Heat acclimation attenuates physiological strain and the HSP72, but not HSP90 , mRNA response to acute normobaric hypoxia

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G O , G, JA, a o L, a , Ma . Heat acclimation attenuates physiological strain and the HSP72, but not HSP90 , mRNA response to acute normobaric hypoxia. *J A Ph* 119: 889–899, 2015. First published July 23, 2015; doi:10.1152/japplphysiol.00332.2015.—Heat acclimation (HA) attenuates physiological strain in hot conditions via phenotypic and cellular adaptation. The aim of this study was to determine whether HA reduced physiological strain, and heat shock protein (HSP) 72 and HSP90 mRNA responses in acute normobaric hypoxia. Sixteen male participants completed ten 90-min sessions of isothermic HA (40°C/40% relative humidity) or exercise training [control (CON); 20°C/40% relative humidity]. HA or CON were preceded (HYP1) and proceeded (HYP2) by a 30-min normobaric hypoxic exposure [inspired O_2 fraction = 0.12; 10-min rest, 10-min cycling at 40% peak O_2 uptake ($\dot{V}O_{2\,peak}$), 10-min cycling at 65% $\dot{V}O_{2\,peak}$]. HA induced greater rectal temperatures, sweat rate, and heart rates (HR) than CON during the training sessions. HA, but not CON, reduced resting rectal temperatures and resting HR and increased sweat rate and plasma volume. Hemoglobin mass did not change following HA nor CON. HSP72 and HSP90 mRNA increased in response to each HA session, but did not change with CON. HR during HYP2 was lower and O_2 saturation higher at 65% $\dot{V}O_{2\,peak}$ following HA, but not CON. O_2 uptake/HR was greater at rest and 65% $\dot{V}O_{2\,peak}$ in HYP2 following HA, but was unchanged after CON. At rest, the respiratory exchange ratio was reduced during HYP2 following HA, but not CON. The increase in HSP72 mRNA during HYP1 did not occur in HYP2 following HA. In CON, HSP72 mRNA expression was unchanged during HYP1 and HYP2. In HA and CON, increases in HSP90 mRNA during HYP1 were maintained in HYP2. HA reduces physiological strain, and the transcription of HSP72, but not HSP90 mRNA in acute normobaric hypoxia.

altitude; cardiovascular; cross-acclimation; cross-tolerance; heat stress; plasma volume

HYPOXIA INCREASES PHYSIOLOGICAL strain both at rest and during exercise (6), with impairment of exercise performance (72), notably during exercise where aerobic metabolism predominates (3). The physiological advantages and disadvantages of repeated hypoxic/altitude exposures for attenuating the negative effects of hypoxia (2) have been summarized in numerous review articles (20, 46). Altitude/hypoxic training methods are varied, with synergistic interactions between simulated and terrestrial, resting or exercise, and continuous and intermittent exposures, each eliciting different magnitudes of adaptation (46). Irrespective of precise application, hypoxic training requires lengthy durations of exposure over prolonged, repeated periods (typically 14-28 days) for meaningful adaptation (27).

Heat acclimation (HA), and acclimatization interventions, carried out by repeated exercise in hot conditions (58), reproducibly reduce physiological strain in hot and cooler conditions (32, 33, 39). Recent reviews support a novel adaptive pathway whereby HA may reduce physiological strain in hypoxia (14, 56, 73). Mechanistic pathways can be subdivided into cross-acclimation, whereby HA attenuates physiological strain (73) and cross-tolerance, whereby cellular responses to HA provide cytoprotection during hypoxia (14). Acute physiological responses to hypoxia (2) can be used as criteria for validating heat-induced cross-acclimation. HA reduces glycolysis and metabolic rates during exercise (34), with plasma volume (PV) expansion (39, 50) and improved myocardial efficiency (38) preserving cardiac output and skeletal muscle blood flow. Muscle oxygenation is also sustained by HA-induced maintenance of central blood volume (BV) via reductions in the core/skin temperature gradient (58) and enhanced evaporative heat loss (51). Improved temperature and hematological regulation facilitate a leftward shift in the oxyhemoglobin saturation curve (37). HA induces expedient and beneficial adaptations within 5–14 daily sessions, demonstrating a greater efficiency of adaptation compared with altitude/hypoxic interventions (23).

Cross-tolerance has been defined as single or repeated sublethal exposures to a stressor, eliciting a positive adaptive effect to a subsequent exposure to a different stressor (35). The cellular pathway for this shares commonality with those seen within in vivo thermotolerance (47). In this model, cellular thermotolerance accompanies the induction of phenotypic adaptations associated with HA (43, 45). Thermotolerance confers cytoprotection against subsequent thermal exposure (45, 74), principally by changes in heat shock proteins (31). Heat shock proteins facilitate important cellular processes as protein chaperones (19) and anti-apoptotic mediators (1). In particular, increases in the inducible proteins heat shock protein (HSP) A1A (HSP72) and HSPC1 (HSP90) mitigate pathophysiological responses to endogenously stressful stimuli. Both HSP72 and HSP90 augment proportionally to increased cellular stress (increased cellular temperature) in response to ex vivo heat shock (45) and have been implicated as important modulators of the adaptive cellular/molecular response to hypoxiamodo34TD

(56, 65, 66); this suggests a shared signaling pathway. Both HSP72 and HSP90 mRNA and protein responses have been used as a marker for identifying the magnitude of stimuli required to initiate the in vivo stress response (45). However, not all of the HSP72 mRNA transcripts are translated to HSP72 protein increase within peripheral blood mononuclear cells following exercise heat stress in humans (44). Basal heat shock protein measurement provides the optimal indication of the acquired capacity to mitigate disruption to cellular homeostasis due to known increases with acclimation (45). The delayed responsiveness of the protein response (16, 17), compared with the within-session heat shock protein mRNA response (25, 44), emphasizes the benefits of the gene transcript as a primary indicator of the magnitude of the stress stimuli and necessity to signal protein transcription should the stimuli be maintained or repeated. Consequently, the mRNA transcription is appropriate to determine whether the HSP72 and HSP90 responses have been attenuated or mitigated, either in response to reductions in physiological strain, or increased basal protein, ultimately highlighting whether cross-tolerance may have been conferred.

HA has been evidenced in improving oxygen saturation and heart rates (HRs) during hypoxic exercise performance (28), with HA also mitigating increases in HSP72 protein in hypoxia, due largely to increased basal concentrations of HSP72 (37). These data support the existence of cross-acclimation/tolerance (37); however, mechanisms for this interaction are presently unknown (39, 45). The aim of this experiment was to determine whether HA would reduce physiological strain and the HSP72 and HSP90 mRNA responses to an acute hypoxic exposure (at rest and at various exercise intensities) compared with exercise training matched for intensity and duration in temperate conditions. It was hypothesized that HA would reduce physiological strain in hypoxia via cardiovascular and thermoregulatory adaptations and that the heat shock protein response to hypoxia would be reduced following HA.

MATERIAL AND METHOD

Participants. Sixteen healthy men, who completed various forms of exercise training between three and six times per week, were assigned to matched groups to perform 10 days of isothermic HA [age 22.5 ± 3.5 yr, nude body mass (NBM) 74.6 ± 7.9 kg, body surface area 1.95 ± 0.13 m^2, peak oxygen uptake ($\dot{V}O_{2\,peak}$) 4.32 ± 0.68 l/min, 58.5 $ml \cdot kg^{-1} \cdot min^{-1}$], or act as a normothermic exercise control (CON; age 26.0 ± 5.0 yr, NBM 74.6 ± 4.8 kg, body surface area 1.93 ± 0.13 m^2, $\dot{V}O_{2\,peak}$ 4.22 ± 0.62 l/min, 56.6 $ml \cdot kg^{-1} \cdot min^{-1}$). Confounding environmental and pharmacological variables were all controlled in line with previous work in the field (24, 25). Urine osmolality was used to confirm hydration in accordance with established guidelines before each experimental/training session [<700 mosmol/kgH_2O (57)]. This experimental control was not violated for any participant for any experimental/training session. All protocols, procedures, and methods were approved by the institutional ethics committee, with participants completing medical questionnaires and written, informed consent following the principles outlined by the Declaration of Helsinki, as revised in 2013.

Preassessment. Before assessment of $\dot{V}O_{2\,peak}$, anthropometric data were collected with NBM measured using digital scales, precise to 0.01 kg (GFK 150, Adam Equipment, Danbury, CT). $\dot{V}O_{2\,peak}$ (l/min) was determined from an incremental test on a cycle ergometer, which was used for all subsequent trials (Monark e724, Monark AB, Varberg, Sweden), at a starting intensity of 80 W, increasing by 24 W/min, in temperate laboratory conditions [20°C, 40% relative humidity (RH)] at sea level (inspired O_2 fraction = 0.2093). $\dot{V}O_{2\,peak}$ was defined as the highest average $\dot{V}O_2$ obtained in any 30-s period, with $\dot{V}O_{2\,peak}$ more appropriately describing the end point of the test due to an absence of $\dot{V}O_2$ plateau in all participants. The confirmation of $\dot{V}O_{2\,peak}$ was made via the attainment of a HR within 10 beats/min of age-predicted maximum, and respiratory exchange ratio (RER) >1.1 in all participants. Expired metabolic gas was measured using breath-by-breath online gas analysis (Metalyser 3B, Cortex, Leipzig, Germany). HR was recorded continually during all experimental/training sessions by telemetry (Polar Electro Oyo, Kempele, Finland).

Hemoglobin mass. Twenty-four hours before hypoxic exposures, hemoglobin mass (Hb_{mass}; g) was measured. Hb_{mass}, BV (ml), and PV (ml) were determined in accordance with the optimized carbon monoxide (CO)-rebreathing method (59). Participants were seated for 20 min, before being connected to a closed glass spirometer, allowing inspiration of a CO bolus of 1.0 ml/kg (68), followed by 2-min rebreathing of a 3.5-liter O_2 bolus. Before and 4 min after CO rebreathing, participants completely exhaled to residual volume into a CO gas meter (Pac 7000, Drager, Pittsb406.2(Pittsb40606.2([(rebreath)-409155

midity were controlled using automated computer feedback (WatFlow control system; TISS, Hampshire, UK). On arrival to the laboratory, participants provided a midflow urine sample for assessment of hydration. Towel-dried NBM was measured before and after the trials, with no fluid consumption permitted between measurements. Sweat rate (SR; l/h) was estimated using the change in NBM from the pre- to postexercise periods. Participants inserted a rectal thermistor (Henleys Medical Supplies, Welwyn Garden City, UK; Meter logger model 401, Yellow Springs Instruments, Yellow Springs, MO) 10 cm past the anal sphincter to measure rectal temperature (T_{rec}) and affixed a HR monitor to the chest. Following a 10-min seated stabilization period in temperate laboratory conditions, at sea level, resting measures [T_{rec}, HR, RPE, and thermal sensation (TSS)] were recorded, and participants immediately entered the environmental chamber (40.2 $\pm$ 0.4°C, 41.0 $\pm$ 6.4% RH) and mounted a cycle ergometer. Participants allocated to the HA group performed ten 90-min sessions involving a combination of cycling exercise and rest in accordance with established isothermic HA protocols (25, 26). HA participants initially exercised, at a workload corresponding to 65% $\dot{V}O_{2\ peak}$, until the isothermic target T_{rec} of $\geq$38.5°C had been achieved, and, upon the attainment of a T_{rec}

tion. Primers 2-microglobulin [National Center for Biotechnology Information (NCBI) accession number: NM_004048; forward: CCGTGTGAACCATGTGACT, reverse: TGCGGCATCTTCAAACCT], HSP72 (NCBI accession number: NM_005345; forward: CGCAACGTGCTCATCTTTGA, reverse: TCGCTTGTTCTGGCTGATGT), and HSP90 [NCBI accession numbers: NM_001017963 (*a a 1*) and NM_005348 (*a a 2*); forward: AAACTGCGCTCCTGTCTTCT, reverse: TGCGTGATGTGTCGTCATCT] were designed using primer design software (Primer Quest and Oligoanalyzer-Integrated DNA Technologies, Coralville, IA) (70). Relative quantification of mRNA expression for each sample was assessed by determining the ratio between the cycling threshold (CT) value of the target mRNA and 2-microglobulin CT values. Fold change in relative mRNA expression was calculated using the 2- CT method.

Sta ca a a . A priori power analysis for key HA-dependent variables selecting conventional (0.05) and (0.20) levels observed that eight participants were required in each experimental group. Before statistical analysis, all outcome variables were checked for normality using Kolmogorov-Smirnov and sphericity tests, using the Greenhouse Geisser method before further analysis. Protocol-specific and physiological data for HA/CON were compared using independent samples *T*-tests. Two-way mixed-design ANOVA was performed to determine differences between HA and CON and *da 1*/pre and *da 10*/post. Two-way mixed-design ANOVA was performed to determine differences between HA and CON, as well as HYP1 with HYP2; thus rest, 40% $\dot{V}O_2$

peak2396-2(ra)e.2396-2(T4ne)-388458.32.8416-d3721260/F2(metp(fc.)]10458.304168.49584261P21-1.11[(ancr(26e.2396-2((560TD15.481D4t5057-248.1(t.11922.18642674

No differences ($P > 0.05$) were observed between HYP1 and HYP2 trials, at rest, 40% $\dot{V}O_{2\,peak}$, or 65% $\dot{V}O_{2\,peak}$ during HA or CON for $\dot{V}O_2$, $\dot{V}_E$, breathing frequency, RPE, or LLQ (Table 3).

HSP72 RNA and HSP90α RNA during exercise. HSP72 mRNA increased during HYP1 ($f = 17.005$; $P = 0.001$, $np^2 = 0.567$). In the HA group, an increase in HSP72 mRNA was observed following HYP1 ($P = 0.006$), but not HYP2 ($P = 0.440$). This was supported by the observation that HSP72 mRNA was greater post-HYP1 compared with HYP2 ($P = 0.021$). No changes in the pattern of HSP72 mRNA expression were observed in CON. HSP90

(f = 17.110; P = 0.001, np^2 = 0.568). However, no

pared with CON (Table 2). No change in Hb_{mass} indicates HA-induced hypervolemia was a response to increases in extracellular fluid, with increases in PV (+446 ml) approximate to the absolute change in BV, reaffirming this as a primary adaptation to heat (61), and an established mechanism for the reduction in HR during exercise. Implementation of isothermic methods (50) for HA are the most probable causes for greater PV expansion (+15%) compared with others utilizing similar participants, protocol length, and environmental conditions [6.5% (39), 9.0% (50), and 11.1% (7)]. It remains to be experimentally elucidated whether maintaining lower intensity exercise, which matches heat production to evaporative heat loss, thus closely controlling T_{rec} at 38.5°C, rather than implementing passive rest following T_{rec} exceeding the target of 38.5°C, would augment even more favorable adaptations resulting from higher SRs and elevated cardiovascular response. As such, despite a large magnitude of adaptation observed within this experiment, this is a potential limitation of

creasing Hb_{mass} (27, 60). Our interventions did not increase Hb_{mass}; thus they did not, or cannot, induce sufficient heat strain and/or training load to stimulate erythropoiesis (27). This disparity from comparable research suggests more data are required to elucidate whether HA can effectively induce changes in Hb_{mass}.

The metabolic response to altitude is a preferential shift

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