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stress response, and subsequent initiation of protein transcription required for increased thermotolerance (Maloyan & Horowitz, 2002). Based upon previous data (Maloyan et al., 1999), HA should increase the measured Hsp72 mRNA transcription, a process primarily regu-

10 90-min sessions cycling continuously at a workload corresponding to 50% $\dot{V}O_{2peak}$ (80 rpm; 50% $\dot{V}O_{2peak} = 1.90 \pm 0.30$ L/min, power at 50% $\dot{V}O_{2peak} = 125 \pm 30$ W). ISO_{CONT} (65% $\dot{V}O_{2peak}$ L/min

Statistical analysis

All outcome variables were first checked for normality using Kolmogorov–Smirnov and sphericity using the Greenhouse Geisser method prior to further analysis. Two-way mixed-design analysis of variance (ANOVA) were performed to determine differences in dependent variables between HA methods for STHA and LTHA timescales (between HA methods and day 1, day 5, and day 10). A three-way mixed-design ANOVA was performed on the Hsp72 mRNA data to determine differences between pre- and post-value (repeated measures – within subjects) on different days (repeated measures – within subjects) from independent HA methods (between subjects). Adjusted Bonferroni comparisons were used as post-hoc analyses, determining where differences existed within ANOVA when a time or interaction was found. Data are reported as mean ± SD, with two-tailed significance was accepted at < 0.05.

Results

Participant characteristics

No differences (> 0.05) existed between groups for descriptive variables height, NBM, BSA, body fat % or $\dot{V}O_{2peak}$. A difference (< 0.05) was observed for age, whereby ISO_{PROG} was older than FIXED (+ 6.5 years).

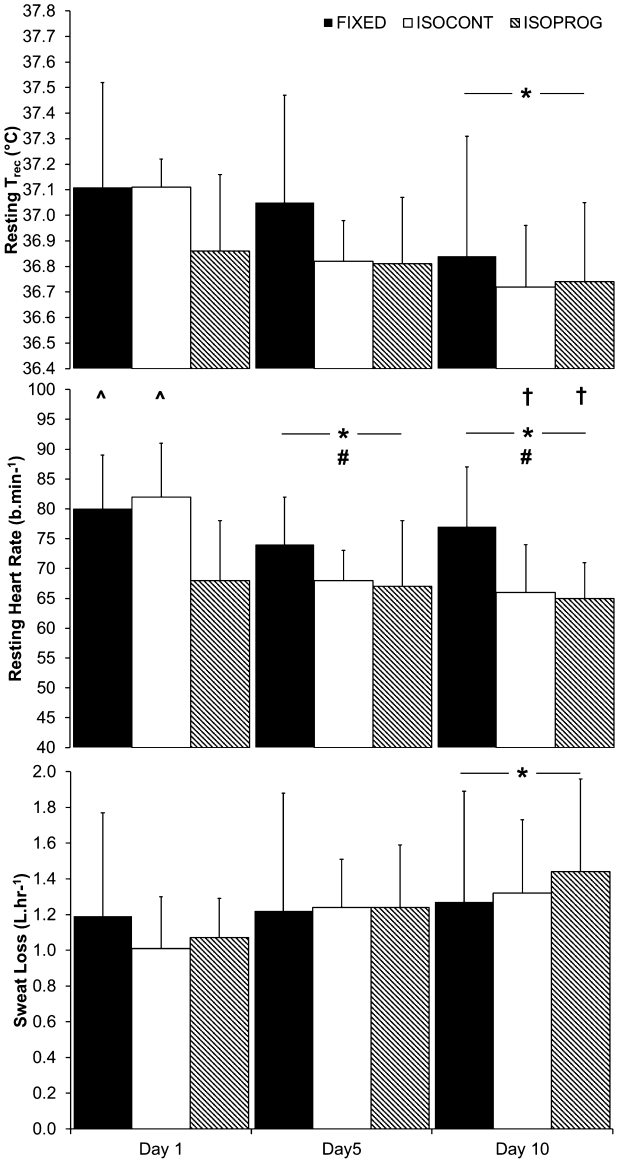
Evidence of HA

Resting T_{rec} was reduced (= 0.002), and sweat loss increased (= 0.002) overall, with a significant reduction between day 1 and day 10 (= 0.003 and = 0.002, respectively); no interaction effects were observed for resting T_{rec} (= 0.592) or sweat loss (= 0.281), Fig. 2. Resting HR demonstrated a significant overall effect (< 0.001) and interaction effect (= 0.009), with significant differences observed between day 1 and day 5 (< 0.001) and day 1 and day 10 (= 0.001) in ISO_{CONT}, and a difference between ISO_{PROG} and FIXED (= 0.043), and ISO_{PROG} and ISO_{CONT} (= 0.015) on day 1, and between FIXED and ISO_{CONT} (= 0.038), and FIXED and ISO_{PROG} (= 0.023) on day 10, Fig. 2.

Session-specific data

Exercising duration (= 0.001), mean session intensity (= 0.002), total work done (< 0.001), mean T_{rec} (= 0.002), duration $T_{rec} \geq 38.5$ °C (= 0.011), mean HR (= 0.019), and peak HR (< 0.001) all demonstrated overall differences between days, no between-day difference was observed for peak T_{rec} (= 0.226) or duration $T_{rec} \geq 39.0$ °C (= 0.245).

Exercising duration (= 0.004), mean session intensity (= 0.000), total work done (= 0.004), mean T_{rec} (= 0.010), peak T_{rec} (= 0.004), duration $T_{rec} \geq$ 38.5 °C (= 0.008), duration $T_{rec} \geq 39.0$ °C (= 0.005) all demonstrated interaction effects; no interaction effect was observed for mean HR (= 0.077) or peak HR (= 0.588). See Table 2 for full post-hoc analysis.



. 2. Mean ± SD changes in resting T_{rec}, resting heart rate and sweat rate following STHA (days 1 to 5) utilizing fixed-intensity (FIXED), continuous isothermic (ISO_{CONT}), and progressive isothermic (ISO_{PROG}) methods.
*Denotes significant difference overall from day 1 (< 0.05).
#Denotes significant difference within group and day (< 0.05).
Denotes significant difference from ISO_{PROG} within group and day (< 0.05).
†Denotes significant difference from FIXED within group and day 1 (< 0.05).

No differences between days or the interaction effect were observed for mean exercising intensity (= 0.124; = 0.061), change T_{rec} (= 0.227; = 0.109).

Hsp72 mRNA responses

No differences in Hsp72 mRNA were observed between days (= 0.236) or across HA methods between days (= 0.167). Hsp72 mRNA did increase pre to post overall (< 0.001), and pre to post over time

Table 2. Mean ± SD protocol, thermoregulatory, and physiological data characterizing exercise heat stress on day 1, day 5, and day 10 of fixed-intensity (FIXED), continuous isothermic (ISO_{CONT}), and progressive isothermic (ISO_{PROG}) methods

	Day 1			Day 5			Day 10		
	FIXED	ISO_{CONT}	ISO_{PROG}	FIXED	ISO_{CONT}	ISO_{PROG}	FIXED	ISO_{CONT}	ISO_{PROG}
Exercising duration (min)	90.0 ± 0.0	61.9 ± 10.7‡	56.3 ± 16.6‡	90.0 ± 0.0	76.3 ± 15.5*	53.1 ± 10.3‡§	90.0 ± 0.0	78.8 ± 15.8*	70.0 ± 9.3*†
Mean session intensity (% $\dot{V}O_{2peak}$)	49.7 ± 0.6	36.6 ± 5.3‡	36.7 ± 11.2‡	50.0 ± 0.0	47.0 ± 8.3*	32.3 ± 8.6‡§	50.0 ± 0.0	50.5 ± 9.5*	45.8 ± 8.0*†
Mean exercising intensity (% $\dot{V}O_{2peak}$)	49.7 ± 0.6	52.6 ± 8.2	58.8 ± 5.1	50.0 ± 0.0	57.4 ± 4.9	56.8 ± 5.9	50.0 ± 0.0	58.7 ± 7.0	58.9 ± 6.2
Total work done (kJ)	656 ± 166	498 ± 81	554 ± 102	673 ± 165	657 ± 100*	500 ± 152	684 ± 164	719 ± 126*	708 ± 176*†
Mean T_{rec} (°C)	38.17 ± 0.17	38.15 ± 0.23	38.21 ± 0.25	37.85 ± 0.22*	38.10 ± 0.19	38.27 ± 0.24‡	37.74 ± 0.19*	38.04 ± 0.23‡	38.18 ± 0.21‡
Peak T_{rec} (°C)	38.92 ± 0.26	38.65 ± 0.32	38.87 ± 0.18	38.52 ± 0.43*	38.66 ± 0.25	38.91 ± 0.24	38.40 ± 0.33*	38.67 ± 0.23	39.06 ± 0.37‡
Δ T_{rec} (°C)	1.81 ± 0.60	1.53 ± 0.37	2.01 ± 0.33	1.47 ± 0.74	1.74 ± 0.20	2.10 ± 0.42	1.56 ± 0.72	1.95 ± 0.32	2.32 ± 0.61‡
Duration T_{rec} ≥ 38.5 °C (min)	32.5 ± 8.5	28.8 ± 15.1	44.4 ± 21.3	13.1 ± 16.0*	22.5 ± 20.7	51.3 ± 18.5‡§	5.0 ± 8.0*	29.4 ± 23.5‡	35.6 ± 18.6‡
Duration T_{rec} ≥ 39.0 °C (min)	5.6 ± 12.1	0.0 ± 0.0	1.9 ± 3.7	1.3 ± 3.5	2.5 ± 7.1	6.9 ± 14.4	0.0 ± 0.0	0.0 ± 0.0	20.0 ± 16.0†‡§
Mean HR (b/min)	159 ± 12	151 ± 13	144 ± 9	149 ± 21	148 ± 9	140 ± 8	146 ± 14	151 ± 8	144 ± 14
Peak HR (b/min)	176 ± 12	183 ± 9	182 ± 11	171 ± 26	172 ± 12	174 ± 8	164 ± 13	174 ± 11	171 ± 13

Exercising duration is cumulative time spent exercising during each of the 90-min sessions. Mean session intensity is calculated from each participant's relative exercise intensity during each 5-min period including rest periods during the given session. Mean exercise intensity is calculated from each participant's relative exercise intensity during each 5-min period excluding rest periods during the given session.

*Denotes difference from day 1 within respective method ($P < 0.05$).

†Denotes difference from day 5 within respective method ($P < 0.05$).

‡Denotes difference from FIXED within respective day ($P < 0.05$).

§Denotes difference from ISO_{CONT} within respective day ($P < 0.05$).

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increases in Hsp72 mRNA indicated that the stress presented at the start of HA, and after STHA and LTHA, all surpassed the minimum required endogenous strain to elicit increased transcription of Hsp72 mRNA in leukocytes across HA methods. The Hsp72 mRNA response provides further evidence of the importance of providing a consistent stressor for adaptation, via the facilitation of consistent or elevations in core temperature throughout STHA and LTHA. Sustained Hsp72

STHA, the time to target core temperature is achieved earlier in the session than in LTHA. A greater duration then remains for heat dissipation and temperature reduction, consequently initiating a resumption of exercise in accordance of the requirements of the protocol. The extended first exercise bout in LTHA reduces the time remaining in the session for resuming exercise and thus participants demonstrate less work/lower average intensity of work later in the session. The greater duration of the initial bout of exercise prior to cessation also rationalizes some of the differences between ISO_{CONT} and ISO_{PROG} during LTHA. The requirement for a greater change in core temperature in ISO_{PROG}, requires participants to exercise for longer initially to attain the higher temperature as such they again perform less work later in the session. These limitations demonstrate the importance of future research optimizing isothermic methods so that a greater consistency of protocol administration, and potentially consistency of Hsp72 mRNA transcription is achieved. A larger sample size may reduce the variability in the protocol administration, and may strengthen the observations of the Hsp72 mRNA particularly trends toward reductions in FIXED, which may become statistically different given prolonged acclimation (i.e., + 10 days) or a greater sample size. It was observed that Hsp72 mRNA post day 5 (= 0.100) and post day 10 (= 0.082) reduced nonsignificantly in comparison to day 1, an observation not true of ISOCONT (post day 1 vs post day 5 = 0.998; post day 1 vs post day 10 = 1.000) or ISOPROG (post day 1 vs post day 5 = 1.000; post day 1 vs post day 10 = 0.677). An explanation for this may relate to the variability in the change in FIXED; physiologically, this might be rationalized by individual differences in acclimation rate, and thus endogenous criteria using this protocol, an element that might be further clarified by a larger sample size.

Future work could involve tissue viability/ experiments to quantify the increased thermotolerance induced between HA methods alongside the measurement of the HSP72 protein; the absence of which is a limitation of the present experiment. Analysis of the acute Hsp72 mRNA response to the first session of progressive isothermic HA would allow analysis of increased hyperthermia from 38.5 to 39 °C to be quantified, although the measurement of mRNA presents a limitation in itself as no data is available to confirm intracellular HSP72 increases, with differential HA methods eliciting different gains in total protein, which may in itself augment a changing mRNA/protein ratio. Cellular thermotolerance is unlikely to be explicit to HSP72 alone, with a number of genes associated with the cellular stress response to hyperthermia. Therefore, a wider genomic and molecular analysis would facilitate further insight into the adaptive mechanisms (Sonna et al., 2002). Data suggests an endogenous threshold/minimum criteria may exist for Hsp72 mRNA or HSP72 protein increases as proposed by others (Amorim et al., 2008; Morton et al., 2009; Magalhães et al., 2010a; Périard et al., 2012; Gibson et al., 2014). Further investigation of precise endogenous signals leading to greatest intracellular Hsp72 mRNA and HSP72 increases in leukocytes and muscle is warranted to enable links between HA and thermotolerance, to be further examined. This could be facilitated by extended HA durations beyond 10 sessions to determine whether in FIXED, further diminished endogenous strain would see a continued attenuation of the post-session mRNA transcription, or via an experiment where either lower isothermic temperatures are targeted, or changes from baseline implemented to elicit graded minimum thresholds. Individual variability associated with metabolic heat production and retention, and the respective effects they may have on Hsp72 mRNA expression could be eliminated by modifying the isothermic method to administer the exercise based upon a fixed relative rate of heat production (Cramer & Jay, 2014), further optimizing acquired cellular thermotolerance through repeated exercise-heat stress at an optimized asymptote of core temperature increase.

Perspectives

Continuous and progressive isothermic HA elicit and sustain similar endogenous systemic strain. This is in contrast to fixed-intensity HA, which elicits less varied, but diminishing thermoregulatory strain following the procurement of STHA and LTHA adaptations. Hsp72 mRNA transcription, a marker of the cellular stress response to hyperthermia and an important component of thermotolerance, demonstrated equal sessional increases utilizing all HA methods. The equal Hsp72 mRNA increases, occurring after equal, reduced, or increased core temperature following STHA and LTHA, suggest that as long as a minimum endogenous criterion is surpassed, additional endogenous thermoregulatory strain is not of further benefit, nor is continual exercise load crucial, so long as hyperthermia is present. These data give confidence that all reported HA methods increase Hsp72 mRNA and are capable of eliciting adaptations toward thermotolerance.

Ke v .d : Thermoregulation, heat stress, cellular stress response, hyperthermia, thermotolerance, heat shock protein 72, heat illness.

Acknowledgements

The authors would like to thank the volunteers for their participation in this investigation. Funding and support was obtained from the University of Brighton and the University of Bedfordshire.

: The authors of this study declare that they have no conflicts of interest.

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