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Effectiveness of an Intermittent Heat Exposure Protocol to Maintain Heat Acclimation

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University of Connecticut, 2015

Background: Heat acclimation (HA) adaptations are temporary and must be sustained for the continued safety of those periodically exerting themselves in hot conditions. **Purpose**: To assess whether an intermittent exercise-heat exposure protocol can mitigate HA decay 25 days after initial acclimation. **Methods**: Sixteen males (VO_{2max}=54.98±5.5 ml·kg^{-1}·min^{-1}) were pair-matched using physical training duration, VO_{2max}, and body surface area then randomly allocated to a no heat (NHE; n=7) or intermittent exercise-heat exposure (IHE; n=9) group. All participants heat acclimated by completing 10-11 days of low-to-moderate intensity exercise (90-240 min) in hot conditions (40℃, 40%RH). Both groups completed a Pre HA and Post HA heat stress test (HST) consisting of two hours of exercise at 45% VO_{2max} in hot conditions to assess HA. After Post HA, participants completed the HST in either a hot (IHE; 40℃, 37%RH) or thermoneutral environment (NHE; 24℃, 21%RH) every fifth day for 25 days with both groups exercising in the hot condition at day 25 (+25d). Thermoregulatory, cardiovascular, and circulating biomarkers of stress were evaluated. Self-led out-of-lab physical activity duration and intensity (heart rate [HR]) were recorded for 25 days after HA. **Results**: Both groups heat acclimated as post-exercise HR and rectal temperature (T_{re}) were lower and sweat rate higher at Post HA versus Pre HA (all p≤0.05). At +25d, post-exercise HR was attenuated in IHE versus NHE (mean difference [NHE-IHE]=28

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bpm (95%CI [8, 48], effect size [ES]=1.41, p=0.01) but sweat rate (0.13 $L \cdot hr^{-1}$, 95%CI [-0.21, 0.46], ES=0.36, p=0.44), skin temperature (0.65℃ (95%CI [-0.17, 1.47], ES=0.85, p=0.11) and T_{re} (0.47℃, 95%CI [-0.24, 1.19], ES=0.68, p=0.18) were similar. Post-exercise cortisol and epinephrine concentrations were higher in NHE versus IHE at +25d (p≤0.046). At +25d, heat adaptation decay was greater in NHE than IHE for T_{re} (87% versus 2.7%), skin temperature (44% versus 18%), and HR (163% versus 53%). Out-of-lab exercise intensity and +25d post-exercise HR were inversely related in IHE (r=-0.89, p=0.017). **Conclusions**: Periodic exercise-heat exposure every five days mitigated rectal temperature decay and cardiovascular strain 25 days after initial HA efforts. Intense exercise in thermoneutral environments in addition to exercise-heat stress after HA aids in minimizing adaptation decay.

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Effectiveness of an Intermittent Heat Exposure Protocol to Maintain Heat Acclimation

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Chapter 1. Review of Literature

Scope of the problem

Hot environments reduce an individual's ability for prolonged exercise. The combination of exercise and heat stress imparts exaggerated strain on the thermoregulatory and cardiovascular systems (32, 56). This is caused by increased skin temperature (T_{sk}) creating a reduced core-to-skin thermal gradient. The vasodilation of skin vascular beds and increased blood flow is needed to transport heat from the core to periphery. The shunted blood flow to the skin creates competition for limited cardiac output between cutaneous and skeletal muscle vascular beds. Cardiac output is sustained (or increased if exercising) through compensatory increases in cardiac frequency. Moreover, exercise in hot conditions leads to losses in total body water and electrolytes due to sweating. Over time hypovolemia occurs, further impairing circulatory and thermoregulatory functions resulting in decreased athletic performance and increased risk of thermal injury if fluids are not replaced (32, 56).

Heat-related injuries, especially exertional heat stroke and myocardial infarct after exercise-heat stress, are serious life-threatening problems in several occupations such as firefighters, military personnel, and athletes of all ages (1, 16, 20, 41, 55). Between 1997 and 2006, heat-related injuries treated in U.S. emergency departments increased 133.5%, accounting for an estimated 54,983 patients (55). Further, in American football, more exertional heat stroke deaths occurred from 2005-09 than any five-year period in the previous 35 years (54). Keeping recreational and occupational athletes safe during physical activity in hot, humid conditions is critically important.

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While strategies have been implemented to mitigate heat-related injury (e.g., work-rest cycles, body cooling, hydration, education), the continued rise in heat-related injuries observed in emergency departments (41, 55) and high school athletes (42) necessitate investigation into physiological adaptations to the heat. Heat acclimation (HA) reduces resting and exercising body temperature, increases sweat rate and sensitivity, and reduces cardiovascular strain (6, 13, 49, 51, 72). Taken together, these physiological adjustments enhance heat dissipation mechanisms that mitigate thermal load, improve exercise-tolerance in the heat, and ultimately contribute in part to the prevention of exertional heat illness (9, 60).

Beyond preventing exertional heat illness, HA has gained popularity among scientists and athletes attempting to improve endurance performance in hot and cool environments (17, 18, 64, 67). Regardless of the intended application, the benefits of HA are temporary (9, 28, 60). It follows that for prolonged protection against thermal injury and mitigation of physiological strain during physical activity, HA associated adaptations must be sustained.

Heat acclimation

Heat acclimation occurs in an artificial (heat chamber) setting while heat acclimatization occurs in a natural (outdoor) setting. Both acclimation and acclimatization are effective modalities (8, 68). Acclimatization in humid heat elicits greater T_{sk}, sweat rates, and circulatory adaptations compared to dry heat. These changes are thought to increase skin wettedness and optimize evaporative heat losses, although sufficient scientific support is for this theory is lacking (73). To facilitate sudomotor (e.g., neural activation of sweat glands) and cardiovascular gains, it may be

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beneficial to train in humid heat during the end of acclimation protocols (67). Perhaps most importantly, acclimation/acclimatization should occur in an environment that emulates the competition setting to enable the athlete to experience the exact nature of the exercise-heat stress (9). In addition to exogenous heat stress, endogenous heat production is an important consideration in HA protocols (see below).

Several consecutive days of exercise-heat exposure induce HA resulting in reduced heat storage (66) and body temperature. These important changes are facilitated by increased sweat rate, sweat gland sensitivity, improved skin blood flow, and cardiovascular adjustments that minimize attenuation of stroke volume and heart rate (HR) elevation (47). The combination of these adaptations reduces perceptual, thermal, and physiological strain. Importantly, the risk of exertional heat illness is lessened and heat tolerance and endurance performance in hot and cool environments improved (9, 46).

Researchers have categorized HA into three divisions defined by the duration of exercise-heat exposure. Short-term HA refers to ≤ 7 days, medium-term is defined as 8-14 days and long-term is considered greater than 14 days of consecutive exercise-heat exposure. The daily exercise-heat exposure duration required to achieve thermal habituation is balanced between session duration, intensity, environment, and fitness/training status of the individual undergoing HA. The optimal synergism between these parameters is unknown, but it is accepted that 100 min/day at approximately 40-50% VO_{2max} for 10-14 days will elicit thermal acclimation (60). However, HA has been induced with high intensity short duration (75% VO_{2max}, 30-35 min) exercise-heat stress bouts for 9 days and isothermal techniques for as little as 5 days (27, 40). More

recently, Taylor (75, 76) contends that elevating T_{re} above 38.5℃ for at least 60 minutes during consecutive days is a sufficient heat adaptation stimulus. While many protocols for inducing HA exist, the adaptive impulse for HA remains exercised-heat induced hyperthermia and hypovolemia sufficient to stress thermoregulatory and circulatory effector organs.

Individuals with high VO_{2max} and regularly exercise require fewer exercise-heat exposures to acclimate to the heat. Thus, short-term HA has been recommended for highly trained athletes (10, 28). The reduced time commitment to achieve HA via short-term protocols fits well with the congested training and competition schedules of elite athletes. However, it is important for clinicians and practitioners to consider that complete acclimation is not realized with short-term HA protocols (< 6 days). It is accepted that 75% of HA adaptations occur within 4-6 days of exercise-heat exposure but full acclimation may require up to 14 days (Table 1.1) (9). Weighing the expected benefits and time required to achieve these benefits via each respective HA protocol is warranted on an individual and competition specific basis.

Table 1.1

"Plateau days" of Physiological Adaptations (Point at Which Approximately 95% of the Adaptation Occurs) During Heat Acclimatization

Adaptation	Days of heat acclimatization
	1 2 3 4 5 6 7 8 9 10 11 12 13 14
Heart rate decrease	
Plasma volume expansion	
Rectal temperature decrease	
Perceived exertion decrease	
Sweat Na^+ and Cl^- concentration decrease*	
Sweat rate increase	
Renal Na^+ and Cl^- concentration decrease	

*While consuming a diet low in NaCl.

Reprinted from Armstrong and Dziados 1986.

Note. The grayed boxed represents the duration and expected adaptations gained from short-term (< 7 days) heat acclimation.

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The physiological changes characterizing a heat acclimated state is induced at varying rates. In their seminal review, Armstrong and Maresh (9) eloquently describe the expected induction rates for the major HA adaptations which are curvilinear in nature (Figure 1.1). Adaptations of cardiovascular origin are the first to occur, usually within 4-6 days followed by reductions in resting and exercising body temperature, plateauing around 5-8 days. Sweat gland adaptations usually take longer to develop (5-14 days) as does electrolyte conservation at the eccrine gland and kidney. This process is mediated by the secretion of aldosterone in response to repeated bouts of exercise-heat stress that induce hypovolemia and blood sodium perturbation. Several physiological changes beyond the classical heat adaptation responses (e.g., HR, sweat rate, and body temperature) have been reported following HA (Table 1.2).

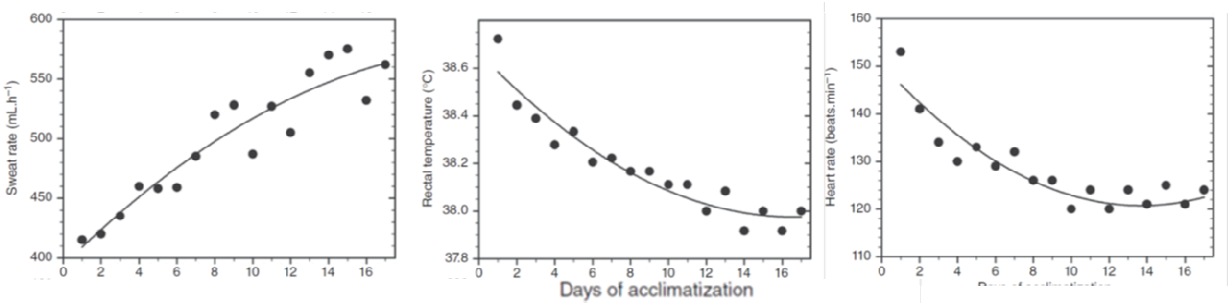

Figure 1.1. Rectal temperature, sweating, and heart rate show rapid initial responses then plateau during successive days of bench stepping (12 steps/min, 4 hours) under 34 C, 80% RH. Data from Wyndham et al. (85).

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Table 1.2. Physiological Adaptations Following Heat Acclimation/Acclimatization

Category	**Increased**	**Decreased**
Thermoregulatory		
	Sweat rate	Sweat electrolyte concentration
	Sweat sensitivity	Resting body temperature
	Skin blood flow	Exercising body temperature
		Exercising skin temperature
		Core-to-skin temperature gradient
Cardiovascular		
	Maximal cardiac output	Sub-maximal heart rate
	Maximal oxygen uptake	
	Plasma volume	
	Total body water	
	Ventricular efficiency	
Metabolic		
		Running economy
		Carbohydrate metabolism
Other		
	Exercise-heat tolerance	Fatigue perception
	Speed at lactate threshold	Effort perception
	Intracellular HSP70	Thermal perception

Table modified from Guy et al. (33)

After HA, on average, one would expect the following degree of adaptation: a post-exercise HR decrease of 16 bpm (range = 10-36), post-exercise body temperature decrease of 0.4°C (0.17-0.80), post-exercise T_{sk} decrease of 0.68°C (0.22-1.89), resting body temperature reduction of 0.2°C (0.01-0.30), and 0.29 $L \cdot hr^{-1}$ (0.00-0.44) increase in

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sweat rate (11, 19, 27, 40, 47, 61, 63, 66, 71, 80). Plasma volume expansion in response to HA appears is variable, increasing 200-500 mL or 3-28% post HA (9, 62, 63). Certainly differences in HA protocol (traditional vs. hyperthermia controlled, number and duration of exercise-heat exposures, frequency, and ambient conditions) and fitness characteristics of the participants affect the magnitude of physiological change derived from HA.

Methodological considerations for inducing heat acclimation

As HA develops, a progressively lower training or adaptive impulse is elicited in traditional constant workload protocols because relative intensity and adaptation are inversely related. It is theorized that this may limit the magnitude of adaptation if precautions are not instituted. For example, increasing exercise duration, intensity, or environmental conditions (76). Authors from international labs advocate that isothermic acclimation protocols are adaptively superior to traditional regimens because exercise intensity is guided by T_{re} responses, not absolute workloads (27, 62, 76). Thus, as adaptation occurs, exercise intensity must increase to achieve similar thermoregulatory strain. However, similar physiological responses between the two techniques were recently observed in a small cohort (n=8) of males with above average aerobic fitness (VO_{2max} range 45-50 $mL \cdot kg^{-1} \cdot min^{-1}$) (30) refuting this superiority claim. Additional evidence is required before definitive conclusions can be drawn, however.

Body temperature measurement equipment and access to artificial indoor conditions pose limitations to the individuals without access to this equipment. Alternatively, Periard et al. (64) suggested using HR to measure relative intensity and acclimation progress. One wonders how effective guiding exercise intensity with HR

would be during exercise-heat stress given the propensity of dehydration and possibility of cardiac drift (53). Alternatively, it has been demonstrated that highly trained runners (8) and team sport athletes (68) can acclimate to the heat outdoors (acclimatization) without affecting team directed training regimens. Importantly, HA induction and decay responses have been shown to vary between individuals (28, 67, 69) and a trial and error period that occurs months before competition is prudent to assess physiological responses.

Relationship between physical training and heat acclimation induction

Endurance training evokes a wide array of cardiovascular, pulmonary, and metabolic adaptations that elevate maximal oxygen consumption (VO_{2max}). Additionally, during acute exercise, large quantities of thermal energy are created, increasing body temperature proportionate to intensity. As a consequence, many of the physiological adaptations elicited following high-intensity endurance training are similar to thermally adapted individuals, suggesting a partial positive cross acclimation or adaptation (38, 76). Similar physiological improvements include lower resting HR and core temperature, expanded blood volume, greater sweat rate and sensitivity, and greater exercise-heat tolerance (76). Many have observed that highly trained athletes appear partially, but not fully, heat acclimated (10, 60, 65). The optimal balance between exercise intensity, duration, and frequency in temperate environments to facilitate HA remains largely unknown.

VO_{2max} has been shown to account for approximately 42-46% of variance in the rate of acclimation measured as the time to rectal temperature (T_{re}) plateau, an accepted measure of a heat acclimated state (59). However, it is not VO_{2max} *per se*, that

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is important, but the physiological changes during the acquisition of higher cardiorespiratory fitness that promotes the quasi-acclimated state. For physical activity to contribute to heat adaptation it appears that increased internal and cutaneous temperature with hypovolemia must occur during exercise. Prospective studies using swimmers (12) and sweatless training (35) established the notion that exercise without a sustained increase in body temperature resulted in insufficient stimulus for HA. Although regular exercise elicits a partial HA state, complete adaptation to the heat requires 5-14 consecutive days of moderate to intense exercise (30-120 minutes) in hot ambient conditions sufficient to elevate body (core and skin) temperature and stress fluid homeostasis.

Heat acclimation decay

Decay of HA induced benefits remains to be fully characterized. Studies examining HA decay are sparse compared to induction. The early HA decay studies of the 1940-60's were rife with inadequacies but pioneering in that they showed the retention of classic HA adaptations varies between individuals and between environments (hot-dry vs. hot-humid) (60). Common shortcomings of these studies include very small sample sizes, incomplete acclimation, or inappropriate measures (3, 60, 84). More recent investigations examining the decline of the heat acclimated phenotype have better characterized the process, but not without disagreement (11, 19, 26, 27, 61, 66, 70, 71, 74, 80, 81). Generally, the classical acclimation criteria of reduced T_{re} and HR persist for approximately 7-21 days (9, 60, 76), although reports of adaptations persisting longer (up to 26 days) are available but few (19, 80). Several questions regarding HA decay, and how to extend acclimation benefits remain. How

much exercise-heat exposure is required to sustain HA adaptations? How long can we expect to extend these physiological adjustments before re-acclimating? After HA, does exercise have to occur in a hot environment to prolong HA adaptations? Is maintaining a fully acclimated state required to reap the prophylactic and ergogenic benefits of HA during exercise-heat stress?

Our current body of HA decay knowledge can provide insight and direction into exploring these important questions. Heat adaptations that occur the earliest also decay the fastest (9, 28, 60). Cardiovascular improvements are gained and lost exponentially in a few days, with and without exercise-heat exposure, respectively (9). Body temperature and sweat rate/sensitivity take longer to develop but also demonstrate a slower decay rate (60).

Taylor (76) purported HA induction and decay is not linear, but cyclical in nature, with decay lasting longer than the time required for acquisition. One of the first descriptions of HA decay was by Dreosti (21) who characterized the physiological losses in indigenous miners after prolonged removal from working in a hot mine, stating, “these workers showed virtually the same rise in body temperature as did natives with no mining experience.” From this initial observation it was clear that adjustments to the heat exist on a continuum and can be modified by exercise-heat exposure.

Although the timeframe of HA decay is generally accepted, there is great inter-individual variability. Individual differences in heat tolerance and acclimation, physical fitness and activity, and the duration, intensity, and frequency of exercise-heat exposures are cited as factors contributing to HA induction and decay variability (3, 11, 19, 26, 27, 61, 66, 70, 71, 74, 80, 81, 84). Although the spread of physiological

responses to HA decay is evident, Givoni and Goldman (31) suggested HA decays at the rate of one day of acclimation for every two days without heat exposure.

Others purport periodic heat exposure delays HA decay. For example, Wyndham and Jacobs (84) suggested one day of heat exposure for every six days removed from the heat. In a more recent review, Taylor (75) proposed a conservative exercise-heat exposure frequency of once every fifth day removed from the heat. It stands to reason that if the aim of periodic exercise-heat exposures after HA is to sustain physiological adaptations, conservative treatment would be prudent. Interventions designed to mitigate HA decay or sustain HA adaptation would be meaningful for individuals periodically exerting themselves in oppressive environmental conditions. In addition to intermittent exercise-heat exposure, investigating factors that contribute to sustaining HA adaptations is warranted.

Heat acclimation appears to be better maintained by individuals who are physically active and have higher aerobic power. Habitual exercise sufficient to elevate body temperature elicits an adaptive stimulus to maintain thermal adaptations (10, 61). In this regard, intense exercise bouts that generate high amounts of metabolic heat may be superior to prolonged, low intensity work bouts. Importantly, exercise with the explicit aim of sustaining a heat acclimated state should occur in at least temperate conditions as exercising in a cool environment appears to exacerbate adaptation loss (71). Saat et al. (71) had one group exercise (60 % VO_{2max}, 60 min) and another rest in cold conditions (18℃, 58% RH) during a 14 day decay period after initial HA (14 days; 31℃, 70% RH; 60 % VO_{2max}, 60 min). The exercised group demonstrated exacerbated decay compared to the control group (HR: 35% vs 17%; T_{re}: 35% vs 9%). When compared to

previous research, this study is an anomaly considering the well-established interaction between physical activity and HA in induction and decay (5, 10). The optimal balance between exercise intensity, duration, and ambient conditions required to sustain HA is unknown and warrants future research.

Re-induction of HA is significantly faster than initial acclimation efforts but still appears variable among individuals. Pandolf et al. (61) heat acclimated soldiers using a traditional constant work rate protocol for nine days (walking 1.34 m/s for 110 min in 49°C and 20% RH) and found minimal adaptation decay 18 days after initial acclimation. Re-induction of HA parameters required just two days to restore previous physiological benefit. The authors believed the well trained status of the participants contributed to the maintenance of the heat adapted phenotype. Habitual physical training during the decay period is an important consideration to the rate of decay as endurance training provides an adaptation stimulus.

Weller et al. (80) used a 10 day hyperthermia-controlled technique (46°C, 18% RH) to induce HA and re-evaluated participants 12 and 26 days later. The authors found that HR and T_{re} improvements were restored to initial levels with just 2 and 4 days of exercise-heat exposure. Post-acclimation physical activity was not controlled or reported in this study but the authors assumed participants were exercising due to their military affiliation.

Most recently, Ashley et al. (11) heat acclimated 10 below average fitness (VO_{2peak} = 33.9 ml/kg/min) subjects using a fixed rate protocol of treadmill walking at 40% VO_{2peak} for 120 min (10 days, 50°C and 20% RH). Following a two, four, or six-week decay period, the authors recommended a re-acclimation period consisting of four

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days of similar exercise-heat after two weeks away from heat-exposure, five days after three weeks, or six days after six weeks.

Undoubtedly, heat adaptations decay over time. Taken as a whole, these data suggest athletes could heat acclimate or re-acclimatize prior to competition or physical exertion in hot ambient conditions. However, the congested training and travel schedules of occupational and elite athletes may not afford several consecutive days of lower intensity exercise-heat exposure to gain (or regain) sufficient HA. In this context, there is merit to exploring more flexible methods such as a single day of period exercise-heat exposure to sustain, rather than re-induce, HA. Table 1.3 shows intermittent heat exercise-heat exposure ratios theorized to sustain or at least minimize HA adaptation decay.

Heat shock protein 72 response to heat acclimation

Heat shock protein 72 (HSP72) is a stress-induced cellular chaperone that improves thermal tolerance, preserves protein and cellular function, structure, and resiliency enhancing the signaling pathways of the cytoprotective mechanisms (39, 44). HSP72 may therefore be central to our understanding of cellular thermotolerance associated with HA (23). Most (4, 49, 51, 87) but not all (37, 79) studies demonstrated that constitutive expression of intracellular HSP70 increases after HA while the heat stress-induced response is blunted in vitro (50, 51) and in human leukocytes (49) and peripheral mononuclear blood cells (87). The dissonance among studies may be attributed to cell type or tissue response of HSP70 (86). Additionally, acclimating subjects may require repeated thermal (T_{re} ≥ 38.5-39.0℃ (29, 49)) and cardiovascular strain before HSP72 kinetics are altered (37). In regards to HA decay, using a rat model

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Michal Horowitz and colleagues suggest that continued epigenetic modifications of the intracellular HSP72-90 prototype act as key factors mediating decay, rapid re-acclimation, and cytoprotection (38, 50, 77, 78). Although intracellular HSP72 appears integral to the HA process, less is understood regarding the extracellular or circulating HSP72 responses to HA.

Table 1.3. Exercise-heat exposure ratios observed after medium-term (8-14 days) heat acclimation to either re-acclimate or sustain heat adaptation

Study	Periodic exercise-heat exposure to prolong acclimation	Consecutive days of exercise-heat exposure to re-acclimate	Physical activity during decay?
Taylor (75) (review)	1:5		N/A
NIOSH (2) (review)		2-3 days upon return to labor in hot conditions	N/A
Weller et al. (80)	1:6	2:12, 4:26	Assumed but not reported
Williams et al. (81)	1:6		No
Ashley et al. (11)	2:7	4:14, 5:28, 6:≥36	No
Saat et al. (71)	1:9		Yes
Pandolf et al. (61)	1:9		Yes
Poirier et al. (66)		7:14	No

Ratios represent the number of exercise-heat exposures to days without heat exposure. Ratios were either recommended (69, 2) or calculated based upon original data observations from each respective study.

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Circulating HSP72 increases after exposure to many stressors including acute bouts of exercise in a thermoneutral (22, 24) and hot environment, by about 33% (49). The pattern of resting and post-exercise circulating HSP72 responses before and after HA is less clear, however. Magalhaes et al. (48) showed circulating HSP70 increased after exercise-heat stress before HA but not after. Kresfelder et al. (45) showed baseline circulating HSP72 decreased after HA and Yamada et al. (87) observed no change after either exercise or HA. One possible reason for these discrepancies is the differences among heat stress tests and HA parameters between studies which affect body temperature responses. Elevated internal temperature is related to increased circulating HSP72 in rats (58) and humans (29). The energetic cost of mounting the HSP72 stress response is great. Thus, elevated post-exercise circulating HSP70 begins to decline after one hour (48) and returns to resting levels within 24 hours (29).

Epinephrine and Cortisol responses to exercise-heat stress

The pituitary-adrenal axis response (i.e., cortisol, epinephrine, and norepinephrine) to exercise is approximately proportional to duration and intensity when intensity is > 50% VO_{2max} (see review by Hackney (34)). Compared to an exercise in a thermoneutral environment, exercise-heat stress substantially increases circulating epinephrine and norepinephrine (15). In a cross-over design, Brenner et al. (15) showed in 11 males that epinephrine, and norepinephrine concentrations in response to two 30-minute exercise bouts at 50% VO_{2max} was significantly higher in 40℃ conditions compared to 23℃ by 66% and 59%, respectively. Cortisol responses did not parallel catecholamine responses in this study likely because the exercise intensity and/or

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duration were too low (34). Indeed, it has been shown that plasma cortisol concentrations rise when internal body temperature increases by at least 1.2℃ (15, 57).

The neuroendocrine response to exercise of a constant intensity level decreases over a given training period as the relative physiological stress imparted by the exercise becomes attenuated (14, 82). Thus, the adaptation of an attenuated neuroendocrine stress response is expected after HA (7, 36). Whether the mitigated post-heat acclimated neuroendocrine response is sustained after 25 days of periodic exercise-heat exposure remains unknown.

Limitations of the current literature

Clear interpretation and synthesis of HA decay findings is difficult due to methodological and quality differences among studies (lack of HA, inappropriate measures, etc.). Many HA decay studies are within subject designs and lack a control group (19, 61, 66, 80). This limits the ability of researchers to parcel out key factors mediating adaptation decay such as heat exposure duration and frequency, and out-of-lab exercise intensity and duration (10). Moreover, most within design HA decay studies either do not control or adequately report out-of-lab physical activity during decay periods. While controlling out-of-lab physical activity may not be feasible, characterizing such activity (e.g., frequency, intensity, and type) will allow researchers to at least addressed the known interaction of between physical activity, HA induction, and decay (10).

Almost all laboratory studies examining HA decay report small homogenous (young, healthy, above average aerobic fitness males) cohorts which reduce the ability for population wide inferences. Indeed, the induction and decay of HA remain largely

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unexplored in many healthy and diseased populations which stand to benefit from the physiological changes derived from HA (e.g., below average fitness capacity (11), diabetes, multiple sclerosis (25), cystic fibrosis, cardiovascular disease, burn victims (83), etc.).

Statement of the problem

HA is a commonly used method to reduce the risk of exertional heat-related injuries and improve performance preparing athletes, civilians, and military personnel to perform physical activity in hot conditions safely (6, 9, 13, 81, 84). However, HA guidelines are not widely used in all settings despite recommendations from several governing bodies, including the American College of Sports Medicine and the National Athletic Trainers' Association (6, 13). Indeed, only 2.5% of high school athletic trainers reported complete compliance to the NATA Inter-Association Task Force heat acclimatization guidelines (43). It is likely that inconvenience, lack of time and logistical support, among others, are barriers athletic trainers, athletes, coaches and military officer's faced when considering implementation of HA (or re-acclimation) for its prophylactic and/or ergogenic benefits. Maintaining initial HA adaptations is critical when opportunities to HA or re-acclimate may not be available due to training, time or logistical constraints. For instance, military personnel (80) or industrial workers (81) between deployments or while on vacation and collegiate or professional athletes returning home or train in cooler climates between national or international competitions in hot, humid environments (52).

The optimal method for retaining the adaptations derived from HA remains uncertain although suggestions have been put forth (11, 75, 80). Surprisingly, no

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suggestion has been scientifically evaluated to assess recommendation efficacy. This is a critical gap in our evidence based practice knowledge regarding the best strategies for successfully preserving a heat acclimated state. This knowledge can help healthcare practitioners reduce the risk of exertional heat illness by prolonging the beneficial adaptations to the heat. This study aims to examine a treatment that will extend the benefits of initial HA efforts. By maintaining the physiological adaptations associated with HA the aforementioned constraints of inducing HA (or re-acclimating) will be significantly diminished or eliminated altogether.

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Chapter 2. Physiological and Circulating Stress Responses to a Heat Acclimation Maintenance Protocol

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Introduction

Heat acclimation (HA) induced by repeated exercise-heat exposures elicits temporary physiological adaptations that improve heat dissipation, lessen thermal load, and reduce cardiovascular strain during strenuous exercise in hot and humid environments. These adaptations improve exercise-heat tolerance, enhance aerobic performance, and most critically, reduce the risk of exertional heat illness (13, 30, 42, 45, 51). However, HA induced benefits are transient and decay within days to weeks depending on whether dry or humid HA was applied (1, 9, 13, 42, 45, 51-53).

The thermoregulatory adjustments derived from HA, namely increased sweat rate and reduced exercising rectal temperature (T_{re}) and heart rate (HR), must be sustained for the continued health and safety of individuals who periodically physically exert themselves in hot environmental conditions. For instance, military personnel (51) or industrial workers (52) employed in hot environmental conditions return home between deployments or vacation in cooler climates. Additionally, collegiate and professional athletes commonly live and train in cooler climates but compete in unaccustomed oppressively hot environments. For example, athletes train in temperate climates but compete(d) in the Beijing or Brazil Olympics, Brazil or Qatar World Cup, or the southern United States periodically during intercollegiate competition (34). Thus, understanding HA decay and methods to extend HA adaptations is needed.

In comparison to HA induction, few authors have investigated the decay of HA, often with unclear or conflicting findings regarding when adaptations are lost (5, 39, 49). Even fewer studies examined re-acclimation to heat using several consecutive days of exercise-heat exposure after a period of decay (9, 40, 51). Sacrificing sequential days

for exercise-heat exposure to re-gain HA may not be feasible for military, occupational, and recreational athletes with demanding training and travel schedules. Alternatively, in a review of literature, Taylor (48) conservatively hypothesized that one day of heat exposure for every five days without should preserve HA associated adjustments after initial HA efforts. The effectiveness of HA maintenance protocols via intermittent exercise-heat bouts remain largely unknown and represent a flexible alternative for those with congested training and/or travel schedules. Therefore, this study aims to investigate the efficacy of an intermittent exercise-heat exposure intervention 25 days after initial HA efforts. This knowledge will guide best practices to mitigate heat adaptation decay for sustained protection against thermal injury following initial HA efforts.

Methods

Experimental design. We used a randomized control trial design to evaluate the effectiveness of a periodic exercise-heat exposure intervention after HA. Before experimental testing, a baseline lab visit occurred to physically characterize our sample population. To control for factors known to affect thermoregulation and HA decay (7), participants were match-paired by self-reported physical activity, maximal oxygen consumption (VO_{2max}), and body surface area at baseline. Matched participants were randomly divided into either an intermittent heat exposure (IHE) or no heat exposure group (NHE). All participants completed the same HA protocol and standardized heat stress tests (HST) were administered before (Pre HA) and after (Post HA) the HA protocol to confirm HA. After HA was induced, participants completed four HSTs in either a hot (IHE; n=9) or thermoneutral (NHE; n=7) environment every fifth day (+5,

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+10, +15, +20d). On day 25 (+25d), both groups performed a final HST in hot environmental conditions to assess the efficacy of the intervention. Twenty-five days represents about the time frame with most HA associated adaptations are expected to decay (5, 39, 41, 49) and agrees with Givoni and Goldman's (23) prediction of HA decay given our protocol duration. Two participants were removed from analysis due to orthopedic injury sustained outside of the study.

Participants. Eighteen recreationally active (physical activity 1-5 times per week with VO_{2max} > 45 ml·kg·min^{-1}) college-aged males volunteered to participate in the study. Participants were assumed to be non-heat acclimated as testing took place from October-March in the northeast United States where ambient conditions averaged 10.4±2.2℃ and 70.5±6.5 %RH. Additionally, no participant reported employment or frequent heavy exercise in hot environmental conditions for one month prior to the study. To be enrolled in the study, participants without metabolic, cardiovascular, respiratory, or musculoskeletal injury limiting exercise, prescription or over-the-counter medication, and no history of exertional heat illness within the past three years. Written informed consent was obtained prior to testing according to University institution review board policy for human subject testing.

Baseline testing. The baseline lab visit included perceptual scale familiarization, self-reported physical activity questionnaire, and measurement of VO_{2max}, body fat, height and body mass. Subjects were familiarized with the following perceptual scales: thermal sensation (19), fatigue, and the OMNI scale of perceived exertion (50). Height and body mass were measured to the nearest 0.1 cm and 0.01 kg (T51P, Ohaus, Pine Brook, NJ), respectively, and used to calculate body surface area (15). We calculated

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percent body fat using a 3 site (chest, abdomen, thigh) skinfold (Lange skinfold calipers, Beta Technology, Inc., Cambridge, MD) (26). VO_{2max} was determined using a ramping protocol on a motorized treadmill monitoring expired gases with open-circuit spirometry (True One 2400® Metabolic Measurement System, Parvo-Medics Inc., Provo UT) and heart rate (HR) telemetry (Model T5K564, Timex Group USA, Inc., Middlebury, CT). VO_{2max} was confirmed if 3/4 following criteria were met: HR within 10 bpm of age predicted max, respiratory exchange ratio ≥1.10, OMNI ≥9, and/or a VO_2 plateau with increased workload (10). VO_{2max} was also assessed after HA and the 25-day intervention.

Heat acclimation protocol. Before all lab visits, participants refrained from alcohol and unaccustomed or strenuous exercise for 24 hours and caffeine for eight hours. Each participant completed 10-11 days of exercise (90-240 min) in a hot environment (40℃ and 40% relative humidity [RH]) within a 12-13 day period. During six of the HA days, a 90 min hyperthermia-controlled technique was employed to provide a progressive overload stimulus theoretically optimizing heat adaptation (18, 49). During these HA days, work load was adjusted so that rectal temperature (T_{re}) reached 38.5℃ within 30 min and remained ≥ 38.5℃ for the remaining hour. For the other HA days, participants performed intermittent treadmill exercise (45-80% VO_{2max}) for 2 or 4 hours with periodic rest breaks totaling 10 or 60 min, respectively. Importantly, regardless of exercise protocol T_{re} was elevated ≥ 38.5℃ for 56.7 ± 16.8 min during each HA trial. The different exercise-heat exposures during HA induction allowed for the inclusion of additional research aims published elsewhere.

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Heat stress test sessions. Subjects performed treadmill exercise at 45% VO_{2max} with a 2% grade for two 60 min bouts separated by 10 minutes of rest. Participants sat for 20 min prior to exercise allowing for physiological variables to stabilize. Expired gases were collected and analyzed to verify relative exercise intensity (45% VO_{2max}) once steady state exercise was achieved (~5 minutes) during Pre HA. If necessary, treadmill speed was adjusted to elicit 45% VO_{2max}. Exercise was terminated if T_{re} = 40.0°C, signs or symptoms of exertional heat illness, or subject volition. Before and after exercise, blood was collected from the antecubital vein after a 10 min seated rest to allow for fluid compartment equilibration. To account for the influence of physical activity on HA decay during the 25-day intervention, HR telemetry units (RaceTrainer™, Timex, Middlebury, CT) and chest straps with exercise logs were provided during the intervention to characterize intensity and duration.

Measurements. For all lab visits, subjects drank 500 mL of water the night before and 250 mL the morning of the visit to ensure euhydration, defined as a urine specific gravity (U_{sg}) ≤ 1.020 determined by handheld refractometer (A300CL, Atago, Bellevue, WA) (8). If subjects were hypohydrated, 500 mL of water was consumed before beginning the trial. T_{re} was measured using a flexible rectal thermometer inserted 10-12 cm beyond the anal sphincter (model 401, Measurement Specialties, Beavercreek, OH). A thermocrom (DS1921G, Embedded Data Systems, Lawrenceburg, KY) was placed on the right chest, deltoid, thigh, and calf with surgical tape to measure regional skin temperature (T_{sk}) and calculate whole body mean T_{sk} (43). Chest mounted telemetry units recorded HR (RaceTrainer™, Timex, Middlebury, CT).

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Pre-exercise body mass was measured after the bladder and bowels were voided. Body mass loss (BML) was calculated as the difference between pre and post nude body mass after accounting for fluid intake, urine output, fecal and respiratory tract water losses. Sweat rate ($L \cdot hr^{-1}$) was determined by dividing BML by the appropriate time interval. Percent gain or loss of HA adaptations was calculated using the following equation from Pandolf et al. (40):

[(+5, 10, 15, 20, or 25 d Avg value - Post HA Avg value) / Pre HA Avg value - Post HA Avg value)] * 100

A positive value indicates decay or loss of HA associated adaptation and a negative result signifies a gain in HA.

Blood collection and biochemical analysis. Venous blood was collected into vacutainer tubes containing EDTA (Vacutainer, Becton Dickinson, Franklin Lakes, NJ). The tubes were inverted and placed on ice while hematocrit was determined by microcentrifugation and hemoglobin by photometric analysis (HB 201+, Hemocue, Lake Forest, CA) in duplicate. Percent plasma volume change (%PVΔ) was estimated by hematocrit and hemoglobin shifts within each trial (14). Vacutainers were then centrifuged at 3000 rpm for 15 min at 4℃. Plasma samples were immediately aliquoted then stored in a -80℃ freezer until analysis.

All plasma samples from a given subject were evaluated under the same analytical run to avoid intra-assay variation within subjects. Lactate (Pointe Scientific, Canton, MI), cortisol (Calbiotech Inc., Spring Valley, CA), heat shock protein 72 (HSP72; Enzo Life Sciences, Farmingdale, NY), and epinephrine (Rocky Mountain Diagnostics, Colorado Springs, CO) were determined by enzyme-linked immunosorbent

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assays in duplicate. Intra-assay CV's for lactate, cortisol, HSP70, and epinephrine were ≤ 4.0, 10.7, 8.4, and 19.3%, respectively.

Statistical analysis. Heat acclimation was deemed to occur if HR, post-exercise T_{re}, post-exercise T_{sk}, and sweat rate were significantly different from Pre HA to Post HA. We used pre-planned t-tests to assess group and time differences. Repeated measures ANOVA was used to assessed changes in biochemical variables. When significant F-values were detected, Tukey's *post hoc* was used to evaluate pairwise comparisons. Pearson product moment correlation was used to assess the relationship between out-of-lab physical activity and physiological responses at +25d. Means ± standard deviations (SD) were calculated for each independent variable. Mean differences and 95% confidence intervals (95%CI) were constructed to identify differences between groups and time points. Effect size (ES) was calculated using Hedges' g equation which is more robust when small sample sizes are employed. Analyses were completed using SPSS version 21.0 (Armonk, NY, IBM Corp.) with an alpha of 0.05.

Outliers were determined using detrended q-q plots. When necessary, outliers, and missing data (< 2%) were replaced with group averages for the respective time point. Epinephrine data were not normally distributed and were natural log transformed prior to statistical analysis to satisfy the normality assumption for parametric testing. Epinephrine concentrations are reported as the absolute change from pre- to post-exercise (pre - post). HSP72 data at baseline (Before HA Pre) were normalized to group baseline values prior to statistical analyses to characterized baseline variability. All other time points were normalized to the individual baseline value.

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Results

Subject characteristics were similar between NHE and IHE (Table 2.1).

Heat acclimation induction. Eight subjects were not able to complete the Pre HA HST; five of the eight subjects exhibited exertional heat illness symptomology and three subjects reached the lab T_{re} cut-off criteria of 40.0℃. This inability to complete the HST prior to HA was expected as our subjects were not acclimated prior to enrollment.

Table 2.1 Subject characteristics

Measure	IHE (n=9)	NHE (n=7)	p-value
Age (y)	23.9 ± 3.7	21.1 ± 2.4	0.11
Body mass (kg)	72.8 ± 6.9	73.1 ± 9.4	0.95
Height (cm)	179.5 ± 4.9	179.1 ± 7.7	0.67
Body surface area (m^2)	1.91 ± 0.10	1.91 ± 0.14	0.89
Body fat (%)	8.8 ± 4.8	9.9 ± 4.4	0.64
VO_{2max} ($ml \cdot kg^{-1} \cdot min^{-1}$)	53.5 ± 5.2	56.9 ± 5.8	0.24
Physical activity ($min \cdot wk^{-1}$)	279.1 ± 138.4	222.0 ± 136.5	0.42

Values are mean ± SD. $p \leq 0.05$ denotes significance (NHE vs. IHE).
IHE = intermittent heat exposure; NHE = no heat exposure.

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After HA, only one subject did not complete Post HA; this subject reached a T_{re} of 40.0℃. Although this individual was not able to complete Pre or Pre HA, suggesting that he did not acclimate, his other physiological responses demonstrate that he did (i.e., HR, T_{sk}, and thermal sensation).

Post-exercise T_{re} decreased after HA in both groups (mean difference (Pre HA-Post HA), 95%CI; NHE = 0.35℃, 95%CI [0.01, 0.70], p = 0.05; IHE = 0.75℃, 95%CI [0.28, 1.22], p = 0.006; Figure 2.1). Post-exercise T_{sk} was lower following HA in NHE (1.34℃, 95%CI [0.81, 1.88], p = 0.001) but not IHE (0.64℃, 95%CI [-0.33, 1.60], p = 0.17; Figure 2.2). HR was reduced in NHE (16 bpm, 95%CI [5, 27], p = 0.011) and IHE (16 bpm, 95% CI [6, 25], p = 0.006; Figure 2.3) following HA. Sweat rate increased after HA in NHE (0.33 $L \cdot hr^{-1}$, 95%CI [0.11, 0.59], p = 0.011) and IHE (0.27 $L \cdot hr^{-1}$, 95%CI [0.02, 0.51], p = 0.038; Figure 2.4). Perceptual responses (thermal sensation, fatigue, and effort perception) were all reduced after HA ($p < 0.05$; Table 2.3).

Intermittent heat exposure period. During the intermittent heat exposure after 10-11 days HA, HSTs were every 4.1 ± 0.8 (mean ± SD) days. On days +5d, +10d, +15d, and +20d, IHE completed the same exercise protocol as NHE, but in stressful environmental conditions (IHE: 39.8 ± 1.3℃, 37.1 ± 5.5 % RH; NHE: 23.8 ± 1.2℃; 21.7 ± 13.5% RH). Comparisons between IHE and NHE for days +5d, +10d, +15d, and +20d are not shown for all variables, but post-exercise T_{re}, T_{sk}, thermal sensation, HR, sweat rate, and %PVΔ were different ($p < 0.05$).

Rectal and skin temperature. Environmental conditions for NHE versus IHE were different ($p < 0.001$) on +5d, +10d, +15d, and +20d as were post-exercise T_{re} (data not shown, $p < 0.05$). Post-exercise T_{re} was not different between NHE and IHE at +25d

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(mean difference (CON-IHE) = 0.47℃ (95%CI [-0.24, 1.19], ES = 0.68, p = 0.18; Figure 2.1). Because we were interested in changes throughout the 25 day intervention, we evaluated within group differences to determine if patterns diverged. Indeed, post-exercise T_{re} at Post HA, +5d, +10d, +15d, and +25d were lower than Pre HA in IHE (Table 2.2). Contrastingly, NHE post-exercise T_{re} was lower (vs. Pre HA) at Post HA, but not at +25d (Figure 2.1).

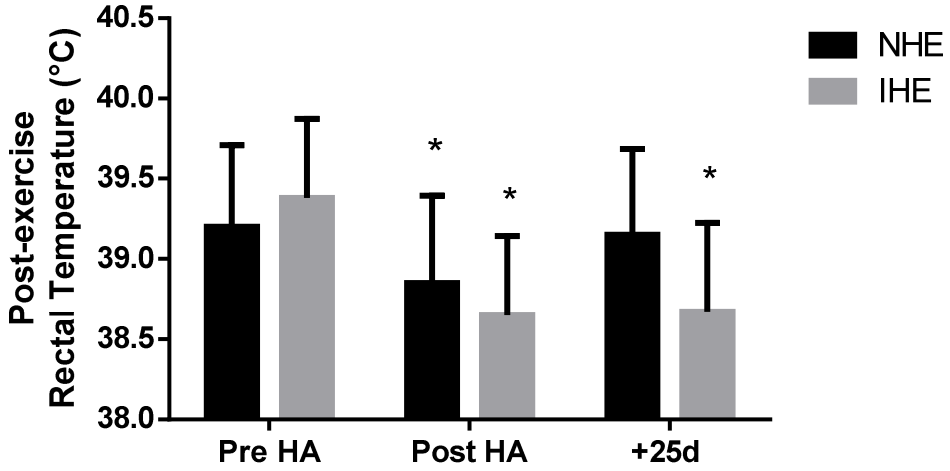

Figure 2.1. Post-exercise rectal temperature response by group over the intervention. * p ≤ 0.03 from Pre HA. NHE = no heat exposure group; IHE = intermittent heat exposure group; HA = heat acclimation. Data are means and 95%CI.

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At +25d, post-exercise T_{sk} was similar between groups (mean difference (CON-IHE) = 0.65℃ (95%CI [-0.17, 1.47], ES = 0.85, p = 0.11; Figure 2.2). Post-exercise T_{sk} was lower only at +5d and +10d compared to Pre HA in IHE (Table 2.2). In NHE, Post HA and +25d post-exercise T_{sk} were lower than Pre HA (p = 0.008).

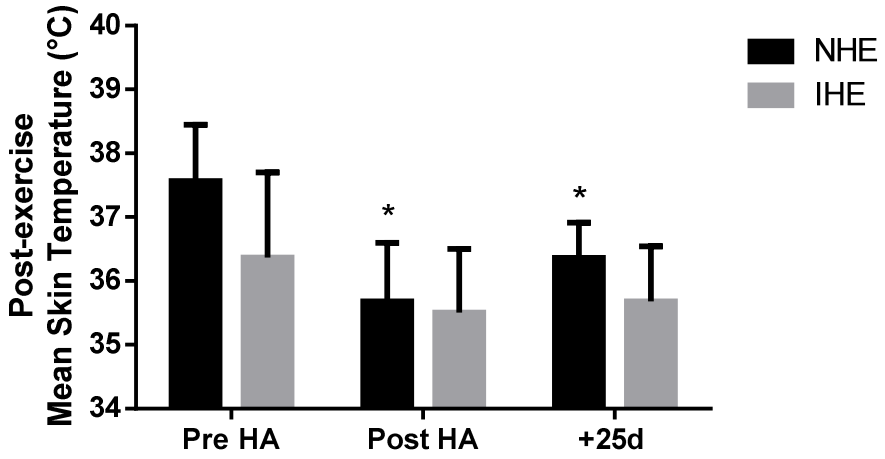

Figure 2.2. Post-exercise skin temperature response by group. * Different from Pre HA, p ≤ 0.008. NHE = no heat exposure group; IHE = intermittent heat exposure group; HA = heat acclimation. Data are means and 95%CI.

Cardiovascular and hydration measures. We measured HR responses as indicators of cardiovascular strain from added environment stress in IHE. Post-exercise HR was 28 bpm (95%CI [8, 48], ES = 1.41, p = 0.01) lower in IHE compared to NHE at +25 d (Figure 2.3). In IHE, +5d and +20d post-exercise HR was higher compared to Post HA, all other time points were similar to Pre and Post HA (p ≥ 0.16) (Table 2.3). In NHE, post-exercise HR was higher on +25d compared to Post HA (p = 0.001) but not Pre HA (p = 0.057).

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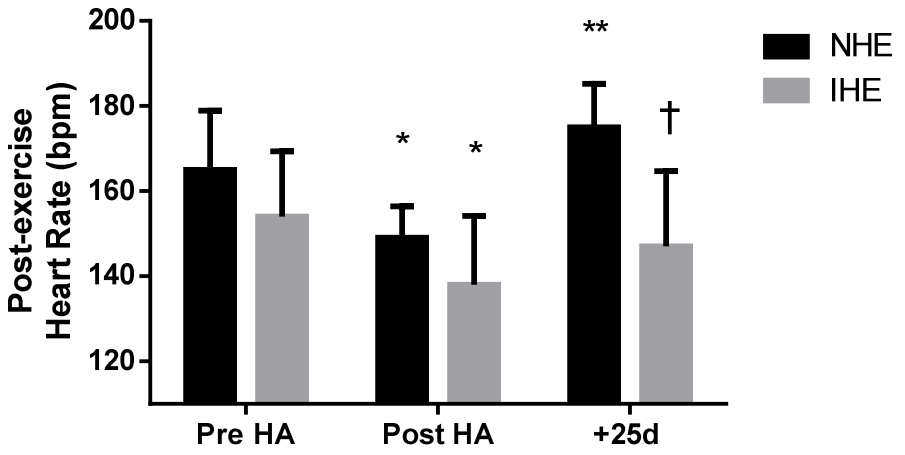

Figure 2.3. Post-exercise heart rate response by group. * p = 0.002 from Pre HA. † p = 0.01 between groups. ** p = 0.001 from Post HA. NHE = no heat exposure group; IHE = intermittent heat exposure Group; HA = heat acclimation. Data are means and 95%CI.

After HA, VO_{2max} increased 6% in IHE (before HA = 53.5 ml·kg^{-1}·min^{-1}, 95%CI [49.5,57.4]; after HA = 56.7 ml·kg^{-1}·min^{-1}, 95%CI [52.7,60.7], p = 0.02) but did not change in NHE (before HA = 56.1 ml·kg^{-1}·min^{-1}, 95%CI [51.4,60.7]; after HA = 57.8 ml·kg^{-1}·min^{-1}, 95%CI [53.2,62.4], p = 0.33). After +25d, VO_{2max} remained unchanged in NHE (57.3 ml·kg^{-1}·min^{-1}, 95%CI [53.8,60.1], p = 0.52) and was not different from before HA in IHE (55.2 ml·kg^{-1}·min^{-}1, 95%CI [50.0,60.3], p = 0.23). VO_{2max} was not different between groups at any time point (p ≥ 0.22).

Relative plasma volume change was not different between NHE (-12.1%, 95%CI [-15.8,-8.4]) and IHE (-10.8%, 95%CI [-15.0,-6.6]) at +25d (mean difference = 1.3%, (95%CI [-4.1, 6.6], p = 0.62). In IHE, %PVΔ at Pre HA (-6.7%, 95%CI [-9.2,-4.1]), Post HA (-7.0%, 95%CI [-10.9,-3.1]), and + 5d (-6.6%, 95%CI [-8.9,-4.1]) were similar (p > 0.05) but greater relative plasma volume losses were observed at +10d (-12.2%, 95%CI

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[-15.4,-9.0]), +15d (-10.9%, 95%CI [-15.7,-6.0]), +20d (-9.4%, 95%CI [-13.7, -5.0]), and +25 d, all $p < 0.05$.

Pre-exercise U_{sg} was not different between groups during any lab trial ($p > 0.09$). Percent body mass loss was not different between NHE and IHE at Pre HA (NHE = 2.52±0.99%; IHE = 3.05±0.47%, $p = 0.18$), Post HA (NHE = 0.95±6.64%; IHE = 3.69±1.24%, $p = 0.24$), or +25d (NHE = 2.78 ±0.72 %; IHE = 3.20±0.63%, $p = 0.21$).

Sweat rate. At +25d, sweat rate was similar between groups (0.13 $L \cdot hr^{-1}$, 95%CI [-0.21, 0.46], ES = 0.36, $p = 0.44$) and was not different from Pre HA or Post HA in both groups (Figure 2.4) ($p \geq 0.15$). Sweat rates at +5d, 10d, 15d, and +20d were similar to Pre and Post HA in IHE ($p \geq 0.15$) (Table 2.3).

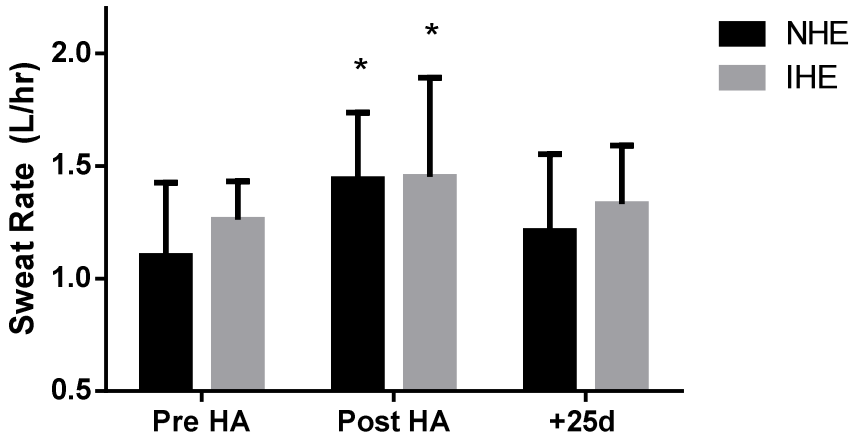

Figure 2.4. Sweat rate response by group over time. * $p = 0.04$ from Pre HA. NHE = no heat exposure group; IHE = intermittent heat exposure group; HA = heat acclimation. Data are means and 95%CI.

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Physical activity and HA decay. Physical activity duration (IHE = 245.6±245.6 min [range = 0 - 600]; NHE = 220.6±159.3 min [range = 0 - 471], p = 0.74) and intensity (IHE = 61.9 10.4% HR_{max} [range = 61.1 - 79.6] versus NHE 68.3±10.6% [range = 51.5 - 68.2] HR_{max}, p = 0.07) recorded outside-of-lab visits during the 25-day intervention was highly varied and not different between groups. Exercise intensity (%HR_{max}) during out-of-lab exercise and +25 d post exercise HR were inversely correlated (r = -0.89, p = 0.017) in IHE only. Exercise intensity (%HR_{max}) during out-of-lab exercise and +25 d post exercise T_{re} approached a significant relationship (r = -0.77, p = 0.072) in IHE only.

To evaluate effectiveness of the intervention to minimize HA adaption loss, the percent decay of HA adaption was calculated (40) after each exercise-heat exposure following HA. Group comparison of HA decay at +25d is illustrated in Figure 2.5. Table 2.2 shows HA adaptation decay within IHE throughout the 25-day intervention.

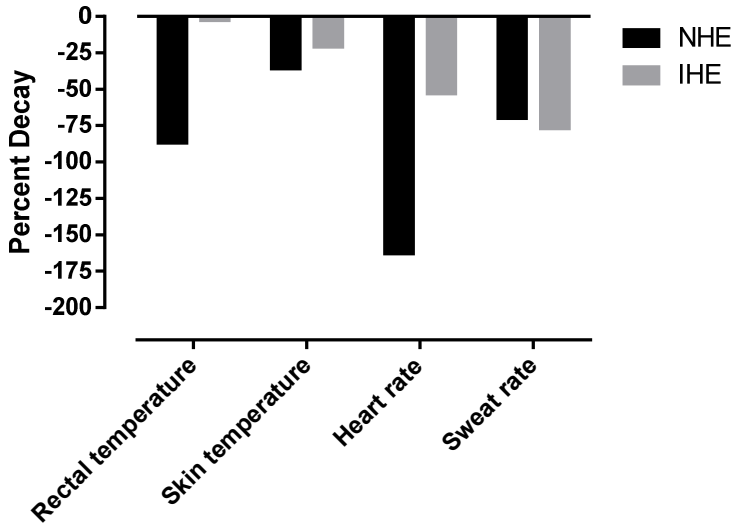

Figure 2.5. Group comparison of adaptation decay 25 days after initial heat acclimation. Negative value denotes a loss of adaptation. NHE = no heat exposure group; IHE = intermittent heat exposure group. % decay calculated using the equation from (40).

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Table 2.2. Decay of selected adaptations in IHE 25 days post heat acclimation

	Pre HA	Post HA	+5d	+10d	+15d	+20d	+25d
Post-exercise T_{re} (℃)	39.38 (38.9,39.9)	38.65* (38.3,39.4)	38.63* (38.1,39.1)	38.66* (38.3,39.0)	38.84 (38.1,39.1)	38.80* (38.4,39.2)	38.67* (38.1,39.2)
% decay			-2.7	1.4	26.0	20.5	2.7
Post-exercise T_{sk} (℃)	36.38 (35.4,37.4)	35.74 (35.2,36.4)	35.46* (34.8,36.1)	35.29* (34.9,35.7)	35.77 (34.9,36.7)	35.72 (34.9,36.5)	35.68 (35.0,36.3)
% decay			4.7	-18.0	34.9	25.1	18.4
Post-exercise HR (bpm)	154 (138,170)	139* (122,155)	154** (139,169)	148 (132,165)	151 (138,164)	152** (140,164)	147 (130,165)
% decay			100.0	60.0	80.0	86.7	53.3
Sweat rate ($L \cdot hr^{-1}$)	1.27 (1.13,1.40)	1.53* (1.24,1.83)	1.40 (1.19,1.62)	1.41 (1.17,1.66)	1.32 (1.09,1.55)	1.42 (1.19,1.66)	1.33 (1.13,1.54)
% decay			50.0	46.2	80.8	42.3	76.9

Data are means (95%CI). Negative value = gain, positive value = loss of adaptation.
* lower than Pre HA, $p \leq 0.042$. ** greater than Post HA, $p \leq 0.032$. T_{re} = rectal temperature, T_{sk} = skin temperature. % decay calculated using the equation from Pandolf et al. (40).

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Perceptual responses. Post-exercise fatigue, OMNI scale of exertion, and thermal sensation on +5d, +10d, +15d, +20d, and +25d were lower than Pre HA ($p \leq 0.022$) but similar to Post HA ($p \geq 0.056$) in IHE (Figure 2.3). Post-exercise +25d OMNI (1, 95%CI [-4, 2], ES = 0.40, p = 0.45), thermal sensation (0.5, 95%CI [-1.5, 0.5], ES = 0.63, p = 0.30), and fatigue (2, 95%CI [-0.5, 4.0], ES = 0.82, p = 0.14) were similar between groups.

Table 2.3. Perceptual responses at the end of each HST by group

	NHE (n=7)			IHE (n=9)		
	Thermal	OMNI	Fatigue	Thermal	OMNI	Fatigue
Pre HA	7.0[§] (6.0,8.0)	6[§] (4,8)	6[§] (4,9)	**6.5**[§] (6.0,7.0)	**7**[§] (5,9)	**6**[§] (4,8)
Post HA	6.0 (5.5,7.0)	4 (2,5)	5 (3,7)	5.5 (5.0,6.0)	3 (2,5)	3 (1,4)
+5d	4.0 (3.0,4.5)	2 (1,3)	2 (0,4)	5.5[†] (5.0,6.0)	3 (1,4)	3 (1,4)
+10d	4.0 (3.5,4.0)	2 (1,3)	2 (1,4)	5.5[†] (5.0,6.5)	3 (1,5)	3 (1,5)
+15d	4.0 (3.5, 4.5)	2 (0,4)	2 (1,4)	5.5[†] (5.0,6.0)	3 (1,5)	3 (1,5)
+20d	4.0 (3.5,4.0)	2 (0,4)	2 (1,4)	6.0[†] (5.5,6.5)	3 (1,4)	3 (1,5)
+25d	6.5 (5.5,7.0)	5 (3,7)	5 (4,7)	6.0 (5.0,6.5)	4 (1,6)	3 (1,5)

Data are mean (95%CI). [§] $p \leq 0.017$ from Post HA. **Bold** signifies $p \leq 0.022$ from all subsequent time points. [†] $p \leq 0.01$ between groups. By design, +5d, +10d, +15d, +20d responses in NHE were different from Pre and Post HA and +25d, $p \leq 0.001$.

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Circulating stress response. Cortisol concentrations were higher at the end of exercise before HA compared to baseline levels in both groups (p = 0.007) but at +25 d post, lower post-exercise concentrations were observed in IHE compared to NHE (p = 0.009) (Figure 2.7). Cortisol was higher at +25 d post compared to +25 d pre in NHE (p = 0.04) but not IHE (p = 0.29).

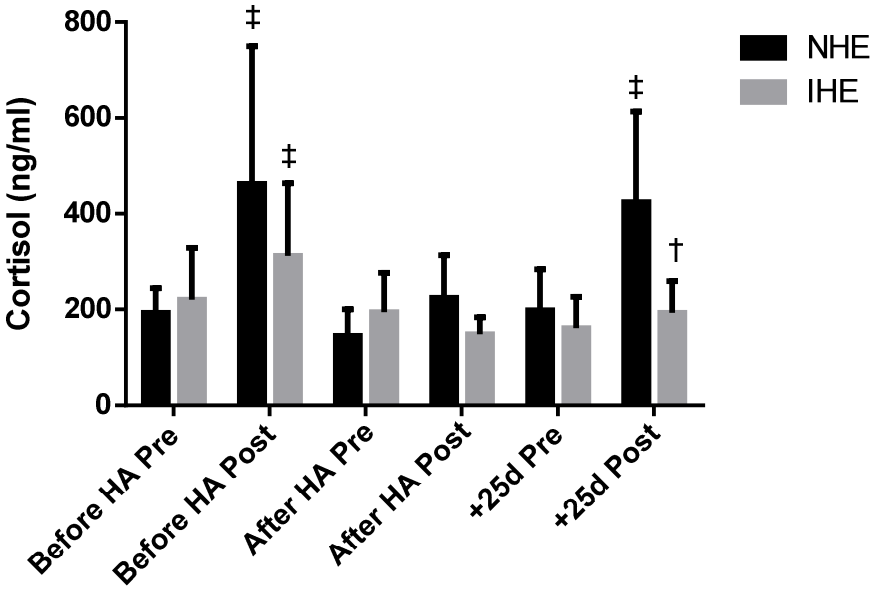

Figure 2.7. Cortisol responses before and after select HSTs. † p ≤ 0.006 between groups. ‡ Different from pre-exercise value of that trial (p ≤ 0.04). NHE = no heat exposure group; IHE = intermittent heat exposure group; HA = heat acclimation. Data are means and 95%CI.

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There were no differences in absolute change score (pre-post) epinephrine concentrations between groups at Pre HA (IHE: 0.22±0.13 nmol·L^{-1} versus NHE: 0.33±0.7 nmol·L^{-1}, p = 0.051). An interaction effect (p = 0.005) for circulating epinephrine was observed whereby epinephrine decreased after HA in NHE (p = 0.048) and trended downward in IHE (p = 0.065). At +25 d epinephrine levels increased only in NHE (Figure 2.8; p = 0.046).

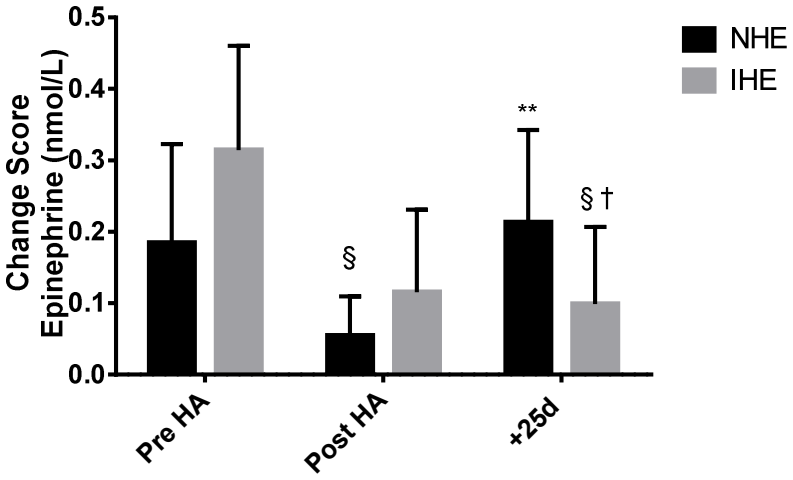

Figure 2.8. Change scores (pre-post exercise) of epinephrine before and after HA and at +25d. § p ≤ 0.048 from Pre HA. † p = 0.046 between groups. ** p = 0.048 from Post HA. NHE = no heat exposure group; IHE = intermittent heat exposure group; HA = heat acclimation. Data are means and 95%CI.

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There were no differences in the raw HSP70 data between IHE (8.8±21.1 ng/mL) and NHE (1.1±1.4 ng/mL) at baseline (p = 0.33). Normalized pre-exercise HSP70 levels were not different at any time point (p = 0.61). HSP70 concentrations increased 22-45% after each HST regardless of group (Figure 2.9; p ≤ 0.032).

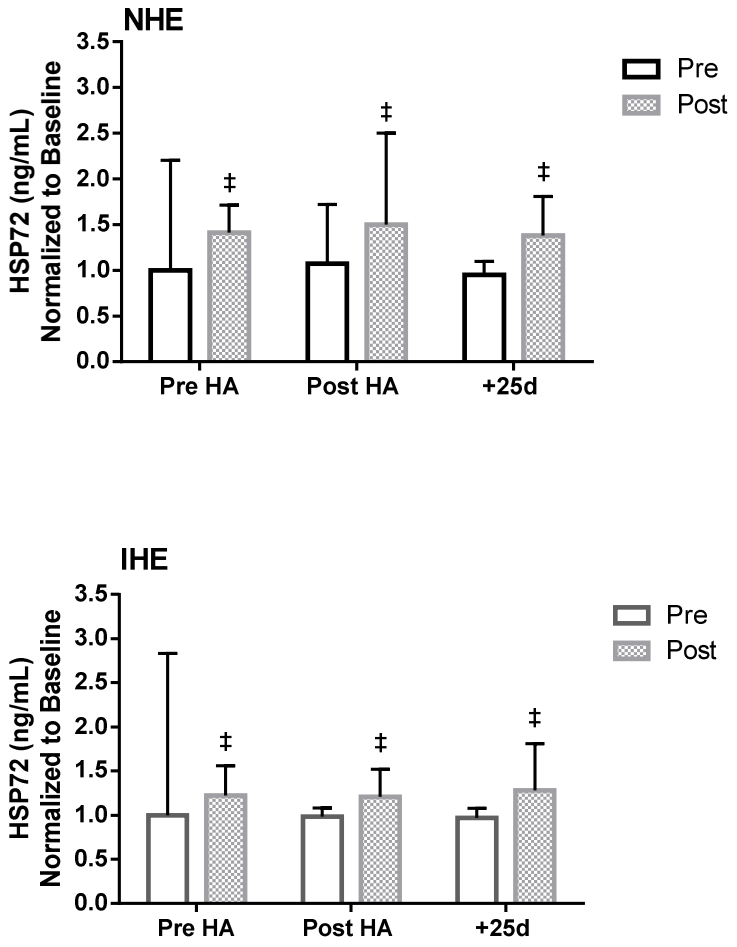

Figure 2.9. Normalized HSP70 concentrations increased similarly after HSTs. ‡ Different from pre-exercise value of the respective trial (p ≤ 0.032). NHE = no heat exposure group; IHE = intermittent heat exposure group. HA = heat acclimation. Data are means and 95%CI.

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Discussion

This study tested the efficacy of an intermittent heat exposure protocol (IHE) aimed at maintaining adaptations to the heat. After HA induction, IHE completed periodic exercise-heat exposure every fifth day while NHE (also heat acclimated) completed a similar protocol in a thermoneutral environment. We observed that post-exercise HR was lower in IHE (vs. NHE) on the 25th day after initial HA. Similarly, post-exercise T_{re} was lower in IHE (vs. NHE) on the 25th day after initial HA. Our findings suggest that intermittent exercise-heat exposure at least every fifth day after HA mitigates some components of HA decay.

Physiological and perceptual responses to +25d. IHE maintained the HA-induced adaptations that reduce physiological strain, 25 days after HA. On day 25, baseline HR was similar between groups but after 20 min of seated rest in the hot chamber NHE (vs. IHE) HR was elevated and this HR difference persisted through the +25d trial ($p \leq 0.009$). Dehydration during exercise and hyperthermia increases HR (6), but our groups were euhydrated ($U_{sg} \leq 1.020$) before and dehydrated similarly during the trial. Although post-exercise T_{sk} and T_{re} were statistically similar, the moderate to large effect sizes ($g = 0.68$-1.14) suggest physiologically important differences existed. It is well known that hyperthermia (increased T_{sk} and T_{re}) and exercise increase cardiovascular strain by shunting blood to cutaneous and muscular vascular beds, away from central blood volume (44). Jose et al. (27) actually defined a mathematical proportion between HR and core temperature, showing that HR increases approximately 7 bpm for every 1°C increase in body temperature. Thermal challenges that raise T_{sk} and subsequently increase skin blood flow create competition for limited

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cardiac output, also elevating HR (12, 28, 54). It is therefore likely, that our, albeit non-significant, group differences in thermal strain (T_{sk} and T_{re}) contributed in part to differential HR responses to +25d.

In addition to thermal strain, post-exercise circulating concentrations of epinephrine were greater in NHE (vs. IHE), also contributing to the group differences in HR at +25d. Indeed, epinephrine is released in response to high levels of exercise-heat stress (11, 20) and, when bound to myocardial β_2-receptors epinephrine causes tachycardia. Although not measured in the current study, HR variability studies attribute lower HR responses in HA individuals to increased parasympathetic activity (16, 17). In sum, the combination of these mechanisms may help to explain the significant HR discrepancy between groups at +25d.

Although post-exercise T_{re} between groups was not statistically significant ($p = 0.18$), the mean difference of 0.47°C may have practical importance given the moderate effect size ($g = 0.68$). Previous studies modulating body temperature with either ice slurry ingestion or water perfused suits have shown that a T_{re} reduction of similar magnitude (~0.50°C) mitigated cardiovascular strain (24), perceived exertion (24, 29) and improved time to exhaustion (24, 29, 47) during exercise in heat. In the present study, the mean difference between post-exercise T_{re} in unacclimated subjects that prematurely terminated versus finished Pre HA was 0.59°C. This difference is comparable to the 0.47°C post-exercise T_{re} group difference at +25d. Thus, a 0.47°C T_{re} reduction appears advantageous, especially in unacclimated hyperthermic athletes, and would aid in avoiding hyperthermia-induced fatigue and exertional heat illness.

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Despite the reduced cardiac strain in IHE at +25d, perceptual responses between groups were similar. Others have also observed dissociation between physiological and perceptual strain at two (45) and four (51) weeks post HA, but some have not (13). Although it is possible that participants in IHE did not perceive reduced thermal sensation or exertion, the truncated thermal (4.0-8.0 unit) and OMNI scale of perceived exertion (1-10 unit) may not have been sensitive enough to discern small but meaningful differences physical strain compared to other commonly used scales (e.g., 6-20 unit Borg scale) (51). Given the growing number of studies observing the dissociation between measures of perceptual and physiological strain weeks after HA, future investigation is warranted to elucidate these relationships.

HA adaptation decay. Because only IHE completed exercise in the heat, we can only assess their HA adaptation decay during the 25 day intervention. Two weeks after HA, post-exercise HR decayed 80%, similar to the 76-87% loss reported by Williams et al. (52) but greater than that observed by more recent studies: 17-35% (45), 20% (40), 33% (51), and 53-58% (42). Similar to IHE HR findings, post-exercise T_{re} decayed 30% two weeks after HA, a result in agreement with the 28-43% loss reported by Poirier et al. (42) but higher than others that observed 15% (51), 18% (40), and 22% (45) decay.

It is generally accepted that HA-induced adaptations are lost roughly three weeks after HA without heat exposure (1, 5, 21, 39, 52, 53). Only three recent studies (including ours) report post-exercise HR and T_{re} decay four weeks after initial HA, with variable findings. Weller et al. (51) reported a 27% decay in HR, Adams et al. (1) observed virtually complete loss, and we found HR decayed 53% in IHE, an improvement compared to the 80% loss observed at +15d. In line with expectations, 25

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days after HA NHE lost nearly all HA-induced HR (163%) and T_{re} (87%) adaptation. In contrast, Weller et al. (51) observed a 9% gain (lowering) in T_{re} 26 days after HA without heat exposure. Conflicting with Weller et al., we observed that after HA and periodic heat exposure every fifth day for 25 days, IHE experienced a 2.7% loss in post-exercise T_{re}. Nonetheless, our data (e.g., NHE vs. IHE) support the contention that intermittent heat exposure at least every fifth day minimized T_{re} decay and cardiovascular strain during exercise-heat stress up to 25 days after initial HA efforts.

It is unclear why, four weeks after HA, post-exercise HR and T_{re} decayed more so in IHE than that observed by Weller et al. (51), who did not employ periodic heat exposure. Differences in HA protocols may explain, in part, these disparate findings. Weller et al. (51) heat acclimated subjects using a constant workload for 60 minutes then increased intensity to maintain T_{re} above 38.5℃ for the remaining 40 minutes. Similarly, Daanen et al. (13) employed a biphasic protocol (constant workload for 60 minutes followed by an incremental load up to additional minutes) and observed 18 days after HA that post-exercise T_{re} further decreased 0.59℃ beyond HST responses observed immediately after HA. The dissonance among HA decay studies 3-4 weeks after HA necessitates additional research to elucidate whether HA-induced adaptations substantially decay (NHE) (1, 52), are sustained (40, 51), or improve (13) when using mixed protocols to induce HA.

At +25d, HR and T_{re} adaptations were better maintained in IHE compared to NHE. The substantial loss of adaptations in NHE is on par with previous studies in manual laborers showing HA adaptation decayed 1-3 weeks after HA but disagrees with others (51). Wyndham and Jacobs (53) observed significant losses in oral temperature

(0.7°F) in 73 acclimated miners after 6 days in cool conditions, although it is uncertain whether complete acclimation was achieved. Williams et al. (52) examined HA losses after 1, 2, and 3 weeks away from heat in 60 heat acclimated miners and found progressive losses overtime with virtually complete HR decay and half of the gain lost in T_{re} at 3 weeks. Adams et al. (1) noted substantial heat tolerance reductions after one week removed from heat exposure in 16 heat acclimated workers and by the 4^{th} week, nearly complete decay. It is important to note that subjects in these studies were not exposed to heat during the decay period, similar to NHE in the present study.

With one exception (51), the differences in decay rates among the earlier (1, 52, 53) and more recent current studies (13, 40, 42, 45) may be that HA adaptation decay was mitigated in studies (13, 40, 42, 45) that periodically assessed physiological responses during the decay period via exercise-heat exposure. These intermittent exercise-heat exposures may have served to elicit adaptive stimuli to effector organs (sweat glands, heart, cutaneous vascular beds) and in turn minimize decay. Periodically assessing physiological responses during HA decay was an unavoidable methodology consequence given the directives of these studies but important in that they support of our data and the contention that intermittent exercise-heat exposure prolongs the beneficial adaptations of HA.

The dramatic decay in post-exercise HR at +25d in NHE (163%) suggests that not only did NHE participants lose the HR benefits of HA, but realized additional losses, possibly due to detraining. Indeed, 2/7 NHE subjects recorded no out-of-lab exercise while 5/7 NHE subjects reduced out-of-lab physical activity compared to activity levels at study genesis. Although VO_{2max} was not different within or between groups at Post

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HA and after the 25 day intervention, it may be the absence of additional adaptive stimuli from habitual out-of-lab exercise and not changes in VO_{2max} per se, that contributed to HA adaptation decay or maintenance (7). In support of this notion, in IHE but not NHE, a strong inverse relationship was observed between out-of-lab exercise intensity and +25d post-exercise HR. Physical activity, even in thermoneutral environments, can elevate body temperature due to metabolic heat production and in turn, impart additional thermal adaptation stimuli to sustain key thermoregulatory and circulatory adjustments (3, 7, 35, 36, 38). Because we did not control out-of-lab exercise intensity, we assessed if out-of-lab exercise or a change in training status (e.g., change in VO_{2max} over the 25 day intervention) influenced +25d post-exercise HR responses between groups. Using these variables as separate co-variates, +25d post-exercise HR was similar between groups ($p \geq 0.12$). This implies that exercise intensity sufficient to induce hyperthermia in addition to intermittent exercise-heat exposure after HA may be prudent practice to reduce HR decay.

Circulating biomarkers. Cortisol and epinephrine concentrations tracked changes in physiological strain before and after HA, as expected (4). At +25d, cortisol and epinephrine concentrations were lower in IHE but not NHE. Cortisol is sensitive to thermal strain (4, 11, 32) and decreases after HA (4). Comparatively, some (11) but not all studies (33) reported increases in epinephrine concentrations after exercise-heat sessions. Discrepancies between relative physiological stress imparted on the subject from exercise and environmental parameters likely explains conflicting reports. Marshall et al. (33) had subjects complete two hours of cycling at 38% VO_{2peak} in 38℃, 60% RH, resulting in a trivial epinephrine increase of 0.11 $nmol \cdot L^{-1}$. Comparatively, Brenner et al.

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(11) exercised participants at ~50% VO_{2max} for 30 minutes in 40℃, 30% RH and observed a significant epinephrine increase of 0.35 nmol·L^{-1}, a result in line with observations from the present study. Cortisol and epinephrine responses at +25d suggest a reduced physiological strain in IHE compared to NHE.

Our subjects achieved classic physiological benefits of adapting to the heat but post-exercise circulating HSP72 concentrations increased uniformly after all HSTs. Studies reporting circulating HSP72 responses to exercise-heat stress are varied with significant increases (22, 31, 55) or no changes observed (22, 25). Several stressors affect circulating HSP72 concentrations, including elevated body temperature to at least 38.5℃ (2, 22, 37). In the present study, post-exercise T_{re} was ≥38.65℃ in all subjects at Pre HA, Post HA, and +25d likely explaining the uniform rise in circulating HSP72 after each HST. Elevated post-exercise circulating HSP72 begins to decline within an hour (31) and returns to resting levels by 24 hours (22) likely due to the high energy requirement of HSP72 translation, transcription, and release into circulation. Thus, it is no surprise that pre-exercise circulating HSP72 concentrations remain unchanged during HA (31, 55), although increased basal concentrations have been reported (46).

Study limitations. A limitation of this study was the extended duration and low intensity of the periodic exercise-heat exposures. Similar HST parameters during the 25 day intervention allowed for the comparison of intervention responses with Pre and Post HA responses. The length of the HST sessions may be problematic to athletes with limited time to devote to sustaining heat adaptation. Importantly, T_{re} decay was substantially reduced in IHE at +25d with 40 hours of periodic low intensity exercise-heat stress. Future research should examine periodic heat-exposure protocols of higher

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intensity and lower duration to determine the appropriate balance of exercise duration and intensity to sustain HA adaptations. Diet and time of day were not controlled which may affect cortisol and T_{re} responses, although no pattern of trial time of day was apparent between groups.

Practical applications. Evidence supporting advice on how long HA is retained and how much exercise-heat stress re-exposure is required to sustain HA is of vital practical importance to athletes, coaches, military and occupational administrators. Our data support the notion of implementing periodic exercise-heat exposure at least once every fifth day to minimize T_{re} decay and cardiovascular strain during exercise-heat stress up to 25 days after initial HA. The exercise-heat regimen examined herein is applicable to individuals who physically exert themselves in oppressively hot environments but train or live in temperate conditions (e.g., military, firefighters, international and collegiate athletes, hazardous material workers). The mitigated decay of T_{re} and reduced cardiac strain following HA implies performance could be enhanced and risk of exertional heat illness reduced for at least one month. Periodic exercise-heat exposures afford logistical flexibility to maintain HA adaptations, contrasting re-acclimation models requiring several consecutive days exercise-heat stress (9, 38, 51), which may place additional burden on training or travel schedules. Undergoing intense physical activity in thermoneutral environments sufficient to elevate body temperature, initiate sweating, and increase cutaneous blood flow may help to sustain some HA adaptations. These data provide initial evidence to help guide evidence based recommendations for practitioners to mitigate HA adaptation decay.

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Appendices

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Univeristy of Connecticut
Department of Kinesiology
Human Performance Laboratory

Consent Form for Participation in a Research Study

Principal Investigator: Douglas J Casa, PhD, ATC
Student Researchers: J. Luke Pryor, Riana R. Pryor, Elizabeth Adams
Study Title: The effect of heat acclimation on repeated bouts of strenuous heat stress, hand cooling efficacy, and the maintenance thereof

Sponsor: Korey Stringer Institute, Center for Health, Injury, and Prevention, Eastern Athletic Trainers' Association, and the National Athletic Trainers' Association

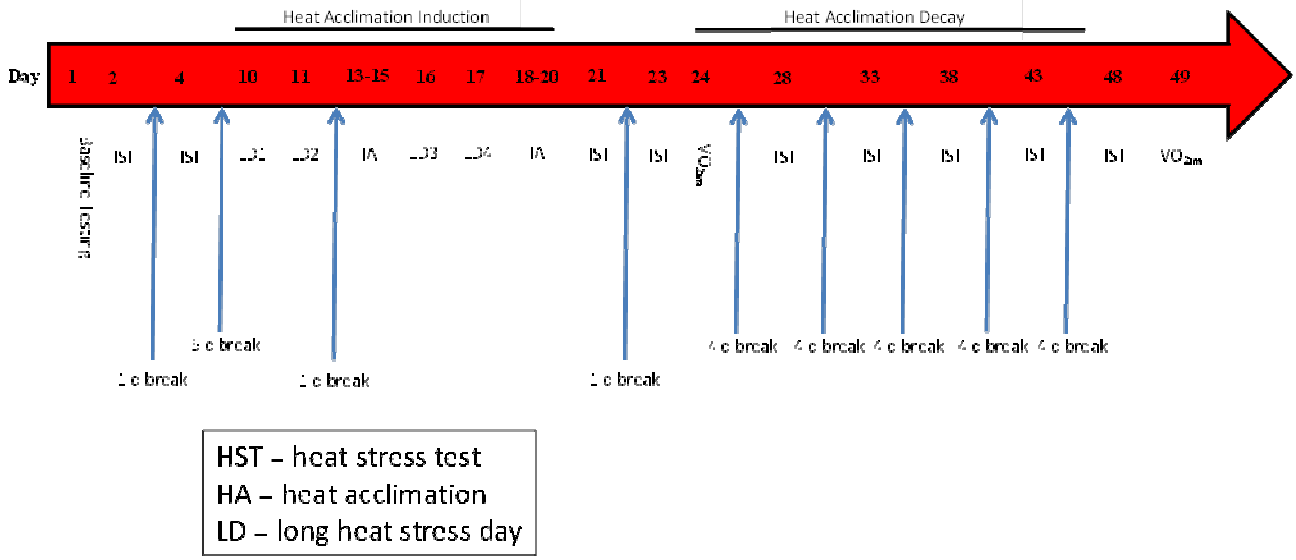
Introduction
You are invited to participate in a research study evaluating the effectiveness of acclimating to the heat on physical activity in the heat, hand cooling efficacy, and the decay heat acclimation adaptations. You are being asked to participate because you are recreationally active, healthy, male, and aged 18-35. This consent form will give you the information you will need to understand why this study is being done and why you are being invited to participate. It will also describe what you will need to do to participate and any known risks, inconveniences or discomforts that you may have while participating. We encourage you to ask questions now and at any time. If you decide to participate, you will be asked to sign this form and it will be a record of your agreement to participate. You will be given a copy of this form.

Why is this study being done?

The purpose of this research study is to evaluate the effect of heat acclimation on repeated bouts of strenuous heat stress, hand cooling efficacy, and the maintenance thereof. It has been shown that heat acclimation reduces the risk of exertional heat illnesses and improves physical activity in the heat. It is unknown if heat acclimation improves hand cooling effectiveness. The lasting effects of long, strenuous days of exercise in the heat have also not been fully studied. Finally, maintaining the beneficial adaptations derived from heat acclimation is important for prolonging the health and safety of those exerting themselves in hot environmental conditions. Collectively, the answers to these research questions will enhance our understanding and utility of heat acclimation in laboratory and field setting.

What are the study procedures? What will I be asked to do?
After signing this informed consent, you will complete a medical history questionnaire to determine whether you meet the inclusion criteria to be part of the study. To allow you

to understand the commitment required to participate in this study, below is a figure illustrating the study timeline and laboratory testing days.



Baseline Testing (BL)

Once you are medically cleared, we will schedule a baseline testing session which will include the following:

1. Complete a training history questionnaire
2. Height, body weight, and body fat percentage determined by skin fold measurement
3. Hand size measurements
4. Grip strength
5. Maximal aerobic capacity (VO_{2max})

Body weight and height will be recorded by a researcher after which percent body fat will be calculated using skinfold measurements at three sites: chest, abdomen, and thigh. Three measurements will be recorded to determine hand size of both hands: hand volume, palm surface area, and finger girth. Hand volume will be measured using water displacement. To determine this you will submerge your hand into a clear cylinder filled to the brim with water. Palm surface area will be measured by tracing the hand as it lies palm side down on a sheet of paper. Lengths from the tips of the fingers and the span of the palm will be used to calculate palm surface area. Finger girth will be measured using a set of finger size measuring rings on both hands. Grip strength will be measured using a hand grip dynamometer. Briefly, you will be seated with elbow flexed to 90°, wrist in a neutral position. You will apply gripping pressure to the dynamometer a total of 3 times to determine average grip strength of both hands.

A graded exercise test on a treadmill using a ramping protocol in a thermoneutral environment will determine your maximal aerobic capacity (VO_{2max}). After a 5 minute warm-up period, you will begin walking at 2% grade. Treadmill speed and/or grade will

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increased each stage until voluntary exhaustion. The VO_{2max} test will be repeated on days 24 and 49.

After baseline testing, you will be randomly assigned to either a heat acclimation or exercise only group. The heat acclimation group will exercise in a heated chamber (104℉, 40% relative humidity) while the exercise only group will undergo the exercise in a cool room (72℉, 40% relative humidity). After the 23rd day of the study (HST 4), the subjects that heat acclimated will be randomly divided into two subsequent groups; a heat exposure group and no heat exposure group. The heat exposure group will exercise in a heated chamber (104℉, 40% relative humidity) while the no heat exposure group will undergo the same exercise but a cool room (72℉, 40% relative humidity) approximately every five days for a 25 day period. You will be afforded the opportunity of a rest day(s) at any point during the study if indicated and will continue the protocol as directed by the researchers.

Prior to all subsequent laboratory visits, you will be instructed to avoid alcohol and strenuous exercise for 24 hours and caffeine for eight hours before testing. You will also drink 500 mL (2 cups) water the night before and 250 mL (1 cup) the morning of this visit to ensure normal hydration upon arrival to the lab. For all laboratory testing, unlimited water will be available for your consumption.

Heat Stress Tests (HST)

To test the effectiveness of a hand cooling device with heat acclimation, you will perform HST in a randomized crossover fashion. For HST 1 and HST 3, you will be randomly assigned to either a hand cooling or control trial, and perform the opposite trial for HST 2 and HST 4. There will be one day between HST 1 and HST 2, as well as one day between HST 3 and HST 4. Following HST 2, there will be approximately five days of rest prior to further testing.

Upon arrival to the lab wearing a T-shirt and shorts, you will provide a urine sample in a clean urine cup to determine hydration. If you are not properly hydrated, you will consume 500 mL (2 cups) water before beginning the test. You will privately insert a rectal thermometer 10 cm past the anal sphincter to ensure your safety during the test. Nude body mass will be recorded whereby you will be weighed behind a door to maintain privacy. Next, you will sit quietly in a chair and fill out an Environmental Symptoms Questionnaire while researchers place small skin temperature buttons placed on your chest, deltoid, calf and thigh to approximate mean skin temperature throughout the exercise bout. A heart rate strap will be applied to your chest and fitted. Resting skin and rectal temperatures, heart rate, and several perceptual scales (thirst, thermal, fatigue, exertion) will be recorded. You will enter the chamber (40℃, 40% relative humidity) and wait for a 20 minute period while your body adjusts to the heat.

Immediately prior to exercise beginning, baseline skin and rectal temperatures, heart rate, perceptual scales will be recorded. You will perform treadmill exercise at 45% VO_{2max} with a 2% grade for two 60-minute bouts with a 10-minute rest in the heat

chamber following each exercise bout. Heart rate, rectal and skin temperature, and perceptuals will be recorded every 10 minutes. A breathable mask will be place over the mouth and nose to collect expired gases for 3-5 minutes to determine oxygen consumption at minute 30 of each 60 minute exercise bout. The researchers will stop exercise for your safety if one of the following occurs: 1) rectal temperature reaches 40.0°C, 2) heart rate > 90% of age predicted maximum for a 5 minute period, 3) you want to stop, 4) unsteady walking gait, or 5) signs or symptoms of heat illness. During the 10 min rest period, you will place your non-dominant hand in the peripheral cooling device (CoreControl, AVAcore Inc.) while seated in the environmental chamber. If you are assigned to the cooling trial, the device will be turned on; for the control trial, the device will remain off. Using a handheld dynamometer, grip strength will be assessed on both hands immediately before and after cooling during the rest periods.

After completing the exercise, you will compete an ESQ and exit the heat chamber and after excess sweat wiped off, a nude body mass will be measured. You will be given a clean urine cup for a post exercise urine sample to determine hydration, as well as a clean bag to place the rectal probe inside while in privacy.

Before and after each HST, a blood draw will be performed (~30 mL). Blood draws will only occur on the days that no hand cooling is performed (days 2 or 4, 21 or 23, 28, 33, 38, 43, 48).

Long Heat Stress Days 1 and 3 (LD 1 and LD 3)

You will record your previous night's sleep and diet the 24 hours prior to long heat stress days. On days 10 and 16 you will complete long heat stress days comprised of exercising for two hours in the heat before and after 2 hours of rest in a cool environment. You will be instructed to drink 500 mL (2 cups) water the night before and 250 mL (1 cup) the morning of this visit to ensure normal hydration. Upon arrival to the lab wearing a T-shirt and shorts, you will provide a urine sample following previously described methods to determine hydration status. You will privately insert a rectal thermometer and nude body mass will be recorded using previously mentioned methods.

Next, you will sit quietly in a chair and complete the Environmental Symptoms Questionnaire (ESQ) to determine signs and symptoms heat illness. After sitting still for 10 minutes, a blood draw will be taken (a little less than 2 tablespoons).

Skin temperature buttons will be placed as previously described. A heart rate strap will be applied and fitted to you. Resting skin and rectal temperature, heart rate, perceived exertion, thirst, thermal, and fatigue scales will be recorded.

You will enter the heat chamber (104°F, 40% relative humidity) and will stand still for 20 minutes to adjust to the environmental conditions. Immediately prior to exercise commencement, baseline skin and rectal temperature, heart rate, perceived exertion,

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thirst, thermal, and fatigue scales will be recorded. You will repeat the exercise durations and intensities described in the table below at 1% grade for 2 hours.

4 min	Jogging (~60% VO2max)
1 min	Running (~80% VO2max)
4 min	Walking
1 min	Running (~80% VO2max)
4 min	Jogging (~60% VO2max)
1 min	Running (~80% VO2max)
5 min	Rest

20 min x 6 cycles = 2 hours

Throughout the test, skin and rectal temperature, heart rate, and perceptual scales will be recorded. Exercise will be terminated if one of the previously mentioned cut-off criteria is met or 2 hours of exercise is completed.

After the 2 hours, you will exit the treadmill and heat chamber and sit in a chair in a cool environment. After 10 minutes, you will complete another ESQ and a blood draw following previously mentioned methods will be performed (a little less than 2 tablespoons). A carbohydrate-electrolyte drink will be provided along with a small meal which we ask that you consume within the 30 minutes for rehydration and refueling purposes. If you have restrictive diets or food allergies, appropriate calorie equivalents will be provided. We encourage you to bring material for entertainment (homework, laptop, music, etc.) during this rest period. The researchers will also provide audio/visual entertainment which may include television or music.

After 2 hours of rest, a blood draw and ESQ will be completed as previously described. You will enter the heat chamber and wait for a 20 minute period, and repeat the exercise duration and intensities shown in the table above for another 2 hours. The same measures will be taken at the same intervals as the previously described exercise bout until one of the termination criteria is met of 2 hours of exercise are completed.

You will leave the heat chamber and after sitting for 10 minutes, a final blood draw will be completed as previously described (a little less than 2 tablespoons). Nude body mass will be recorded to determine sweat rate and a urine sample will be measured to determine hydration. The ESQ will be completed and you will be monitored until your rectal temperature drops below 38.5℃, as a safety precaution.

Throughout all exercise and recovery bouts on Long Day 1, you will have unlimited access to water. The times and amount of water will be recorded so on Long Days 2, 3, and 4 the same amount of water will be proved at the same time periods for consistency purposes.

Long Heat Stress 2 and 4 (LD 2 and LD 4)

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Upon arrival to the lab, a recovery scale and muscle soreness scale will be recorded. The rest break and second 2 hour exercise bout will not be included during these days. All other procedures during these lab visits are identical to the long heat stress days 1 and 3. The day after long heat stress 4, you will return to the lab only for a blood draw (a little less than 2 tablespoons).

Heat Acclimation Days (HA)

On days 13-15 and 18-20, you will become heat acclimated through a heat acclimation (HA) specific protocol. Before and after HA exercise, a urine sample and nude body mass will be measured with previously described methods. Only on the first day of HA before exercise, a blood draw (a little less than 2 tablespoons) will be performed after 10 min of sitting. A rectal thermometer, heart rate monitor, and skin temperate buttons will be placed following previous mentioned methods.

You will enter the heat chamber and baseline measures for skin and rectal temperature, heart rate, and perceptual scales will be measured. You will alternate riding on bike and ambulating on a treadmill continuously for 90 minutes in a hot, humid environment (40℃, 40% relative humidity). During the first 30 minutes of the protocol, the goal will be to increase rectal temperature to 38.5℃. The goal of the remaining 60 minutes is to maintain rectal temperature between 38.5-39.99℃. To achieve these goals, exercise intensity will vary. During this time skin and rectal temperature, heart rate, and perceptual scales will be measured every 15 minutes. Exercise will be terminated if one of the previously described cut-off criteria is met.

After completing the exercise, you will exit the heat chamber and after excess sweat wiped off, a nude body mass will be measured.

Heat Acclimation Maintenance Protocol (days 28, 33, 38, 43, 48)

The heat acclimation maintenance intervention days will follow the same protocols as the HST sessions described previously. If you are randomly assigned to the heat exposure group, you will conduct the exercise portion of this lab session in a hot chamber (104℉, 40% relative humidity). If you are randomly assigned to the no heat exposure group you will conduct the exercise portion of the lab session in a cool room (72℉, 40% relative humidity). After the day 23rd of the study (HST 4), you will report to the lab every fifth day for a 25-day period and perform the HST to document the decay/maintenance of adaptations.

During days 28-48, you will be given a heart rate monitor and watch to wear if you choose to perform physical activity outside of the lab to record duration and intensity (heart rate). You will also be asked questions regarding your training time and venue, such as what time of day and whether the training occurred indoors or outdoors to help characterize your normal training routine.

<u>What are the risks or inconveniences of the study?</u>

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There are no known risks of using the hand cooling device. The risks of participation in this study are as follows: (a) a musculoskeletal injury such as a muscle strain, ligament sprain, or bone fracture, (b) delayed onset muscle soreness, (c) a fall during the treadmill walking or performance tests, (d) although very unlikely, a disturbance of heart rhythm during exercise, (e) exertional heat illnesses (f) discomfort involving the insertion, removal, and movement of the rectal thermometer, (g) redness, irritation, or infection from blood draws, and (h) there is a risk associated with blood draws such as infection at the site of skin puncture. To minimize these risks, universal precautions will be utilized and a HPL trained researcher will perform each blood draw. Over the course of the 49 day study, 22 blood draws will be done equating to 560 mL (about 2.5 cups) of blood in total. This is comparable to the volume of blood given during American Red Cross donations except that it will occur over a 2 month period. You will be asked to refrain from blood donations during the length you participate in this study to further mitigate any risks. The time devoted to study participation (57-70 hours over a 2 month period) may be considered an inconvenience. The table below is a breakdown of time commitment per day.

The inconveniences of participating in this study are as follows: (a) refraining from alcohol and strenuous exercise 24 hours prior to testing (b) refraining from caffeine 8 hours prior to testing (c) possible discomfort from wearing a heart rate monitor and rectal thermistor during exercise (d) large time commitment to complete this study and (e) mimicking of diet 24 hours prior to each long heat stress day.

Overall Time Commitment per Day

Day	Time (hr)
VO_{2max}	0.75
Heat Stress Test Days	2.5
Long Heat Stress Day 1/3	8
Long Heat Stress Day 2/4	4
Heat Acclimation Days	2

What are the benefits of the study?

The proposed study will increase knowledge of the effects of heat acclimation on body cooling and thermoregulation. This may improve athletic performance in athletes and help medical professionals prevent heat related injuries. The results of this study may influence and encourage further education of health care providers regarding the importance of heat acclimation before intense exercise in hot environments. Your participation in this study may benefit the general population, by allowing researchers, athletic trainers and coaches better implement heat acclimation protocols to improve performance and mitigate the risk of exertional heat illnesses. Understanding how the body responds to long bouts of exercise-heat stress the following day is vital in

preventing exertional heat illness. Exploring whether heat acclimation improves hand cooling effectiveness will enhance our utilization of this modality in occupational settings (military, industry, athletic, etc.) to prevent heat-related injury. Finally, evaluating the efficacy of the proposed intervention (exercise-heat exposure every 5 days for a 25-day period after initial heat acclimation efforts) will benefit those individuals required to perform periodic physical activity in hot, humid environments such as athletes, firefighters, and military troops. Upon request, the researchers will provide body fat percentage and VO_2 max information to you upon completion of the study.

Will I receive payment for participation? Are there costs to participate?
There are no costs for participating in this study. Accumulation of payment will be prorated and spread out throughout the course of the lab visits as described in the table below. In total, if you are randomly assigned to the group that heat acclimates and finish the study in its entirety, you will receive $800. The group that does not heat acclimate (exercise only) will receive $500 upon finishing the study. If you decide you leave the study for any reason, your payment will be prorated.

Groups	Sample Size	VO_{2max} Days	VO_{2max} ($5/d)	HA/EX days	HA/EX ($15/d)	Heat Test Days	Heat Test Days ($25/day)	Finishing Bonus	Total Compen-sation	Total Hrs
HA (Question 2), HA or EX (Question 3)	24	3	15	6	90	13	325	370	800	70.75
EX (Question 2)	8	2	10	6	90	8	200	200	500	57.5

Note. HA = heat acclimated group; EX = exercise only group.

How will my personal information be protected?
The following procedures will be used to protect the confidentiality of the data collected from you. You will be assigned a random identification number that will be used on all data. The researchers will keep all study records (including any codes to your data) locked in a secure location. A master key that links names and identification numbers will be maintained in a separate and secure location. The master key and data will be destroyed after 3 years after all associated publications have been published. All electronic files (e.g., database, spreadsheet, etc.) containing identifiable information will be password protected. Any computer hosting such files will also have password protection to prevent access by unauthorized users. Additionally, de-identified data from lab testing may be stored in the cloud using secure, password protected platforms. Only the members of the research staff will have access to the passwords. Data that will be shared with others will be coded as described above to help protect your identity. All blood samples will be immediately de-identified upon collection and stored in the Department of Kinesiology for three years. These samples may be retained for a longer period of time, to be analyzed when funding becomes available. At the conclusion of this study, the researchers may publish their findings. Information will be presented in summary format and you will not be

identified in any publications or presentations. We will do our best to protect the confidentiality of the information we gather from you, but we cannot guarantee 100% confidentiality.

You should also know that the UConn Institutional Review Board (IRB) and the Office of Research Compliance may inspect study records as part of its auditing program, but these reviews will only focus on the researchers and not on your responses or involvement. The IRB is a group of people who review research studies to protect the rights and welfare of research participants.

Can I stop being in the study and what are my rights?
You do not have to be in this study if you do not want to participate. If you give consent to be in the study, but later change your mind, you may withdraw at any time. You may also choose to withdraw yourself from the study at any time. There are no penalties or consequences of any kind if you withdraw from the study or choose not to participate.

Whom do I contact if I have questions about the study?

Take as long as you like before you make a decision. We will be happy to answer any questions you have about this study. If you have further questions about this study or if you have a research-related problem, you may contact the principal investigator, Dr. Douglas J Casa, at 860-486-3624 or a student investigator, Mr. Luke Pryor, at 860-895-7613 or Mrs. Riana Pryor at 860-486-3222. If you have any questions concerning your rights as a research participant, you may contact the University of Connecticut Institutional Review Board (IRB) at 860-486-8802.

Consent Form for Participation in a Research Study

University of Connecticut

Principal Investigator: Douglas J Casa, PhD, ATC

Study Title: The effect of heat acclimation on repeated bouts of strenuous heat stress, hand cooling efficacy, and the maintenance thereof

Documentation of Permission:
I have read this form and decided that I will give permission to participate in the study described above. Its general purposes, the particulars of my involvement and possible risks and inconveniences have been explained to my satisfaction. I understand that I can withdraw at any time. My signature also indicates that I have received a copy of this permission form. Please return this form to the principal investigator.

_____	_____	_____
Signature:	Print Name:	Date:
_____	_____	_____
Research Assistant	Print Name:	Date:

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Training History Questionnaire

1. Please describe your typical weekly **resistance training** routine:
Days/week Sets Reps Intensity (typical loads or %1RM)

For how many years have you been resistance training?

2. Please describe your typical weekly **endurance training** routine:
Days/week Duration (miles or time) Intensity (min/mi or speed)

Typically, what time of day do you complete this endurance training?

What percentage of this activity is conducted outdoors?

For how many years have you been endurance training?

3. List any recreational activities or sports that you devote time to on a weekly basis

Activity	**Times per week**	**Session Duration (min)**	**Other Notes (Denote if Intramural Sport, Club Sport, Rec-League, etc.)**

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Post Heat Acclimation Training Log

Date	Location	Exercise Type	Duration (min)	HR strap on?
	□ Indoors □ Outdoors	□ Weight training □ Running □ Other		□ Yes □ No
	□ Indoors □ Outdoors	□ Weight training □ Running □ Other		□ Yes □ No
	□ Indoors □ Outdoors	□ Weight training □ Running □ Other		□ Yes □ No
	□ Indoors □ Outdoors	□ Weight training □ Running □ Other		□ Yes □ No
	□ Indoors □ Outdoors	□ Weight training □ Running □ Other		□ Yes □ No
	□ Indoors □ Outdoors	□ Weight training □ Running □ Other		□ Yes □ No

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Timex Run Trainer Instructions

Please wear the Timex watch and heart rate strap any time you exercise outside of the lab for the remainder of the study. Also, be sure to record your exercise on the Post Heat Acclimation Training Log.

Instructions for using the watch:

1. Put on the heart rate strap. The strap should be snug but not restrictive.
2. Press mode until ‘chrono’ appears.
3. Press ‘start’ on the watch when you begin exercise.

IMPORTANT – if the heart rate does not appear within a minute or two, press the ‘heart rate’ button. If this does not fix the problem, contact the researchers.

4. When you stop exercising, press the ‘stp/rst/set’ button.
5. Save the exercise bout by pressing and holding the ‘stp/rst/set’ button until you hear a beep and the watch says ‘workout saved’.

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Perceptual forms

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Environmental Symptoms Questionnaire

How Do You Feel Questionnaire

1. Place an X in the box to explain HOW YOU HAVE BEEN FEELING TODAY.
2. PLEASE ANSWER EVERY ITEM.
3. If you did not have the symptom, say NOT AT ALL.

Symptoms	Not At All	A Little	Somewhat	Moderate	A Lot	Extreme
I feel lightheaded						
I have a headache						
I feel dizzy						
I feel thirsty						
I feel weak						
I feel grumpy						
It is hard to breathe						
I will playing at my best						
I have a muscle cramp						
I feel tired						
I feel sick to my stomach (nauseous)						
I feel hot						
I have trouble concentrating						
I have “goose bumps” or chills						

Source: modified from Sampson et al. (1980) and validated by Stearns et al. (2014).

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Thermal Perception

0.0	Unbearably Cold
0.5	
1.0	Very Cold
1.5	
2.0	Cold
2.5	
3.0	Cool
3.5	
4.0	Comfortable
4.5	
5.0	Warm
5.5	
6.0	Hot
6.5	
7.0	Very Hot
7.5	
8.0	Unbearably Hot

Source: Young AJ, Sawka MN, Epstein Y, Decristofano B, and Pandolf KB. Cooling different body surfaces during upper and lower body exercise. J Appl Physiol 63:1218-1223, 1987.

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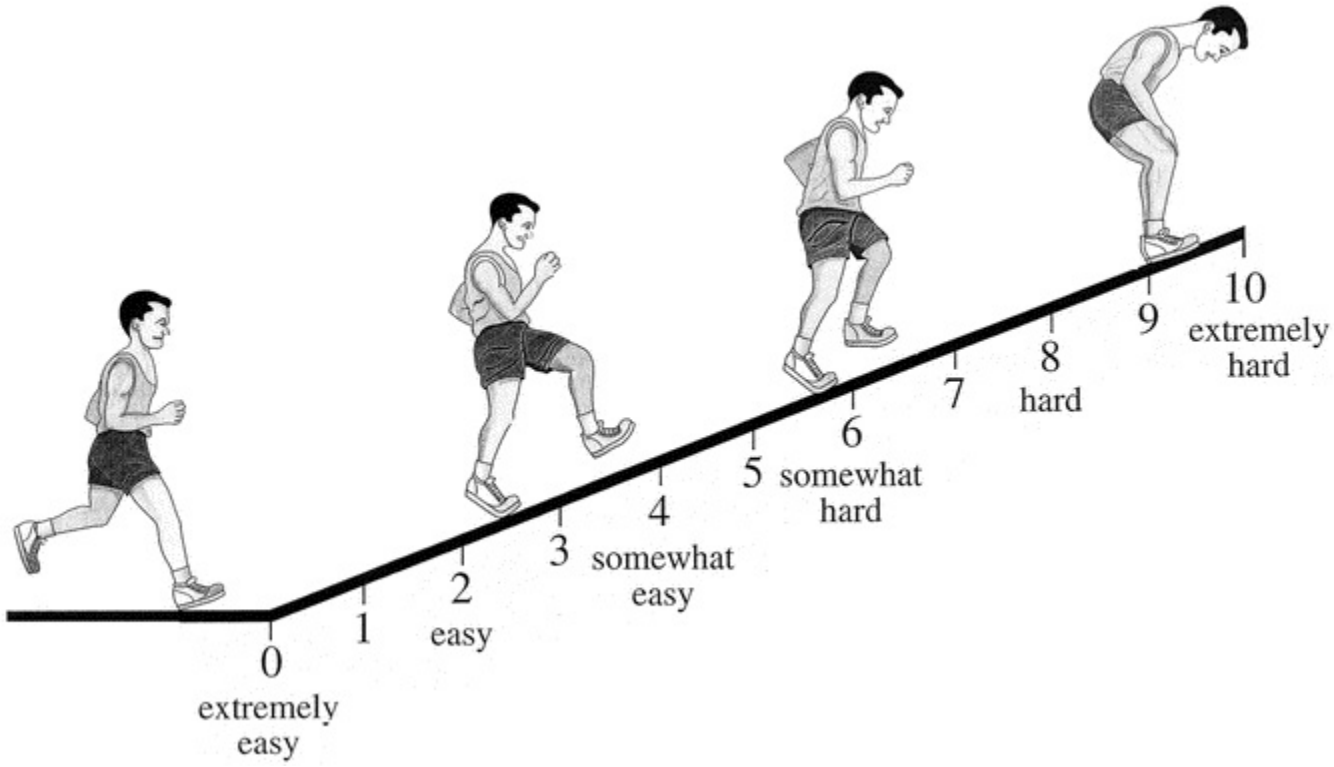
OMNI Scale of Perceived Exertion



Source: Robertson RJ, Goss FL, Dube J et al. Validation of the adult OMNI scale of perceived exertion for cycle ergometer exercise. *Med Sci Sports Exerc* 36(1):102-8, 2004.

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Thirst Scale

1	Not Thirsty At All
2	
3	A Little Thirsty
4	
5	Moderately Thirsty
6	
7	Very Thirsty
8	
9	Very, Very Thirsty

Source: Engell DB, Maller O, Sawka MN, Francesconi RN, Drolet L, and Young AJ. Thirst and fluid intake following graded hypohydration levels in humans. Physiol Behav 40:229-236, 1987.

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Fatigue Scale

INDICATE YOUR LEVEL OF OVERALL FATIGUE <u>RIGHT NOW</u>

0	No Fatigue At All
1	Very Small Amount of Fatigue
2	Small Amount of Fatigue
3	Moderately Fatigued
4	Somewhat Fatigued
5	Fatigued
6	
7	Very Fatigued
8	
9	Extremely Fatigued
10	Completely Fatigued

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Ancillary data

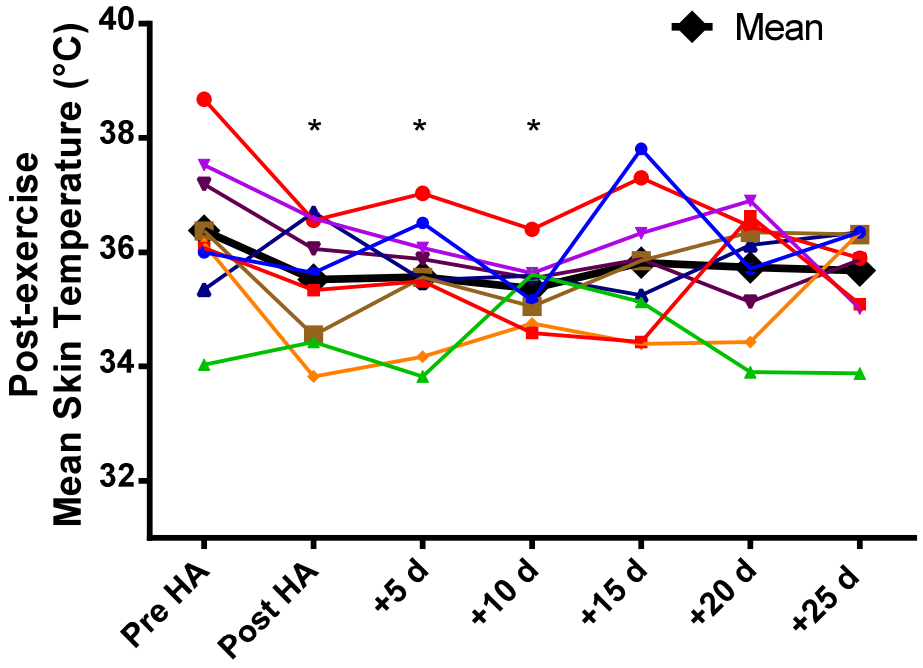

Figure 1. Individual post-exercise T_{sk} responses in IHE. * Group mean was different from Pre HA, $p < 0.038$.

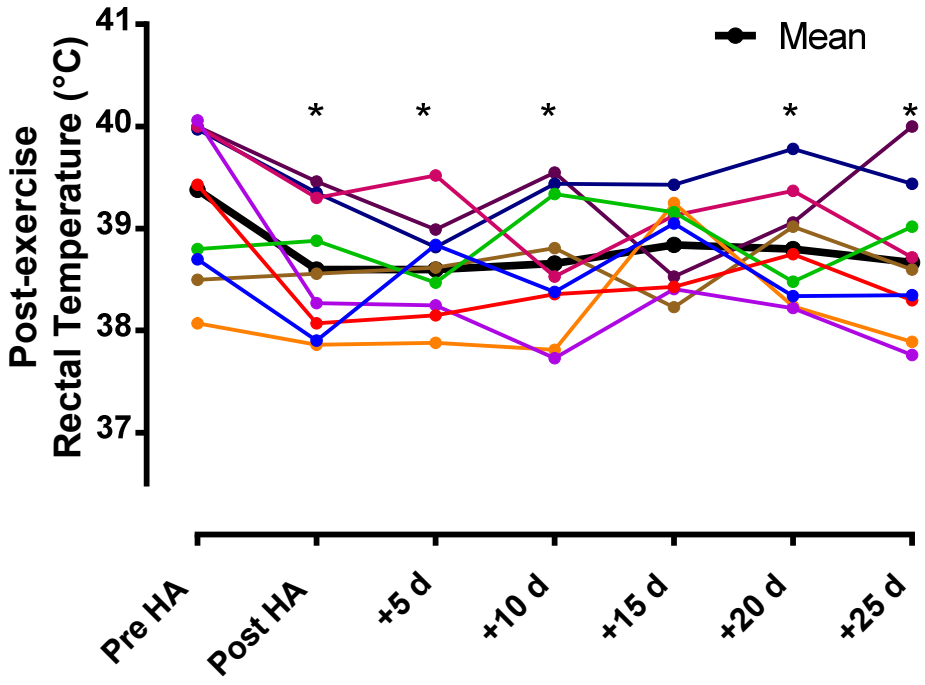

Figure 2. Individual post-exercise T_{re} responses in IHE. * Group mean was different from Pre HA, $p = 0.042$.

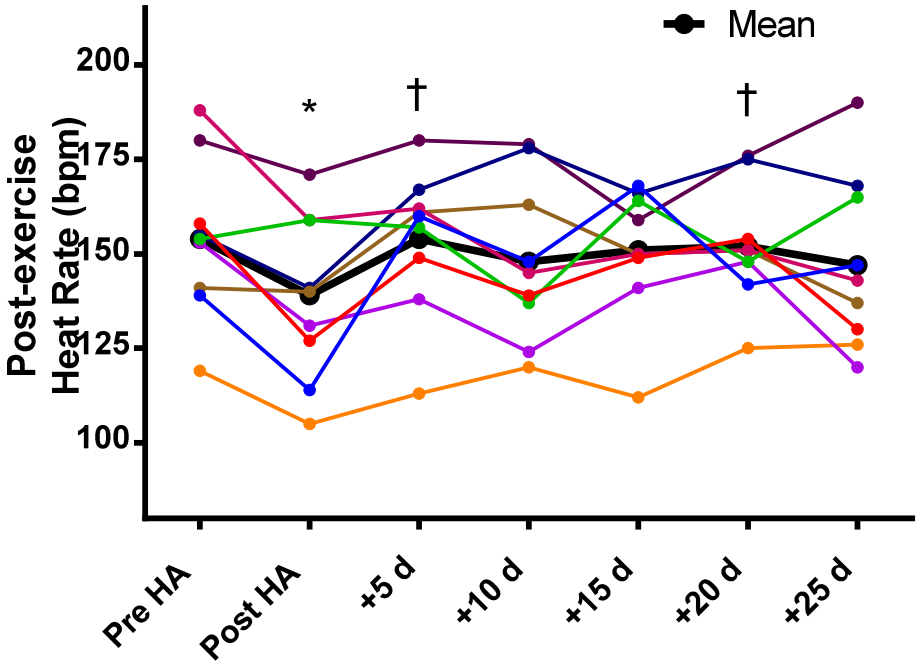

Figure 3. Individual heart rate responses in IHE. * Group mean was lower than Pre HA, p = 0.01. † Group mean greater than Post HA, p ≤ 0.032.

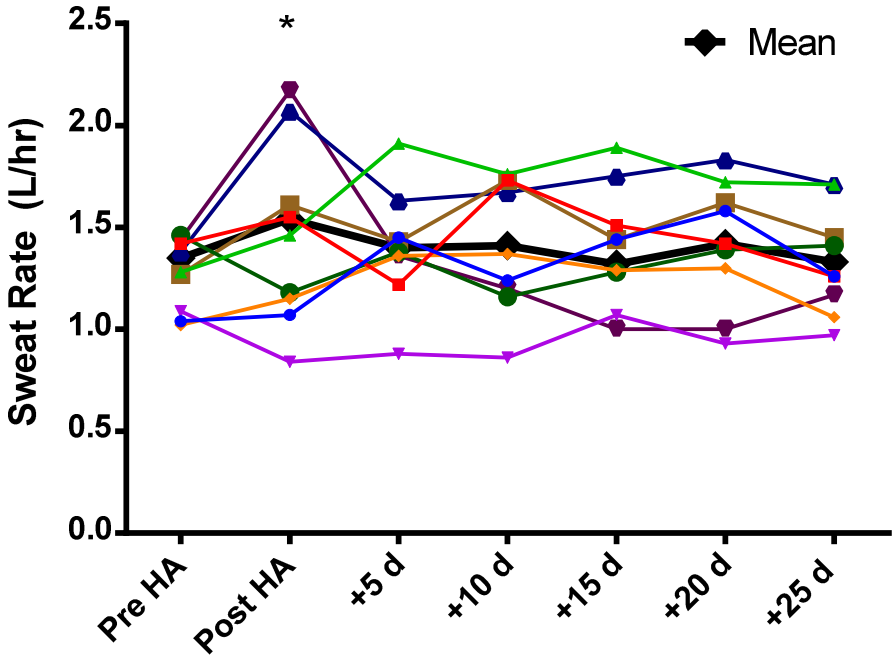

Figure 4. Individual sweat rate responses in IHE. * Group mean was different from Pre HA, p < 0.04.

Table 1. Maximal oxygen consumption (ml/kg/min) throughout the study by group

	NHE (n=6)	IHE (n=9)	Combined (n=15)
Pre HA	56.1 (51.4,60.7)	53.5 (49.5,57.4)	54.5 (51.8,57.2)
Post HA	57.8 (53.2,62.4)	56.7* (52.7,60.7)	57.1* (54.5,59.8)
Post Intervention	57.3 (53.8,60.1)	55.2 (50.0,60.3)	--

HA = heat acclimation, NHE = no heat exposure, IHE = intermittent heat exposure group. Data are mean (95%CI). * signifies p ≤ 0.02 from Pre HA. IHE and combined groups gained 6.0% and 4.6%, respectively. Metabolic cart flow and gas concentration calibration error <2.0%.

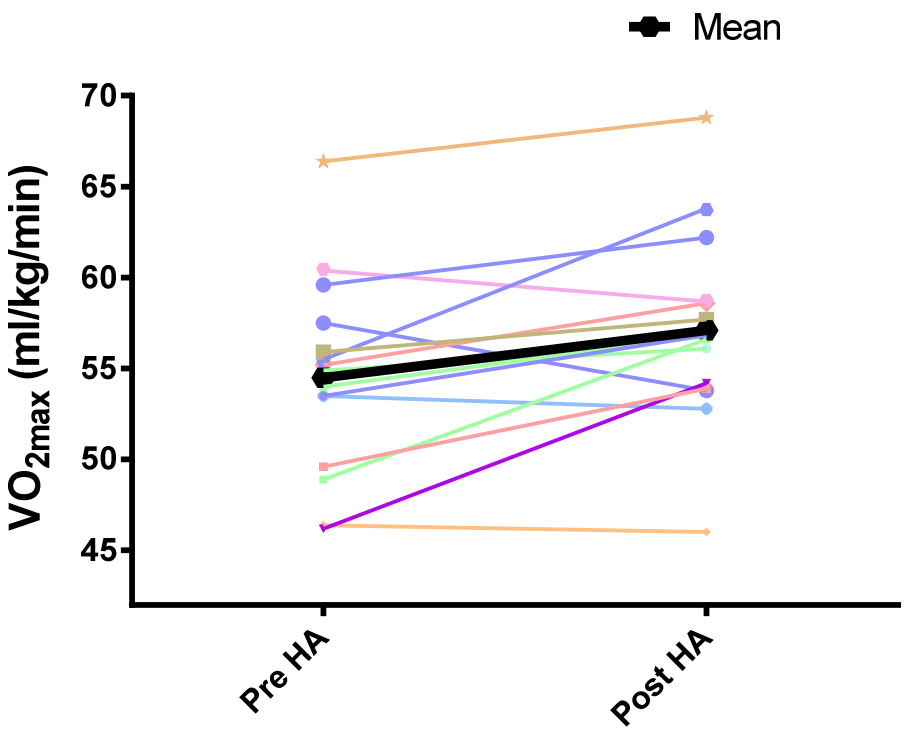

Figure 5. Individual VO_{2max} responses before and after heat acclimation (HA). Mean VO_{2max} increased 4.8% after HA (p = 0.012, n=15).

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