption subsequently.

It has often been suggested that the adaptive response to heat is augmented in those with a high $\text{VO}_{2\text{max}}$ (e.g. Armstrong and Maresh, 1991; Casadio *et al.*, 2017), although closer inspection of the extant literature suggests that this assertion is based on a limited number of observations (Pandolf *et al.*, 1977). Likewise, it has been proposed that individuals with a high $\text{VO}_{2\text{max}}$ are partially heat acclimated compared to those with lower $\text{VO}_{2\text{max}}$ (e.g. Aoyagi *et al.*, 1997; Shvartz *et al.*, 1977). However, the present study has shown that baseline $\text{VO}_{2\text{max}}$ (absolute or relative) was not related to the initial thermo-physiological responses to exercise

in the heat, nor to the magnitude of the adaptive responses following the HA intervention. The reasons for these discrepant findings are unclear, although at the genomic level, transcriptome profile data from rodent models has shown that heat and exercise each induce specific transcriptional programmes (Kodesh *et al.*, 2011). Alternatively because, both the baseline $\text{VO}_{2\text{max}}$ and the adaptive response of $\text{VO}_{2\text{max}}$ to training have a considerable genetic component (Bouchard *et al.*, 2011a,b) the use of $\text{VO}_{2\text{max}}$ as surrogate of training level and by extension the extent to which elevated thermal strain is encountered through habitual training will, at best, provide a crude estimate. Future studies investigating this topic should consider analyses of in-depth training data, rather than relying on measurement of $\text{VO}_{2\text{max}}$ as a surrogate of training level. Importantly, our observations have practical relevance; irrespective of baseline $\text{VO}_{2\text{max}}$, individuals required to exercise in high ambient temperatures should consider undertaking a HA intervention and those with a high $\text{VO}_{2\text{max}}$ should not consider themselves partially heat-acclimated. Indeed, the belief that a $\text{VO}_{2\text{max}}$ confers some HA may, in part, explain the recent report that only 15% of athletes at the 2015 Athletics world Championship employed an HA programme prior to competition (Périard *et al.*, 2017).

We also hypothesised that individuals who had undergone prior HA might demonstrate an augmented acclimatory response. However, our analyses indicated that there were no significant differences in the adaptive response of those individuals who had undertaken a prior HA intervention. This finding is somewhat at odds with data showing that the magnitude of some aspects of the acclimation response are increased with re-acclimation (Saat *et al.*, 2005). Indeed, a recent meta-analysis concluded that the process of re-acclimation to heat was faster than the initial acclimation, at least in terms of reduction in deep body temperature and cardiovascular adaptations (Daanen *et al.*, 2018). Likewise, data from rodent studies has demonstrated the presence of a cellular cytoprotective acclimation

memory (Horowitz, 2016), although the relevance of these observations for the whole-organism acclimation response is not yet clear. Indeed, we were not able to measure aspects of cellular tolerance in the present study and so cannot draw comparisons with Horowitz *et al.* (2016), whilst closer inspection of the meta-analytic data indicates that in many of the primary studies the re-acclimation process took place after a relatively short decay (*e.g.* Saat *et al.*, 2005) and some of the effects are likely due to a baseline influence caused by retention of some of the initial adaptation to HA. Importantly, our data indicate that the baseline HST responses of those who had undergone prior HA were not different from those who were undertaking HA for the first time suggesting that the elapsed period between the acclimation was sufficient to enable full decay.

In the present study we used a controlled hyperthermia HA intervention in which work rate was adjusted in order to maintain a target T_{re} of 38.5°C-38.7°C on each day. In contrast to traditional approaches, which typically use the same daily work-rate (*e.g.* Lind and Bass 1963; Pandolf *et al.*, 1977; Senay *et al.*, 1976) and may, therefore, result in a diminishing thermal forcing-function over the HA intervention, this approach maintains the thermal forcing-function. Whilst our data indicate that the thermal ‘dose’ was well maintained over the course of the HA intervention (no time effect for time $T_{re} > 38.5^{\circ}\text{C}$) there were notable inter-participant differences in the total time accumulated above this thermal threshold. Previous research suggests that the magnitude of adaptive response during HA is diminished when T_{re} is $<38.5^{\circ}\text{C}$ (Fox *et al.*, 1963), but there is no additional benefit when T_{re} is raised to 39.0°C (Gibson *et al.*, 2015). Likewise, Lind and Bass (1963) demonstrate that the adaptive response with 1×100 min daily exercise-heat exposure was greater than 2×50 minute daily exercise-heat exposures, which they attributed to the greater amount of time spent elevating tissue temperature with the multiple exposure protocol, whereas Fox *et al.*, (1963)

demonstrated that the adaptive response was greatest in individuals spending the most time with a T_{re} of $\sim 38.5^{\circ}\text{C}$. In contrast, our data indicate that the indices of acclimation were not related to the time spent with a $T_{re} > 38.5^{\circ}\text{C}$. The reason for this apparently discrepant finding is not clear. However, T_{re} may not be the most appropriate index of thermal strain and $\bar{T}_b$ might represent a better index because it incorporates a measure of central and peripheral tissue temperature, which is important for HA (Regan *et al.*, 1996), whereas the thermoeffector stimulus may be more closely related to other parameters, such as E_{req} (Gagnon *et al.*, 2013).

Inter-individual variability in the adaptive response to heat has led to the suggestion that individuals might be classified as ‘responders’ or ‘non-responders’ to heat (Racinais *et al.*, 2012), as is the case with adaption to other stressors such as altitude (Chapman *et al.*, 1998), or exercise (Bouchard *et al.*, 2011b). However, in some instances this classification has been based upon a single reference parameter, such as plasma volume expansion (Racinais *et al.*, 2012) and when a range of adaptation indices are presented it is unclear whether the response profile is consistent across indices (Racinais *et al.*, 2014). A moderate correlation was demonstrated between the increase in WBSR and the magnitude of the reduction in the within HST ΔT_{re} , however, on the whole, the various aspects of the adaptive response were not correlated. Thus, the adaptive response to heat not only varies between individuals, but also between indices of adaptation. For example, the magnitude of blood volume expansion was not related to the changes in thermal indices of adaptation, WBSR, or exercise heart rate, thus an individual who demonstrates a high adaptive response for blood volume may demonstrate a low adaptive response for sudomotor, cardiovascular or thermal aspects of adaption. The basis for this between-indices variation is unknown, although independence between aspects of the adaptive response to heat has been demonstrated previously (Garrett *et al.*, 2009; Neal

et al., 2016a; Périard *et al.*, 2015). Baseline differences might contribute to some of the variation. For instance, some individuals have a naturally-occurring high blood volume (Martino *et al.*, 2002), which might limit the scope for hypervolemia, with less influence on other indices of adaptations. Alternatively, a low response for a given parameter may simply be a consequence of an insufficient stimulus for adaption for that parameter; recent studies examining heterogeneity in the training response have demonstrated that ‘non-responders’ to a standard exercise training programme demonstrate a training response when the exercise ‘dose’ is increased (Montero and Lundby, 2017).

Some of the physiological responses during the initial HST were related to the magnitude of subsequent adaptive response. For instance, the $\Delta\bar{T}_b$ and $\Delta\bar{T}_{sk}$ recorded in the pre-HA HST was related to the reduction in end-exercise $\bar{T}_b$, and the reduction in the within HST ΔT_{re} and $\Delta\bar{T}_b$ following the HA intervention. Thus, a large increase in $\bar{T}_{sk}$ or $\bar{T}_b$ during a standard HST might be a useful index for assessing baseline HA status and in identifying those individuals who will most benefit from HA. Likewise, the reduction in end exercise T_{re} and $\bar{T}_b$ over the HA intervention was related to the end-exercise T_{re} in the pre-HA HST, indicating that individuals with the greatest end exercise T_{re} in the first HST also had the greatest reduction in end-exercise T_{re} in $\bar{T}_b$ after HA intervention. However, we urge some caution with interpretation of this data. We hypothesised that the highest T_{re} and WBSR in the initial HST would be observed in individuals with the highest H_{prod} and E_{req} , respectively, and that these high baseline responses would provide greater scope for evidencing adaptation. Indeed, our analysis confirmed that H_{prod} (in W or $W \cdot kg^{-1}$) was related to the T_{re} and $\bar{T}_b$ responses during the first HST, whereas the largest amount of variance in WBSR was explained by E_{req} and E_{req}/E_{max} . These finding are consistent with the Cramer and Jay (2015) and indicate that some of the response to the initial HST is determined by protocol design, rather than basal HA

state. Whilst this was not unexpected, most studies of HA do not attempt to standardise heat production during the HST (*e.g.* Garrett *et al.*, 2009; Gibson *et al.*, 2015; Neal *et al.*, 2016ab; Pandolf *et al.*, 1977; Rendell *et al.*, 2017; Senay *et al.*, 1976; Shvartz *et al.*, 1977) because it is typically assumed to be of little relevance for within-participants design so long as the same external work-rates are used post-HA. However, our analysis also demonstrated a moderate negative correlation between the reduction in the within HST ΔT_{re} following HA and the absolute H_{prod} (W) in the initial HST, suggesting that the design of the initial HST may also affect the subsequent response. Although we acknowledge that correlation is not evidence of causality, we propose that this represents a potential baseline effect whereby those demonstrating low-baseline HST response (due to the low H_{prod}) have less scope for evidencing an adaptive response. The precise design of any HST will depend upon the nature of the research question. However, future studies examining the variability in the adaptive response to HA should consider standardisation of H_{prod} when assessing basal acclimation status and the subsequent adaptive responses to a HA programme, particularly when there are differences in participant VO_{2max} . Importantly, none of the other adaptation indices were related to the HST design and the majority of the variance in the HA response remains unaccounted for; subsequent studies should examine the influence of genetic and epigenetic factors on the variability in the HA response.

5.0 Conclusion

At the cohort-level, there was clear evidence of HA following the 10-day HA intervention, but pronounced variation was evident at the individual-level. This inter-participant variation was not related to factors that have putatively been proposed to influence the adaptive response to heat, including VO_{2max} , a history of prior HA, and the thermal ‘dose’ accrued during the HA intervention. The magnitude of adaptive response is, in the main, specific to

the index of adaption; individuals who demonstrate a high, or low, adaptive response on one index of HA do not automatically demonstrate a similar response across the spectrum of HA indices. Some of the thermo-physiological responses during the initial HST were related to the magnitude of subsequent adaptive response, indicating that the initial response to a standard HST may have utility in identifying those individuals who will obtain the greatest adaptations from HA. However, some of the initial thermo-physiological responses may also have been influenced by the design of the HST; ΔT_{re} was strongly related to H_{prod} and WBSR was strongly related to E_{req} . Moreover, the reduction in the within session ΔT_{re} following HA was related to the H_{prod} in the initial HST, indicating that the design of the HST may also have influenced the scope for demonstrating adaption. Nevertheless, the substantial majority of the inter-individual variance in the adaptive response to heat remains unaccounted for and future studies should seek to increase understanding of the factors contributing to this variance.

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8.0 DECLARATION OF INTERESTS

The authors have no conflicts of Interest to Declare

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10 FIGURE CAPTIONS

Figure 1. a) relationship between pre heat acclimation (HA) haemoglobin concentration and the reduction in end-exercise T_{re} following HA; b) relationship between $\Delta \bar{T}_{sk}$ in the pre HA Heat Stress Test (HST) and the reduction in the within HST ΔT_{re} following HA; c) relationship between $\Delta \bar{T}_b$ in the pre HA HST and the reduction in the end-exercise $\bar{T}_b$ following HA; d) relationship between $\Delta \bar{T}_{sk}$ in the pre HA HST and the reduction in the within HST $\Delta \bar{T}_b$ following HA; e) relationship between average exercise $\bar{T}_{sk}$ in the pre HA HST and the increase in whole body sweat rate following HA; f) relationship between end-exercise heart rate in the pre HA HST and the reduction in the average exercise heart rate following HA.

Figure 2. Correlation coefficients for associations between thermoregulatory responses during the initial heat stress test and relevant independent variables: a) end exercise T_{re} ($^{\circ}\text{C}$) = light grey bars, ΔT_{re} ($^{\circ}\text{C}$) = dark grey bars); b) end-exercise $\bar{T}_b$ ($^{\circ}\text{C}$) = light grey bars), $\Delta \bar{T}_b$ ($^{\circ}\text{C}$) = dark grey bars; c) whole body sweat rate ($\text{L}\cdot\text{hr}^{-1}$); d) average exercise heart rate ($\text{beats}\cdot\text{min}^{-1}$). * $P < 0.05$; ** $P < 0.01$. BSA = body surface area; $\text{VO}_{2\text{max}}$ = maximum rate of oxygen uptake; H_{prod} = heat production; E_{req} = evaporative requirement for heat balance; E_{max} = maximum rate of evaporation to the environment; ND = no denomination.

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Dr Jo Corbett is an Associate Head in the Department of Sport and Exercise Science at the University of Portsmouth, UK. He is a member of the Extreme Environments Laboratory, where his research examines the effect of environmental stressors, alone and in combination, on human performance and health.

Dr Rebecca Rendell (née Neal) completed her Ph.D. at the University of Portsmouth in 2017 after receiving her Master's degree in Human and Applied Physiology from King's College London in 2013, and undergraduate degree in Sport and Exercise Science from the University of Birmingham in 2012. Rebecca is now a Lecturer in Exercise Physiology at Bournemouth University and conducts research in the areas of exercise and extreme environmental physiology and sports performance.

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Dr Joseph Costello is a Senior Lecturer in exercise physiology and a member of the Extreme Environments Laboratory at the University of Portsmouth, UK. His research interests are directed towards i) understanding the physiological effects of various stressors (e.g. exercise, extreme environments, clothing) on human performance and ii) establishing evidence-based practice in sport and exercise science through the publication of high quality systematic reviews and meta-analyses.

Prof Mike Tipton is a professor of human & applied physiology with an interest in the physiological and pathophysiological response to extreme environments, and the selection, preparation and protection of those entering such environments