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Lasting Effects of Exercise in Heat on Subsequent Exercise and Thermoregulation

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Introduction: Best practice recommendations suggest five days of short term heat acclimation (STHA) prior to multi-bout exercise days to reduce risk of exertional heat illnesses. It is unknown if five days of STHA is sufficient to mitigate thermal strain and protect against EHI. Purpose: To determine lasting physiological effects of exercise in heat on subsequent exercise before and after STHA. Methods: Eighteen men (age: 22 ± 3 y, height: 180.0 ± 6.0 cm, weight: 74.24 ± 7.42 kg, body fat: 9.4 ± 4.1%, maximal oxygen consumption: 54.6 ± 5.1 $ml \cdot kg^{-1} \cdot min^{-1}$) performed two intermittent treadmill exercise sessions two hours apart, followed by one session the following day (40°C, 40% relative humidity). Three additional days of STHA consisted of 90 minutes of exercise in the same environment. Subjects again completed a double exercise session followed by a single session the following day. Heart rate (HR), rectal temperature (T_{re}), and perceptual scales were assessed throughout exercise. Environmental symptoms and blood variables were assessed Pre- and Post-exercise. Results: Before STHA, resting HR and T_{re} were lower before Bout 1 (80 ± 12 bpm, 36.79 ± 0.44°C) compared to Bout 2 (103 ± 17 bpm, $p < 0.001$; 37.06 ± 0.50°C, $p = 0.038$) but were similar to Day 2 (81 ± 13 bpm, $p = 0.728$; 36.80 ± 0.32°C, $p = 0.924$), respectively. Exercising HR and T_{re} were similar between Bouts 1 and 2 despite a shorter exercise duration during Bout 2 (93 ± 27 min) than Bout 1 (120 ± 0 min, $p < 0.001$). Rate of T_{re} rise was greater during Bout 2 (0.031 ± 0.011$°C \cdot min^{-1}$) than Bout 1 (0.023 ± 0.004$°C \cdot min^{-1}$, $p = 0.011$). The STHA protocol induced a partially acclimated state, indicated by decreased exercising T_{re}, HR, thermal sensation, and perceived exertion ($p < 0.05$). Both Pre- and Post-STHA, many participants were unable to complete the full exercise protocol due to elevated T_{re}, environmental symptoms, and fatigue. Conclusion: Multi-bout exercise on the first of two sequential days led to a higher resting level of fatigue

and premature cessation of exercise during the second bout of exercise both Pre- and Post-STHA.

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Lasting Effects of Exercise in Heat on Subsequent Exercise and Thermoregulation

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CHAPTER 1

Review of Literature

Many high school, collegiate, and professional athletes begin preseason training in the hottest summer months of July and August, resulting in many cases of exertional heat illnesses (EHI) (1-10). Multiple exercise sessions in one day are common practice in team sports such as American football. With teams having limitations on official start dates and practice times, coaches introduce multi-bout exercise early in the preseason, which may lead to increased fatigue, thermal strain, and risk of EHI (11-13).

Heat acclimation effectively mitigates risk of EHI (5, 14-18). A series of adaptations occur via the body habituating to external stressors of heat and humidity, resulting in decreased internal temperature, skin temperature, and heart rate (HR), as well as increased plasma volume, sweat sensitivity and rate, and altered sweat concentration (19). The combination of these adaptations enhances heat loss to the environment, allowing for decreased thermal strain after acclimation. Full acclimation benefits are seen around days 10-14 (19), however shorter protocols such as five days of heat acclimation, termed short term heat acclimation (STHA) can effectively provide some, but not full, benefits for safety and performance (20-25). The effectiveness of STHA on protecting against the stressor of multi-bout exercise in heat is a gap in the literature.

Multi-Bout Exercise Heat Stress

Multiple bouts of exercise in one day is common practice in military training, occupational work (e.g. fire suppression), and athletics (e.g. two halves of a game, preseason practices). When these activities are performed in hot conditions, core temperature can reach over 39°C (26) with the second bout of exercise compromised due to cumulative thermal load (13, 27-32). Thus, it is important to understand if there is prolonged physiological strain from the first bout of activity affecting the second bout. By understanding

typical responses to multi-bout activity, prevention strategies can be created to mitigate thermal load and fatigue seen during the second exercise bout.

The ideal length of rest breaks to provide a full recovery from physical activity is not currently known. Short rest breaks such as 15 minutes are common during half times of athletic competitions and rehabilitation between bouts of fire suppression. Fifteen minutes of rest results in incomplete recovery from the first exercise bout, shown by Kenny et al. who studied cycling in warm conditions (30°C, 30% relative humidity (RH)) with subjects resting in the same environment (27). Although skin temperature returned to baseline within 15 minutes, the second 30 minute exercise session resulted in elevated HR and esophageal temperature immediately prior to activity (Figure 1.1). It should be noted that body temperature did not drastically rise throughout exercise and peaked around 37.7°C, only slightly higher than normal resting body temperature.

Figure 1.1 (27).

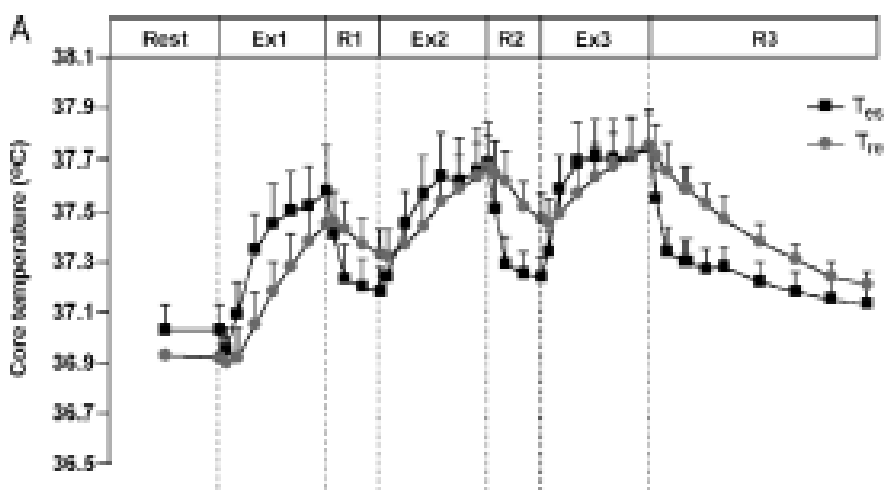

FIGURE 4—Mean changes in core [(esophageal, T_{es}; rectal, T_{re}) top panel, A], muscle [(vastus lateralis, T_{vl}; upper trapezius, T_{ut}; triceps brachii; T_{tb}) middle panel, B], and mean skin temperatures [($\bar{T}_{sk}$) bottom panel, C] during preexercise resting, exercise (Ex1, Ex2, and Ex3), and postexercise rest (R1, R2, and R3) periods for the three successive exercise/rest cycles. Final recovery period (R3) extended to 60 min in duration. Error bars indicate SD.

Rest breaks of slightly longer duration (30 minutes) also resulted in poor recovery. This is illustrated by exacerbated initial rectal temperature (T_{re}) rate of rise (13), and exercising T_{re} (28, 29), oxygen consumption (13), blood lactate (28) and HR (13, 28, 29) during succeeding exercise compared to the first exercise session. This indicates that 30 minutes of rest between activity is not sufficient for a full return to resting values, possibly due to insufficient removal of metabolic byproducts, increased circulating catecholamines, and incomplete replenishment of energy stores, all resulting in excess post-exercise oxygen consumption (33). One study reported similar skin temperature between bouts of activity, however, exercise was performed in only moderately warm (22°C) environmental conditions and maximum exercising T_{re} only reached 37.5°C (Figure 1.2). Hotter ambient conditions as seen during rotations of fire suppression resulted in T_{re} reaching 38.72°C (29), somewhat higher than the previously described studies, but again, body temperature is not comparable to athletes undergoing extreme hyperthermia (>39°C) (26).

Figure 1.2 (13).

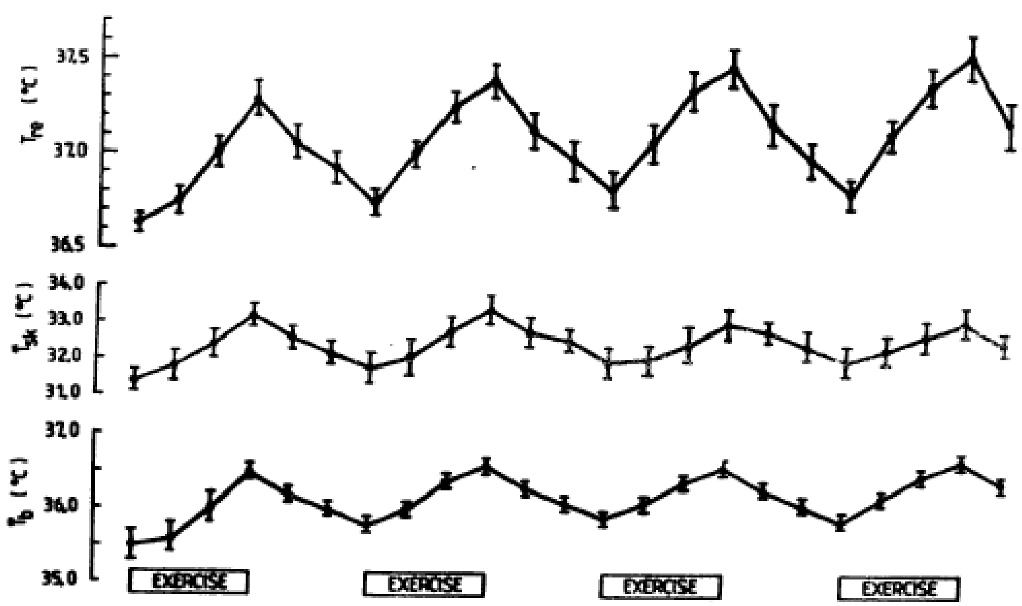

Fig. 1. Changes in rectal (Tre), mean skin ($\overline{\text{Tsk}}$) and mean body ($\overline{\text{Tb}}$) temperatures during four consecutive, 30 min exercise-bouts of the same intensity and post-exercise rest intervals. The values are means ± SE. The significance of differences between the first and consecutive exercise-bouts as well as between the pre--exercise and consecutive resting periods is marked by: x — $p < 0.05$, xx — $p < 0.01$, xxx — $p < 0.001$

Doubling rest breaks to 60 minutes in length allowed enough time for T_{re} and HR to return to resting values following fire suppression (30). In a separate study, children completed 80 minute exercise sessions in 33°C before and after 1 hour of rest in a thermoneutral environment (31). Similar exercising T_{re} and HR but higher rating of perceived exertion in both young (12-13 y) and older (16-17 y) boys and girls were reported (Figure 1.3). This indicates there was similar cardiovascular and thermal strain during exercise bouts, suggesting a full recovery during the rest break. This may have been accomplished partly due to appropriately replacing sweat losses by rehydrating during rest as indicated by similar pre-exercise urine specific gravity. Again, both of these protocols resulted in only moderately elevated T_{re} (~ 38.5°C) due to warm ambient conditions and moderate intensities, allowing for a quick recovery during rest.

Figure 1.3 (31)

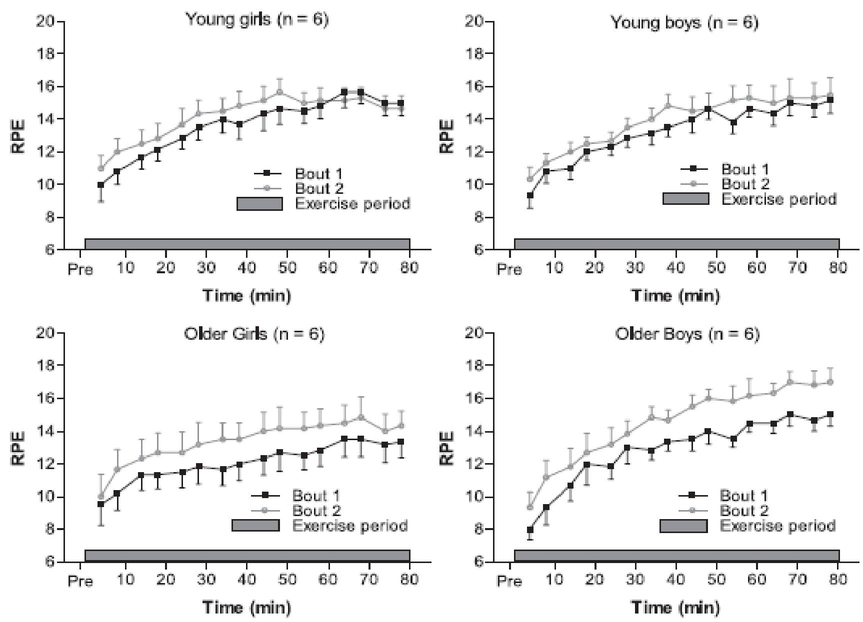

Fig. 5. Rating of perceived exertion (RPE) responses during the 2 exercise bouts in the heat for each age and sex subgroup. Values are means ± SE.

One study incorporated 90 minutes of rest after light intensity (30% VO_{2max}), long duration (3 hours) heat stress (50°C) and prior to a 15 min cycling time trial in heat (40°C) (34). Complementing other studies (30, 31), T_{re} reached 38.25°C during heat stress and returned to resting levels during the rest break. Similar perceived exertion, HR, and T_{re} were measured during the time trial. Another study

incorporating 90 minutes of rest exercised individuals at a much greater intensity. Aerobically trained men underwent two 80 minute high intensity (70% maximal oxygen consumption (VO_{2max})) treadmill runs in a warm (22-25°C) environment separated by 90 minutes of rest (32). Rectal temperature was higher throughout the second exercise bout despite a similar starting temperature and similar oxygen uptake. Heart rate did not fully recover during the rest period and remained higher throughout the second exercise trial. These results may partially be due to fluid restriction of 100mL of water during each exercise bout, decreasing blood volume and elevating HR (35) and T_{re} (14).

In summary, thermal strain during subsequent activity depends not only on the length of rest breaks, but also intensity of exercise, ambient conditions, and training status of subjects. Based on similar studies involving lower intensity exercise in milder conditions, a rest break of more than 90 minutes is needed to mitigate thermal strain during subsequent exercise when undergoing extensive, intense exercise in a hot environment. These conditions are yet to be studied and warrant further investigation.

Multi-Day Exercise Heat Stress

Many athletes, soldiers, and laborers are required to endure exercise for sequential days in hot, humid environments. Concern for these individuals' safety is paramount as EHI are most likely to occur under these oppressive conditions. Additionally, multi-day activity can become uncompensable when protective equipment or uniforms are worn during activity, additionally burdening exercising individuals (36, 37). Although this type of activity is common for many physically active populations, few studies investigated if a stressful exercise session affects physiological responses during a subsequent exercise the following day.

The Marine Corps is known for creating rigorous training schedules for new recruits to best prepare them for combat. Due to the high intensity of exercise and training in the summer months in the southern United States, EHI risk is high (38). To prevent these potential illnesses, wet bulb globe temperature (WBGT) readings are utilized to determine activity modifications or cancellations, in line with American

College of Sports Medicine and National Athletic Trainers' Association suggested policies (2, 15). Even with the Marine Corps following these guidelines, 83% of EHI during basic training occurred when ambient conditions indicated the assumed lowest level of risk (39). This indicates a need to revisit and possibly revise WBGT policies to determine if risk categories should be amended.

Wallace et al. determined that EHI could best be predicted when accounting for both current WBGT at the time of the incident and the average WBGT from the day previous to the EHI incident (39). This suggested there is a cumulative effect of environmental conditions of the previous and current day on EHI risk, as indicated in Table 1.1. This information is supported by Ferrara et al. who reported the highest incidence of EHI is on the second day of practice in National Collegiate Athletic Association Division I American football athletes (unpublished data). The mechanism of this cumulative load and possible disruption of thermoregulation, leading to a greater risk of EHI remains to be elucidated. This study was the first of its kind to attempt to predict and define the risk of EHI based on WBGT readings in the southeast United States. It may be prudent to re-examine current WBGT guidelines to modify or cancel practices based on a combination of both current and previous day environmental conditions.

Table 1.1 (39)

TABLE 4. Cumulative effects of heat on EHI risk: odds ratios for combinations of current hour and previous day average WBGT*.

		Odds Ratio of EHI Risk								
Current Day WBGT (°F)	100	*3.4*	*3.6*	*3.9*	*4.2*	*4.5*	*4.8*	*5.1*	*5.5*	*5.9*
	95	*2.9*	*3.1*	*3.3*	*3.5*	*3.8*	*4.0*	*4.3*	*4.6*	*4.9*
	90	*2.5*	*2.7*	*2.8*	*3.0*	*3.2*	*3.4*	*3.6*	*3.9*	*4.1*
	85	*2.1*	*2.3*	*2.4*	*2.5*	*2.7*	*2.9*	*3.0*	*3.2*	*3.4*
	80	1.8	1.9	*2.0*	*2.2*	*2.3*	*2.4*	*2.6*	*2.7*	*2.9*
	75	1.6	1.7	1.7	1.8	1.9	*2.0*	*2.1*	*2.3*	*2.4*
	70	1.3	1.4	1.5	1.6	1.6	1.7	1.8	1.9	*2.0*
	65	1.2	1.2	1.3	1.3	1.4	1.4	1.5	1.6	1.7
	60	1.0	1.0	1.1	1.1	1.2	1.2	1.3	1.3	1.4
		60	**65**	**70**	**75**	**80**	**85**	**90**	**95**	**100**
		Previous Day Average WBGT* (°F)								

* Average of the hours from 7:00 a.m. to 4:00 p.m.
Italics indicate odds ratio ≥2.0.

Multi-day exercise was studied to determine changes in hydration status throughout August preseason practices in American football players. Hydration markers such as urine specific gravity and change in body mass indicated that athletes were hypohydrated by the second day compared to the first day of

practice, indicating insufficient replacement of fluids between practices (12) (Table 1.2). This may contribute to exacerbated responses to subsequent exercise on the second day of activity as seen in a previous study (unpublished data), considering that rate of rectal temperature (T_{re}) rise is exacerbated by approximately 0.22°C·min^{-1} and HR approximately three beats per minute for each percent an individual begin exercise hypohydrated (14, 35). If athletes are able to maintain euhydration either during practice or replenish fluid losses before the next practice, it stands to reason that incidence of EHI during subsequent exercise could be less likely.

Table 1.2. (12)

TABLE 3
Changes in Body Weight, Blood, and Urine Measures in Football Players[a]

	Change in BW from BL, kg	Hct	Hb	USG	UNa, mmol × L^{-1}	UK, mmol × L^{-1}
BL		44.76 ± 0.98	15.2 ± 0.54	1.0175 ± .006	196.4 ± 61.41	69.35 ± 42.14
Day 2						
Pre-AM	–0.80 ± 0.83	45.75 ± 2.05	15.83 ± 0.98	1.0230 ± 0.007	34.87 ± 16.23	36.16 ± 27.3[b]
Post-AM	–2.23 ± 1.2	47.79 ± 1.5	16.21 ± 0.51	1.0281 ± 0.006[c]	22.93 ± 16.85[c,d]	90.1 ± 54.9

Understanding how exercise sessions on the first day of exercise affects physiological strain the following day is essential to quantify typical strain endured by athletes during preseason practices. McLellen et al. investigated these effects in both warm (22.5°C WBGT) and hot (26.5°C WBGT) environmental conditions with single or double exercise sessions on Day 1 in males and females (40). Both single and double exercise sessions in either environment on Day 1 resulted in similar sweat rate, rate of sweat evaporation, and exercising HR, T_{re}, skin temperature, perceived exertion and thermal sensation on Day 2. Despite similarities in these variables, exercise participation under hot conditions on either Day 1 or Day 2 resulted in premature cessation of exercise in eight of the 14 total trials. Only three of the seven participants were able to complete the entire exercise protocol for both of their trials in hot conditions. Participants ended exercise early, citing fatigue, difficulty breathing, nausea, and dizziness despite a final T_{re} of only approximately 38.3°C. It is important to note that participants wore a

lightweight cotton combat jacket and pants, resulting in uncompensable heat stress during exercise in hot conditions, leading to exacerbated responses to heated exercise.

In summary, exercising for consecutive days in extreme environmental conditions can increase thermal strain during subsequent exercise sessions. Performance can decrease and the risk of developing a heat illness increases during ensuing exercise. Both field and controlled laboratory studies should explore this unique exercise construct with varying environmental conditions, lengths of rest breaks and practices, equipment and clothing, and intensities of exercise.

Short Term Heat Acclimation

While full heat acclimation takes approximately 10-14 days, short term heat acclimation (STHA) of approximately five days also provides partial, but not always complete, physiological benefits (19). Nearly full physiological adaptations of certain cardiovascular variables can be accomplished in this short timeframe to provide protection from EHI. It is for this reason that the National Athletic Trainers' Association suggests implementing five days of STHA prior to beginning double practice sessions during summer preseason sport practices (17).

Typical STHA protocols consist of 70-90 minutes of exercise in 37-43°C, 12-60% relative humidity environmental conditions (21, 22, 24, 25, 41, 42). Intensity varies from 30-58% VO_{2max} with the goal of increasing and sustaining a core temperature of at least 38.5°C. Cycling (21, 22, 24, 41, 42), treadmill exercise (41), and team sport play (25) protocols have been proven effective in inducing adaptations. Sport specific STHA protocols allow for a unique opportunity for players to adjust to hot conditions as a team while also further developing skills. This is different than typical acclimation protocols that require a heated room and aerobic equipment to simply increase body temperature for an extended period of time.

Beneficial thermoregulatory adaptations such as decreased resting (22, 41) and exercising (22, 24, 41, 42) core temperature, exercising skin temperature (24), and renal and sweat sodium and chloride concentration (19, 25), and increased sweat rate and loss (24, 25, 41), occur during STHA. Resting and

exercising T_{re} can be reduced up to 0.23°C and 0.60°C in as little as six days of STHA, respectively. Body temperature is lowered due to enhanced evaporative cooling via enhanced sweat rate and skin blood flow. Evaporative cooling is enhanced due to lowered cutaneous vasodilation and sweating thresholds following STHA, allowing for sweating to occur sooner, mitigating rise in T_{re} (22).

Concurrently, cardiovascular adaptations such as decreased resting (22) and exercising heart rate (19, 21, 22, 24, 41, 42), mean arterial pressure (22) and increased plasma volume (19, 25), forearm vascular conductance (22), and skin blood flow (21) are experienced during days 3-6 of STHA. Heart rate is decreased at the same relative intensity largely due to plasma volume expansion, increasing stroke volume and allowing for similar blood distribution in fewer beats (43). Plasma volume expansion is seen due to expansion of extracellular fluid, aiding in distributing blood to the skin for evaporative cooling purposes (44). Concurrently, sympathetic nervous activity, measured by plasma norepinephrine levels, is reduced following only three days heat acclimation, aiding the decrease in HR (45).

Time to exhaustion (21) and total work performed (42) have both shown improvements following STHA protocols. Perceptual variables such as perceived exertion (19, 41) and thermal sensation (24) may also decrease following STHA, while fatigue and thirst remain to be explored.

Exercising core temperature is mitigated more quickly in lean (≤ 20% body fat) versus obese (≥ 28% body fat) boys (9-12 y) and both exhibited adaptations within the first six days of STHA (41). Similarly, resting core temperature adapted more quickly in lean boys, with both populations acclimating within five days of STHA. Obese boys had higher pre-acclimation resting core temperatures and decreased temperature to the Pre-STHA level of lean boys. This indicates that heavier individuals may require closer monitoring due to the relative higher temperature even after STHA. Sweat rate was reduced in both groups, but was slightly faster to change in lean (3 days) versus obese (4 days) individuals. Lean boys also sweat more relative to body size than larger individuals. Heart rate was reduced on day six of STHA in lean but not obese boys (41). These characteristics should be further explored in adult men and women to determine if certain morphology is best adept at acclimating efficiently. This information

could specifically benefit sports such as American football that has players of many sizes undergoing similar STHA protocols.

Many thermoregulatory, cardiovascular, performance, and perceptual adaptations can occur within just a few heat acclimation sessions. This provides evidence that heat acclimation may mitigate risk of developing EHI during the first couple weeks of summer preseason training, even after just a few days of exercising in heat. Team sport athletes can benefit from sport specific acclimatization protocols that do not require access to a heated room for extended periods of time, as typically seen during acclimation. Therefore, it is suggested that a STHA protocol is followed prior to beginning multi-bout exercise sessions in a hot environment.

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CHAPTER 2

Physiological Responses to Repeated Exercise Bouts in Heat

ABSTRACT

Introduction: Exertional heat illnesses (EHI) occur during summer preseason practices when multi-bout exercise is common. Current policies suggest conducting only one practice on the first five days of preseason practice, and not begin multiple exercise sessions until the sixth day of practice but these recommendations are not always followed. Multi-bout exercise may contribute to increased thermal strain and risk of EHI if conducted on the first practice day, although there are a limited number of studies on this topic. Purpose: To determine lasting physiological effects of exercise in heat on subsequent exercise. Methods: Eighteen healthy men (age: 22 ± 3 y, height: 180.0 ± 6.0 cm, weight: 74.24 ± 7.42 kg, body fat: 9.4 ± 4.1%, maximal oxygen consumption: 54.6 ± 5.1 $ml \cdot kg^{-1} \cdot min^{-1}$) underwent two exercise sessions one day (Bout 1 and 2), followed by one session the following day (Day 2) in 40°C, 40% relative humidity environmental conditions. Exercise bouts consisted of two hours of treadmill intermittent aerobic exercise while consuming water *ad libitum*. On the first exercise day, the bouts were separated by two hours of rest in a thermoneutral environment. Heart rate (HR), rectal temperature (T_{re}), and perceptual scales were assessed throughout exercise. Environmental symptoms and blood variables were assessed prior to and following each exercise bout. Planned paired samples t-tests compared variables between bouts at similar time points. Results: Resting HR and T_{re} were greater before Bout 2 than Bout 1 ($p < 0.05$) but were similar to Day 2 ($p > 0.05$). Rate of T_{re} rise was 35% greater during Bout 2 (0.031 ± 0.011 $°C \cdot min^{-1}$) than Bout 1 (0.023 ± 0.004 $°C \cdot min^{-1}$, $p = 0.011$). Immediately post exercise HR, T_{re}, thirst, fatigue, and perceived exertion were similar between Bouts 1 and 2 despite a shorter exercise duration during Bout 2 (93 ± 27 min) compared to Bout 1 (120 ± 0 min, $p < 0.001$). 100%, 39%, and 72% of participants completed the full exercise protocol during Bout 1, Bout 2, and Day 2, respectively. Kidney and liver enzymes were slightly elevated at the end of all exercise bouts ($p < 0.05$) and return to baseline prior to exercise the following day. Conclusion: Multi-bout exercise on the first of two sequential days of exercise leads to a higher resting level of fatigue and premature cessation of exercise on Bout 2 and Day 2 compared to Bout 1.

Introduction

In the late summer, high school and collegiate fall sport preseason practices commence, introducing intense, long duration exercise to often sedentary individuals unaccustomed to exercising in heat. This sets the framework for the dramatic rise in exertional heat illnesses (EHI) including heat cramps, heat syncope, heat exhaustion, and heat stroke during the summer months (1-5). Cooper et al. reported a 15.29 incidence rate per 1000 athlete exposures during the first week of collegiate American football practice compared to an overall August average of 8.95 (2).

It is common that during the first week of pre-season practice multi-bout practices commence. This not only increases the time during which the body undergoes thermal strain, but limited time between bouts may also increase fatigue and not allow for proper rehydration and nutrient replenishment. Recent epidemiological evidence suggests a spike in EHI on the second day of collegiate pre-season football practice, although the cause is yet to be determined (unpublished data).

Another population in which EHI are common is military recruits. From 1982-1991, Marine Corps recruits experienced approximately 145 EHI per year (6). The combination of peak and average wet bulb globe temperatures (WBGT) reached on the day prior to a heat illness were more predictive of the illness than the WBGT the day the heat illness occurred (7). Accounting for both the current and previous days' WBGT resulted in the greatest predictability of EHI. Recruits were most likely to have EHI if they exercised in high temperatures the previous day (7). This suggests a cumulative load whereby individuals are at greatest risk of heat injuries the second day of exercise in hot environments. Although the circumstances surrounding this unfortunate trend have been proposed, the physiological underpinnings of this phenomenon remain largely unknown.

Therefore, the purposes of this study were to 1) determine how one bout of strenuous exercise in heat affects thermal and cardiovascular strain during a second exercise bout and 2) determine how day of strenuous multi-bout exercise in the heat affects thermal and cardiovascular strain during exercise the subsequent day. We hypothesized that thermal and cardiovascular strain would be greater during both the

second bout (Bout 2) and second day (Day 2) of exercise compared to the first bout (Bout 1) before, but not after, STHA.

Methods

Subjects

Eighteen healthy men (age: 22 ± 3 y, height: 180.0 ± 6.0 cm, weight: 74.24 ± 7.42 kg, body fat: 9.4 ± 4.1%, maximal oxygen consumption (VO_{2max}): 54.6 ± 5.1 $ml \cdot kg^{-1} \cdot min^{-1}$) participated in this study, which was approved by the university Institutional Review Board. All were in good health without current musculoskeletal or chronic health problems, free of cardiovascular, metabolic and respiratory disease, and had no history of exertional heat stroke within the past 3 years. Subjects provided written informed consent prior to participation.

Preliminary Testing

A study physician determined subject eligibility using a medical history questionnaire. Subjects were asked to refrain from alcohol and strenuous exercise 24 hours and caffeine 8 hours prior to testing. Height was measured to the nearest 0.5 cm using a wall mounted stadiometer. Body mass was measured to the nearest 0.01 kg using a floor weight scale (T51P, Ohaus, Pine Brook, NJ). Body fat percentage was estimated using previously described methods (8). Skinfold thickness was measured at three sites (chest, abdomen, and thigh) in duplicate using a Lange skinfold caliper (BetaTechnology Inc, Cambridge, MD).

Subjects performed a graded exercise test on a treadmill following a ramping protocol in a thermoneutral environment. Open circuit spirometry (TrueOne 2400 Metabolic Measurement System, Parvomedics, Sandy, UT) measured oxygen consumption throughout the test. Individuals with a VO_{2max} $\geq$ 45.0 $ml \cdot kg^{-1} \cdot min^{-1}$ were approved to participate.

Protocol

Subjects were instructed to drink 500mL water the night before and 250mL the morning of each laboratory visit. Subjects provided a urine sample to measure urine specific gravity via refractometer (A300CL, Atago, Bellevue, WA). If subjects were deemed hypohydrated (USG > 1.020) subjects consumed 500ml water prior to proceeding with the protocol. Subjects privately inserted a rectal thermistor (401, Measurement Specialties, Beavercreek, OH) and nude body mass was recorded. A heart rate (HR) monitor (Race Trainer, Timex, Middlebury, CT) with chest strap was donned.

Subjects entered an environmental chamber (4284-2L-W, Minute-Eleven, Weymouth, MA) regulated at 40°C, 40% relative humidity, and sat quietly for a 20 minute equilibration period. A modified Environmental Symptoms Questionnaire (ESQ) was completed by subjects to determine signs and symptoms of heat illness (9, 10). After 10 minutes of seated rest, a blood draw was completed. Immediately prior to exercise commencement, rectal temperature (T_{re}), HR, and perceptual scales were recorded. Perceptual scales included 11-point OMNI run-walk and fatigue scales, a 9-point thirst scale, and 17-point thermal sensation scale.

Exercise consisted of 2-hour bouts of interval aerobic exercise on a treadmill as described in Table 2.1. Intensities were calculated from the VO_{2max} determined during preliminary testing and mimic previously determined typical pre-season American football intensities (11). Subjects completed the exercise durations and intensities at 1% grade. To ensure the first bout of exercise (Bout 1) was completed in its entirety, running and/or jogging speed was lowered for individuals with an exceptionally fast T_{re} rate of rise to ensure one of the termination criteria was not met: 1) $T_{re} \geq 40.0$°C, 2) unsteady gait making exercise unsafe, or 3) subject request. The following exercise bouts (Bout 2 and Day 2) mimicked the speeds of Bout 1. Throughout exercise, rectal temperature, heart rate, and perceptual scales were recorded and subjects drank water *ad libitum*.

Table 2.1. Exercise protocol per exercise bout.

4 min	Jogging (~60% VO_{2max})
1 min	Running (~80% VO_{2max})
4 min	Walking (4.83 km·h^{-1})
1 min	Running (~80% VO_{2max})
4 min	Jogging (~60% VO_{2max})
1 min	Running (~80% VO_{2max})
5 min	Rest
20 min x 6 cycles = 2 hour bout of exercise	

Upon the completion of 2 hours of exercise, subjects completed an ESQ, and rested quietly in a thermoneutral environment. After 10 minutes of equilibration, a blood draw was performed. Subjects replenished fluids with water and a carbohydrate-electrolyte drink *ad libitum*, and consumed a small meal (~800 kcal) within 30 minutes. After two hours rest, the ESQ was again assessed, a blood draw was completed, and nude body mass was recorded. Subjects then completed Bout 2 until two hours was completed or one of the termination criteria was met. Upon completion of exercise, subjects again completed an ESQ and venipuncture. Nude body mass was recorded to determine sweat rate and a urine sample was provided to assess hydration.

Approximately 24 hours later, an identical 2 hour exercise bout was completed.

Blood Collection and Analysis

All blood draws consisted of an antecubital venipuncture completed using an aseptic technique. Whole blood was allowed to clot after which samples were centrifuged at 3,000 rpm for 15 min at 4°C, aliquoted, and stored at -80° C until analysis. Cortisol concentrations were determined by an enzyme-linked immunosorbent assay (CalBiotech Inc, Spring Valley, CA). The intra-assay coefficient of variation was 3.5%. Complete blood counts and comprehensive metabolic panels were completed with serum samples by a commercial biochemical laboratory (Quest Diagnostics, Wallingford, CT).

Statistical Analysis

Planned paired samples t-tests assessed variables at similar time points between bouts. Missing biomarker data (< 1% of data) were replaced with the mean of that time point. Cohen's *d* effect size was calculated for rate of T_{re} rise. To determine statistical significance the α-level was set at 0.05. All data were analyzed in SPSS software version 21.0 (IBM SPSS Statistics, Chicago, IL). Data are presented as mean ± SD.

Results

Comparison of Bout 1 and Bout 2

Despite a two hour rest break, resting T_{re} (37.06 ± 0.50°C) and HR (103 ± 17 bpm) were higher before Bout 2 compared to Bout 1 (36.79 ± 0.44°C, 80 ± 12 bpm, $p = 0.038$, $p < 0.001$, respectively) (Table 2.2). Rate of T_{re} rise was also greater for Bout 1 (0.023 ± 0.004°C $\cdot min^{-1}$) than Bout 2 (0.031 ± 0.011°C $\cdot min^{-1}$, $p = 0.011$, $d = 0.918$). Immediate post-exercise (IPE) T_{re} was similar ($p = 0.370$) despite a shorter Bout 2 exercise duration. All subjects completed the full 115 min Bout 1 but averaged 93 ± 27 min during Bout 2 ($p < 0.001$). Eleven subjects ended exercise early due to: T_{re} reaching the laboratory cut-off (5/11), symptoms of EHI (4/11), joint pain (1/11), and fatigue (1/11).

Table 2.2. Baseline and immediately post-exercise values between bouts of exercise.

	Pre-Exercise			Immediately Post-Exercise		
	Bout 1	Bout 2	p-value	Bout 1	Bout 2	p-value
Rectal Temperature (°C)	36.79 ± 0.44	37.06 ± 0.50	0.038	39.49 ± 0.31	39.60 ± 0.43	0.370
Rate of T_{re} Rise (°C·min^{-1})	---	---	---	0.023 ± 0.004	0.031 ± 0.011	0.011
Heart Rate (bpm)	80 ± 12	103 ± 17	0.001	170 ± 19	178 ± 19	0.223
Thirst	2 ± 1	2 ± 1	0.529	4 ± 1	4 ± 2	0.859
Thermal Sensation	5.0 ± 0.5	5.0 ± 0.5	0.144	6.5 ± 1.5	7.0 ± 0.5	0.043
Fatigue	1 ± 1	2 ± 1	0.001	6 ± 3	7 ± 3	0.165
Perceived Exertion	---	---	---	6 ± 3	8 ± 2	0.042
Exercise Time (min)	---	---	---	120 ± 0	93 ± 27	0.001

Note. "---" indicates variable was not collected. T_{re} = rectal temperature. Bpm = beats per minute.

Bout 2 resulted in a similar sweat rate (1.45 ± 0.46 L·hr^{-1}) as Bout 1 (1.43 ± 0.36 L·hr^{-1}, p = 0.921). Subjects lost 2.85 ± 0.73 kg sweat in Bout 1 and body mass was slightly lower before Bout 2 (73.88 ± 7.50 kg) than Bout 1 (74.48 ± 7.72 kg, p = 0.006), indicating slight dehydration prior to Bout 2.

Subjects reported greater fatigue ($p < 0.001$) immediately before Bout 2 (2 ± 1) than Bout 1 (1 ± 1). Thermal sensation (p = 0.043) and perceived exertion (p = 0.042) were greater following Bout 2 (Table 2.4). Subjects reported similar changes in environmental symptoms (p = 0.488) and IPE thirst (p = 0.859), and fatigue (p = 0.165) between exercise bouts.

Serum cortisol concentration was similar at rest before Bout 1 (235 ± 101 ng·mL^{-1}) and Bout 2 (210 ± 77 ng·mL^{-1}, p = 0.310) and IPE during Bout 1 (467 ± 185) and Bout 2 (517 ± 241, p = 0.296). Both kidney and liver enzymes were elevated following exercise (Table 2.3). Despite a 2 hour rest break, blood urea nitrogen, creatinine, aspartate transaminase (AST), and alanine transaminase (ALT) remained elevated before Bout 2 ($p < 0.05$) compared Bout 1.

Table 2.3. Biomarkers of kidney and liver function during the first day of exercise.

	Bout 1		Bout 2	
	Pre	Post	Pre	Post
BUN ($mg \cdot dL^{-1}$)	16 ± 6†‡§	18 ± 6*§	18 ± 5*§	21 ± 5*†‡
Creatinine ($mg \cdot dL^{-1}$)	0.95 ± 0.09†‡§	1.24 ± 0.22*§	1.18 ± 0.16*§	1.40 ± 0.33*†‡
BUN:Creatinine	17 ± 6†‡	15 ± 6*	16 ± 6*	15 ± 5
AST ($U \cdot L^{-1}$)	23 ± 9†‡§	29 ± 11*‡	28 ± 10*†§	30 ± 11*‡
ALT ($U \cdot L^{-1}$)	19 ± 7†‡§	22 ± 9*	22 ± 9*	22 ± 9*

Note. BUN = Blood urea nitrogen. AST = aspartate transaminase. ALT = alanine transaminase.
* indicates different than Bout 1 Pre value. † indicates different than Bout 1 Post value. ‡ indicates different than Bout 2 Pre value. § indicates different than Bout 2 Post value.

White Blood Cell (WBC) count rose over time with the most amount of circulating WBCs occurring after Bout 2 (Figure 2.1). Neutrophils, the largest component of WBCs, followed the same pattern. Lymphocytes, the second largest component of WBCs, were significantly lower immediately before Bout 2 compared to all other time points ($p < 0.05$).

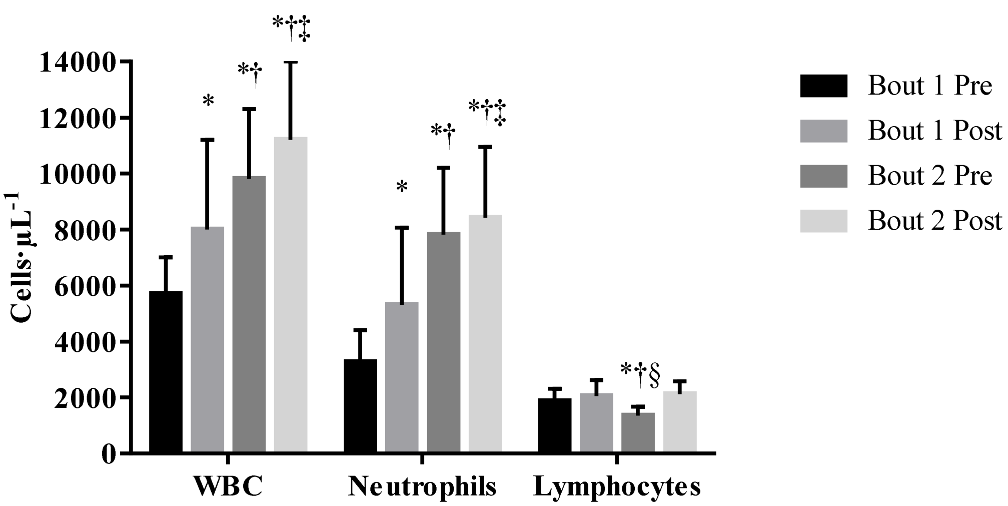

Figure 2.1. Blood cell counts during the first day of exercise. WBC = White Blood Cells. * indicates different than Bout 1 Pre value. † indicates different than Bout 1 Post value. ‡ indicates different than Bout 2 Pre value. § indicates different than Bout 2 Post value.

Comparison of Days 1 and 2 of Exercise

Heart rate and T_{re} were similar ($p > 0.05$) both before and IPE Bout 1 and Day 2 (Table 2.4). Eighteen subjects completed the full 120 min exercise on Bout 1 compared to 13 subjects on Day 2 (113 ± 12 min, $p = 0.032$) (Figure 2.2). Of the participants who ended exercise early on Day 2, 3/5 stopped due to T_{re} reaching the laboratory cut-off, 1/5 stopped due to symptoms of EHI, and 1/5 stopped due to fatigue.

Fatigue was greater at the beginning of Day 2 (2 ± 1) compared to Bout 1 (1 ± 1, $p = 0.012$) but was similar by the end of exercise (6 ± 2, 6 ± 3, $p = 0.954$). Exercise on both days resulted in similar sweat rate (1.43 ± 0.36 $L \cdot hr^{-1}$, 1.47 ± 0.36 $L \cdot hr^{-1}$, $p = 0.497$). Urine specific gravity indicated slight hypohydration upon arrival to the laboratory before Day 2 (1.021 ± 0.009) compared to Bout 1 (1.016 ± 0.008, $p = 0.027$). Subjects reported a greater increase in environmental symptoms after Bout 1 (13 ± 10 units) than Day 2 (9 ± 7 units, $p = 0.045$).

Table 2.4. Baseline and immediately post-exercise values between bouts of exercise.

	Pre-Exercise			Immediately Post-Exercise		
	Bout 1	Day 2	p-value	Bout 1	Day 2	p-value
Rectal Temperature (°C)	36.79 ± 0.44	36.80 ± 0.32	0.924	39.49 ± 0.31	39.46 ± 0.46	0.812
Heart Rate (bpm)	80 ± 12	81 ± 13	0.728	170 ± 19	170 ± 21	0.987
Thirst	2 ± 1	2 ± 1	1.000	4 ± 1	3 ± 1	0.149
Thermal Sensation	5.0 ± 0.5	5.0 ± 0.5	0.130	6.5 ± 1.5	7.0 ± 0.5	0.185
Fatigue	1 ± 1	2 ± 1	0.012	6 ± 3	6 ± 2	0.954
Perceived Exertion	---	---	---	6 ± 3	6 ± 2	0.837

Note. "---" indicates variable was not collected. Bpm = beats per minute.

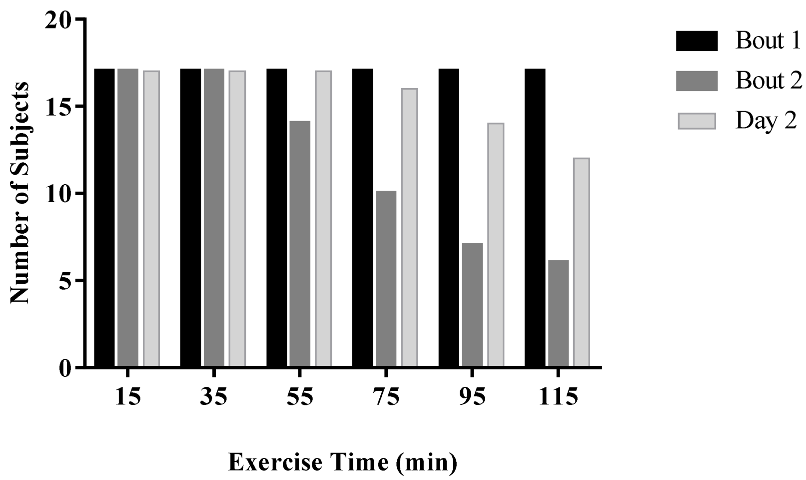

Figure 2.2. Number of participants continuing exercising during each exercise bout.

Serum cortisol concentration was similar following Bout 1 (467 ± 185 ng·mL^{-1}) and Day 2 (445 ± 215 ng·mL^{-1}, p = 0.535). BUN and AST were elevated at the beginning of Day 2 compared to Bout 1 (p = 0.034, p = 0.036, respectively) (Table 2.5). At the end of exercise, BUN was further elevated on Day 2 (22 ± 5 mg·dL^{-1}) compared to Bout 1 (18 ± 6 mg·dL^{-1}, p = 0.050).

Table 2.5. Biomarkers of kidney and liver function during two days of exercise.

	Bout 1		Day 2	
	Pre	Post	Pre	Post
BUN (mg·dL^{-1})	16 ± 6†‡§	18 ± 6*§	20 ± 5*§	22 ± 5*†‡
Creatinine (mg·dL^{-1})	0.95 ± 0.09†§	1.24 ± 0.22*‡	1.01 ± 0.13†§	1.24 ± 0.24*‡
BUN: Creatinine	17 ± 6†	15 ± 6*‡	20 ± 6†§	18 ± 4‡
AST (U·L^{-1})	23 ± 9†‡§	29 ± 11*	27 ± 10*§	33 ± 12*‡
ALT (U·L^{-1})	19 ± 7†§	22 ± 9*‡	20 ± 8†§	22 ± 8*‡

Note. BUN = Blood urea nitrogen. AST = aspartate transaminase. ALT = alanine transaminase. * indicates different than Bout 1 Pre value. † indicates different than Bout 1 Post value. ‡ indicates different than Day 2 Pre value. § indicates different than Day 2 Post value.

Circulating WBCs and neutrophils were similar at baseline of each day ($p > 0.05$) and peaked IPE (Figure 2.3). Lymphocytes were slightly higher IPE on Day 2 (2201 ± 534 cells·μL^{-1}) compared to Bout 1 (2052 ± 571 cells·μL^{-1}, $p = 0.041$).

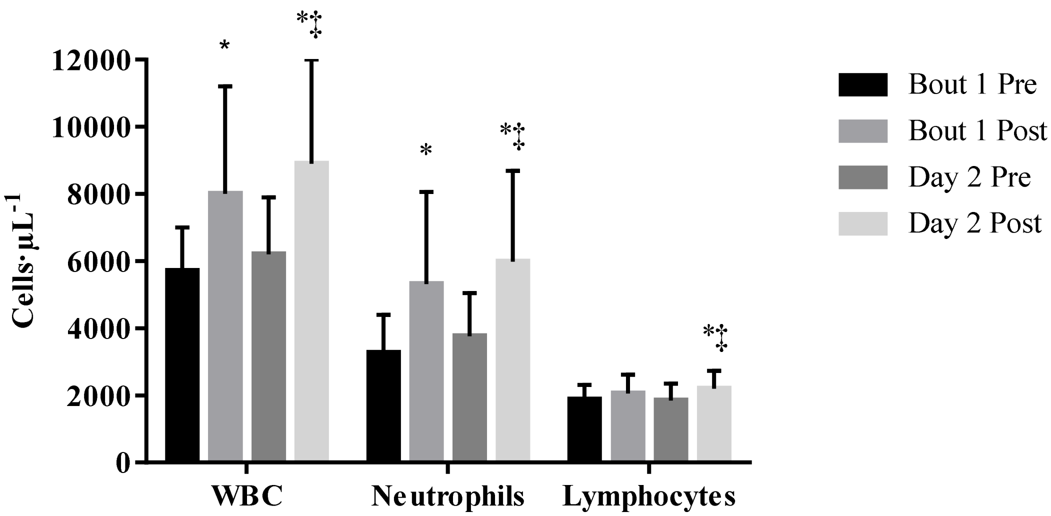

Figure 2.3. Blood cell counts during first two days of exercise. WBC = White Blood Cells. * indicates different than Bout 1 Pre value. ‡ indicates different than Day 2 Pre value.

Discussion

The purpose of this investigation was to determine if a single exercise session impacts a second bout, and if this double exercise session affects physiological responses during exercise the subsequent day. The findings reveal that physiological strain is increased during a second bout of exercise compared to the first bout, despite a 2 hour rest break. Identical exercise during a second exercise session resulted in only 39% of participants able to complete the protocol, and only 72% of participants the following day. This is similar to a previous study that noted many individuals unable to complete all exercise bouts (12). This demonstrates that prior exercise in a hot, humid environment predisposes individuals to physiological strain the following day, resulting in premature cessation of exercise. These findings validate the NATA and ACSM guidelines that urge against multi-bout exercise on the first day of sport practices (1, 13) and

supports the notion that exercise in a hot environment may predispose individuals to increased thermal strain and EHI the following day (7).

Two hours of rest did not allow for HR and T_{re} to return to baseline values following Bout 1. Similar results have been shown in 30 minutes of cycling in less stressful environments with 30 or 60 minutes of recovery (14, 15). Sawka et al. reported no differences in starting HR or T_{re} after 90 minutes of rest following 80 minutes of treadmill running at 70% VO_{2max} (16). These results are likely different due to lower ambient condition (22-25°C, 45-60% relative humidity) and studying marathon runners instead of recreationally active men as in the present study, as athletes of higher aerobic fitness have a more rapid recovery HR, lower O_2 deficit and O_2 debt, and improved lactate clearance (17, 18).

Similar end of exercise HR, T_{re}, thirst, and fatigue were reported after two hours of rest. It was determined, however, that two hours of rest did not allow for full recovery as perceived exertion and thermal sensation were higher following Bout 2 compared to Bout 1 and many participants requested to prematurely terminate exercise. Concurrently, rate of T_{re} rise was 35% greater during Bout 2 than Bout 1. A large effect size ($d = 0.918$) indicates the difference is substantial and clinically important. Athletes may be at greater risk of EHI during a second bout of exercise compared to the first bout due to this accelerated rise in temperature. Additional research into the effectiveness of extended rest breaks between exercise bouts is warranted to determine if the current guidelines that suggest three hours rest between exercise sessions is sufficient to mitigate risk of EHI. It is possible that more than three hours of rest after initial exercise is necessary to allow for a full recovery and not affect subsequent exercise.

Few studies have investigated the effects of multi-day exercise in hot environmental conditions. McLellen et al. compared multi-day treadmill walking in a warm environment (22.5°C WBGT), mimicking military basic training drills (12). Researchers concluded that HR, T_{re}, thermal sensation, and perceived exertion were not affected by exercise the previous day in similar environmental conditions. These results complement our findings, despite a lower environmental temperature and lower work rate in the previous study. In higher environmental conditions (26.5°C WBGT) only three of the seven

participants were able to complete the full 2 hour walking exercise, supporting our results of early cessation of exercise due to fatigue, EHI symptoms and high T_{re} in five subjects on Day 2.

Collegiate American football players became significantly dehydrated during preseason double session practices without adequately replacing fluids prior to the following day's practice (19). The present study determined individuals were hypohydrated prior to exercise on Day 2, although they remained hydrated throughout the first day of exercise, drinking *ad libitum*. This difference in results may be attributed to study settings (field vs lab) and drinking restrictions (structured rest breaks vs constant access).

In the field setting, exercising individuals do not always commence exercise euhydrated as observed in the present study (19, 20). Hypohydrated individuals have demonstrated higher core temperature rates of rise by approximately $0.21°C·min^{-1}$ compared to euhydrated individuals (21). In settings in which athletes must continue exercise at a predetermined intensity, such as military marches in which all individuals must complete a task in a certain timeframe, T_{re} could reach dangerous levels more quickly during following activity. While no subjects ended exercise prematurely during the first bout of exercise, five reached a T_{re} of 40°C during the second bout, and three reached 40°C during the second day of exercise. Similar IPE T_{re} was attained during each bout despite shorter exercise times during Bout 2 and Day 2, indicating higher rates of rise during subsequent exercise. Had all participants continued exercise for the full 115 min, greater increases in symptoms of EHI would become more likely and exertional heat stroke may have resulted.

Robson et al. found no differences in circulating cortisol in trained cyclists over the course of six consecutive days of exercise (22). This supports our findings of similar serum cortisol during the subsequent day, although exercise mode (endurance cycling vs intermittent treadmill exercise) is different. This indicates that overall physiological stress is likely similar throughout each exercise bout. One must consider if the need to end exercise early stems from perceptual differences during the second exercise bout. Indeed, greater EHI symptomology and fatigue perception was observed in Bout 2 compared to Bout 1.

Physical activity is known to cause disturbances in circulating white blood cell counts (23), with ambient heat additionally altering cell counts (24). These changes caused by exhaustive exercise typically return to resting values within 6-24 hours of perturbation (24-26). In the present study, circulating white blood cells were elevated following exercise in heat and recovered within 24 hours.

It should be noted that exercise intensity was identical between exercise bouts and days. Performing exercise at varying or different work rates may reveal different results. Treadmill intensities were chosen to mimic typical work rates during National Collegiate Athletic Association Division I American football preseason sport practices (11). Future studies should investigate self-paced exercise during multi-bout, multi-day exercise, as seen during individual sports such as cross country, to determine EHI risk.

In conclusion, this study has shown that intense exercise in oppressive heat impacts exercise during subsequent exercise bouts and days. Multi-bout exercise should not be performed on the first day of sport practice in a hot environment, as this may increase risk of exertional heat illnesses. Medical personnel should be cognizant that heat illness risk is greater during subsequent exercise when exercise intensity is maintained.

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CHAPTER 3

Short Term Heat Acclimation Mitigates Physiological Strain During Repeated Exercise Bouts in Heat

ABSTRACT

Introduction: Multi-bout exercise performed during summer preseason practices may increase the risk of exertional heat illnesses. Short term heat acclimation (STHA) may mitigate this risk, although few studies have investigated this concept. Purpose: To determine if a five day STHA protocol decreases physiological strain during multi-bout exercise compared to before acclimation. Methods: Seventeen men (age: 22 ± 3y, height: 179.5 ± 6.0 cm, weight: 74.20 ± 7.66 kg, body fat: 9.5 ± 4.2%, maximal oxygen consumption: 54.7 ± 5.3 $ml{\cdot}kg^{-1}{\cdot}min^{-1}$) completed two exercise sessions one day (Bout 1, Bout 2), followed by one session the following day (Day 2) in 40°C, 40% relative humidity environmental conditions before and after three additional days of heat acclimation. Two hours of intermittent treadmill aerobic exercise comprised each exercise bout. During multi-bout exercise on the first and sixth day of exercise day, the two bouts were separated by two hours of rest in a thermoneutral environment. Days three through five consisted of 90 minutes of treadmill activity or cycling. Heart rate (HR), rectal temperature (T_{re}), and perceptual scales were assessed throughout exercise while environmental symptoms and blood variables were assessed before and after each exercise bout. Planned paired samples t-tests compared variables between bouts at similar time points. Results: The STHA protocol induced an acclimated state as indicated by decreased exercising T_{re} and HR on day six compared to day one. Exercising HR, T_{re}, and perceptual scales were decreased following Bout 1 after STHA compared to Bout 1 before SHTA ($p < 0.05$). Absolute lymphocytes were reduced both before and after Bout 1 following STHA compared to Bout 1 before SHTA ($p < 0.05$). In Bout 2, exercising HR, T_{re}, thermal sensation and perceived exertion were reduced after STHA compared to Bout 2 before SHTA ($p < 0.05$). Despite enduring acclimation, only 18% of subjects completed the entire Bout 2 protocol, compared to 35% before acclimation. On Day 2 both before and after STHA, only 13 of the 17 subjects completed the entire exercise bout. Conclusion: Five days of STHA decreased thermal strain during multi-bout exercise. Despite partial heat acclimation, the majority of individuals still terminated exercise early during a second bout of exercise due to high T_{re}, environmental symptoms, and fatigue.

Introduction

Strenuous physical activity and oppressive environments represent arguably the greatest cardiovascular stressors in humans (1). Athletes and occupational workers such as firefighters, members of the military, law enforcement officers, and industrial workers are required to frequently exert themselves in hot, humid environments. Annually, heat stress experienced during preseason American football practices can lead to a high incidence of exertional heat illnesses (EHI), especially in the late summer months (2-4). Cooper et al. reported a 15.29 incidence rate per 1000 athlete exposures during the first week of American football practice compared to an August average of 8.95 (5). It is proposed that the combination of multiple-session, strenuous exercise and hot environmental conditions during the first few days of activity predisposes individuals to EHI, however, all physiological responses and mechanisms are not yet known.

The current scientific evidence suggests heat acclimation (HA) is perhaps the most effective method to mitigate EHI and improve physical performance in heat. HA adaptations include improvements in cardiovascular function such as increased plasma volume and stroke volume, and decreased heart rate (6, 7). Heat dissipation mechanisms such as cutaneous vascular function, sweat rate and onset, and reductions in core temperature are also improved resulting in reduced thermal load (6, 8). Short term heat acclimation (STHA), five or less days of HA, also incurs benefits including decreased rectal temperature (T_{re}) and heart rate (HR) and increased plasma volume, sweat rate, and work capacity (9-13).

Organizational bodies including the American College of Sports Medicine and National Athletic Trainers' Association suggest implementing preseason HA prior to beginning multi-bout exercise sessions and introducing athletic equipment (14-16). Few studies have investigated if STHA mitigates thermal strain during intense multi-bout, multi-day exercise as typically seen in preseason American football practice. Therefore, the purpose of this study was to determine if STHA mitigates thermal strain during strenuous multi-bout, multi-day exercise in heat.

Methods

Subjects

Seventeen healthy men (age: 22 ± 3y, height: 179.5 ± 6.0 cm, weight: 74.20 ± 7.66 kg, body fat: 9.5 ± 4.2%, maximal oxygen consumption (VO_{2max}): 54.7 ± 5.3 $ml\cdot kg^{-1}\cdot min^{-1}$) voluntarily participated in this study, after providing written informed consent which was approved by the University of Connecticut Institutional Review Board. All were free of current musculoskeletal or chronic health problems, cardiovascular, metabolic and respiratory disease, and had no history of exertional heat stroke within the past three years.

Preliminary Testing

Prior to all testing sessions, subjects were asked to refrain from alcohol and strenuous exercise for 24 hours and caffeine for 8 hours. Height was measured to the nearest 0.5 cm and body mass to the nearest 0.02 kg (T51P, Ohaus, Pine Brook, NJ). Body fat percentage was estimated using previously described methods (17) using skinfold thickness measured at three sites (chest, abdomen, and thigh) in duplicate using a Lange skinfold caliper (BetaTechnology Inc, Cambridge, MD).

A treadmill graded exercise test and open circuit spirometry (TrueOne 2400 Metabolic Measurement System, Parvomedics, Sandy, UT) measured maximal oxygen consumption (VO_{2max}) in a thermoneutral environment. Individuals with $VO_{2max} \geq 45.0$ $ml\cdot kg^{-1}\cdot min^{-1}$ were accepted to participate.

Protocol

Subjects were instructed to drink 500mL water the night before and 250mL the morning of each laboratory visit to ensure euhydration, confirmed with urine specific gravity (USG) via refractometer

(A300CL, Atago, Bellevue, WA) upon arrival to the lab. Hypohydrated (USG > 1.020) subjects consumed 500ml water prior to proceeding with the protocol.

Subjects inserted a rectal thermistor 10-12 cm beyond the anal sphincter (401, Measurement Specialties, Beavercreek, OH), a HR monitor (Race Trainer, Timex, Middlebury, CT) was applied, and nude body mass was recorded. Subjects entered an environmental chamber (4284-2L-W, Minute-Eleven, Weymouth, MA) regulated at 40°C, 40% relative humidity, and sat quietly for a 20 minute equilibration period. After 10 minutes of seated rest, a blood draw was completed.

Immediately prior to exercise commencement, a modified Environmental Symptoms Questionnaire (ESQ) was completed to determine signs and symptoms heat illness, and T_{re}, HR, and perceptual scales were recorded (18, 19). Perceptual scales included 11-point OMNI run-walk and fatigue scales, a 9-point thirst scale, and 17-point thermal sensation scale.

The exercise protocol consisted of 2-hour bouts of interval aerobic treadmill exercise as described in Table 3.1. Intensities were chosen based on a previous study that determined typical preseason American football practices intensities and were calculated from preliminary testing VO_{2max} (20). Running and/or jogging speed was lowered for individuals with an exceptionally fast T_{re} rate of rise to ensure the first exercise (Bout 1) was completed in its entirety one of the termination criteria was not met: 1) $T_{re} \geq$ 40.0°C, 2) unsteady gait making exercise unsafe, or 3) subject request. All following exercise bouts were identical to Bout 1. Throughout exercise, T_{re}, HR, and perceptual scales were recorded. Subjects drank water ad libitum throughout all exercise and rest periods.

Table 3.1. Exercise protocol per exercise bout at 1% grade.

4 min	Jogging (~60% VO_{2max})
1 min	Running (~80% VO_{2max})
4 min	Walking (4.83 $km{\cdot}h^{-1}$)
1 min	Running (~80% VO_{2max})
4 min	Jogging (~60% VO_{2max})
1 min	Running (~80% VO_{2max})
5 min	Rest
20 min x 6 cycles = 2 hour bout of exercise	

Note. VO_{2max} = maximal oxygen consumption.

Upon the completion of 2 hours of exercise, subjects completed an ESQ then rested quietly in a thermoneutral environment. After 10 minutes of seated rest, a blood draw was performed. Subjects replenished fluids with water and a carbohydrate-electrolyte drink ad libitum, as well as ate a small meal (~800 kcal). After 1.5 hours of rest, the ESQ was again assessed, a blood draw was completed, and nude body mass was assessed. Two hours after ceasing Bout 1, subjects began Bout 2 until two hours was completed or one of the termination criteria was met. Upon completion of exercise, subjects again completed an ESQ and venipuncture. Nude body mass was recorded and a urine sample was provided.

Upon arrival to the laboratory on Day 2, delayed onset of muscle soreness (DOMS) was assessed with a visual analog scale with the 0 cm anchor labelled “No Soreness” and 10 cm anchor labelled “Unbearable Soreness”. Overall recovery was assessed with a 15-point scale with a 6 indicating “No Recovery” and 20 indicating “Maximum Recovery”. Exercise consisted of an identical 2-hour exercise bout. On Days 6 and 7, the protocols from Days 1 and 2 were repeated.

Heat Acclimation (HA) Protocol (Days 3-5)

Subjects underwent a hyperthermia-controlled STHA protocol for three days. Subjects completed 90 minutes of cycling and treadmill exercise in a hot, humid environment (40°C, 40% relative humidity). During the first 30 minutes of exercise, subjects exercised at a self-chosen intensity that

elevated T_{re} to 38.5°C. The remaining 60 minutes maintained T_{re} between 38.5-39.99°C. Water was consumed a*d libitum*.

Blood Collection and Analysis

Blood draws consisted of an antecubital venipuncture. Whole blood was allowed to clot after which samples were centrifuged at 3,000 rpm for 15 min at 4°C. Serum samples were analyzed by a biochemical laboratory, Quest Diagnostics (Wallingford, CT) for metabolic and cell variables. Additional serum was transferred into microcentrifuge tubes and stored at -80°C to later analyze cortisol via enzyme-linked immunosorbent assays (CalBiotech Inc, Spring Valley, CA). The intra-assay coefficient of variation was 3.5%.

Statistical Analysis

Planned paired samples t-tests assessed biomarkers, physiological, and perceptual variables Pre- and Post-HA. Missing biomarker data was replaced with the mean of that time point. Rectal temperature rate of rise was calculated using the IPE value of the shorter exercise bout and comparing to the similar time point of the other exercise session. The α-level was set at 0.05. All data were analyzed in SPSS software version 21.0 (IBM SPSS Statistics, Chicago, IL). Data are presented as mean ± SD.

Results

Induction of Short Term Heat Acclimation

Five days of HA induced changes in immediate post-exercise (IPE) T_{re} and HR (Table 3.2). Resting T_{re} and sweat rate were similar Pre- and Post-HA.

Table 3.2. Short term heat acclimation characteristics.

	Pre-HA	Post-HA	p-value
Resting rectal temperature (°C)	36.77 ± 0.44	36.70 ± 0.47	0.630
Post-exercise rectal temperature (°C)	39.50 ± 0.32	39.07 ± 0.54	0.012
Post-exercise heart rate (bpm)	169 ± 20	160 ± 21	0.003
Sweat Rate ($L\cdot hr^{-1}$)	1.48 ± 0.31	1.40 ± 0.71	0.696

Note. HA = heat acclimation. Bpm = beats per minute.

Single Sessions

Short term heat acclimation resulted in decreased thermal strain during exercise. While IPE thermal sensation was similar Pre- and Post-HA ($p = 0.747$), T_{re}, HR, perceived exertion, thirst, and fatigue were reduced Post-HA (Table 3.3). Subjects reported a smaller increase in environmental symptoms Post-HA (6 ± 6 units) compared to Pre-HA (14 ± 10 units, $p = 0.007$).

Table 3.3. Physiological and perceptual changes following Bout 1 Pre- and Post-Heat Acclimation.

	Pre-HA	Post-HA	p-value
Rectal temperature (°C)	39.50 ± 0.32	39.07 ± 0.54	0.012
Heart rate (bpm)	169 ± 20	160 ± 21	0.003
Perceived Exertion	6 ± 3	5 ± 3	0.003
Thirst	4 ± 2	2 ± 1	0.003
Fatigue	6 ± 3	4 ± 3	0.003
Thermal Sensation	6.5 ± 1.5	6.5 ± 0.5	0.747
Total Exercise Time (min)	120 ± 0	119 ± 5	0.333

Note. HA = heat acclimation. Bpm = beats per minute.

Lymphocyte count was reduced Post-HA both before (1687 ± 407 cells·μL^{-1}) and after (1840 ± 440 cells·μL^{-1}) Bout 1 compared to Pre-HA (1883 ± 453 cells·μL^{-1}, 2046 ± 594 cells·μL^{-1}, p = 0.006, p = 0.039, respectively). Aspartate Transaminase (AST) was slightly elevated before Bout 1 Post-HA (26 ± 9 U·L^{-1}) compared to Pre-HA (24 ± 10 U·L^{-1}, p = 0.029), but were similar IPE (p = 0.748). White Blood Cells (WBCs), Neutrophils, Blood Urea Nitrogen (BUN), Creatinine, BUN:Creatinine, and Alanine Transaminase (ALT) all responded to Bout 1 similarly Pre- and Post-HA (p > 0.05).

Double Sessions

Short term heat acclimation decreased thermal strain during double exercise sessions. Rectal temperature, HR, OMNI, and thermal sensation at the end of Bout 2 were mitigated Post-HA (Table 3.4). Heat acclimation did not affect IPE thirst (p = 0.352) or fatigue (p = 0.353), or change in environmental symptoms (p = 0.058).

Table 3.4. Physiological and perceptual variables following Bout 2 Pre- and Post-Heat Acclimation.

	Pre-HA	Post-HA	p-value
Rectal Temperature (°C)	39.62 ± 0.43	39.27 ± 0.65	0.030
Heart rate (bpm)	180 ± 19	159 ± 24	0.004
Perceived Exertion	8 ± 2	6 ± 3	0.011
Thermal Sensation	7.0 ± 0.5	6.5 ± 1.0	0.013
Thirst	4 ± 2	3 ± 2	0.352
Fatigue	7 ± 1	7 ± 1	0.353

Note. HA = heat acclimation. Bpm = beats per minute.

Six subjects completed the full 115 min Bout 2 Pre-HA with all subjects averaging 92 ± 28 min compared to only 3 subjects Post-HA with all subjects averaging 89 ± 26 min (p = 0.648) (Figure 3.1). Pre-HA, 11 subjects ended exercise early due to: T_{re} reaching the laboratory cut-off (5/11), symptoms of heat illness (4/11), joint pain (1/11), and fatigue (1/11). Post-HA, 14 subjects ended early due to: reaching the laboratory cut-off (5/14), symptoms of heat illness (4/14), joint pain (2/14), and fatigue (3/14).

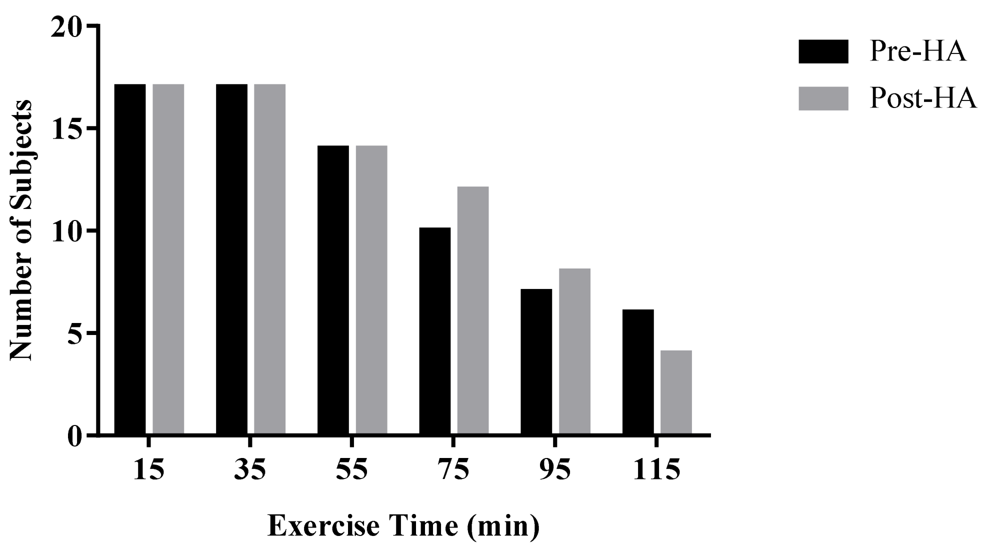

Figure 3.1. Number of participants exercising throughout Bout 2 Pre- and Post-Heat Acclimation. HA = heat acclimation.

During double sessions, IPE serum cortisol concentration was mitigated by HA (361 ± 221 ng·mL^{-1}) compared to Pre-HA (517 ± 241 ng·mL^{-1}, p = 0.002). Neutrophils were reduced Post-HA both before (5494 ± 2274 cells·µL^{-1}) and after (6085 ± 1378 cells·µL^{-1}) Bout 2 compared to Pre-HA (7496 ± 2131 cells·µL^{-1}, 8020 ± 2102 cells·µL^{-1}, p = 0.007, p < 0.001, respectively) (Figure 3.2). Similarly, WBCs were reduced Post-HA both before (7646 ± 2164 cells·µL^{-1}) and after (8559 ± 2198 cells·µL^{-1}) Bout 2 compared to Pre-HA (9392 ± 2207 cells·µL^{-1}, 1075 ± 2292 cells·µL^{-1}, p = 0.005, p = 0.002, respectively). Lymphocytes, Blood Urea Nitrogen (BUN), Creatinine, BUN:Creatinine, AST, and ALT all responded to Bout 2 similarly Pre- and Post-HA (p > 0.05).

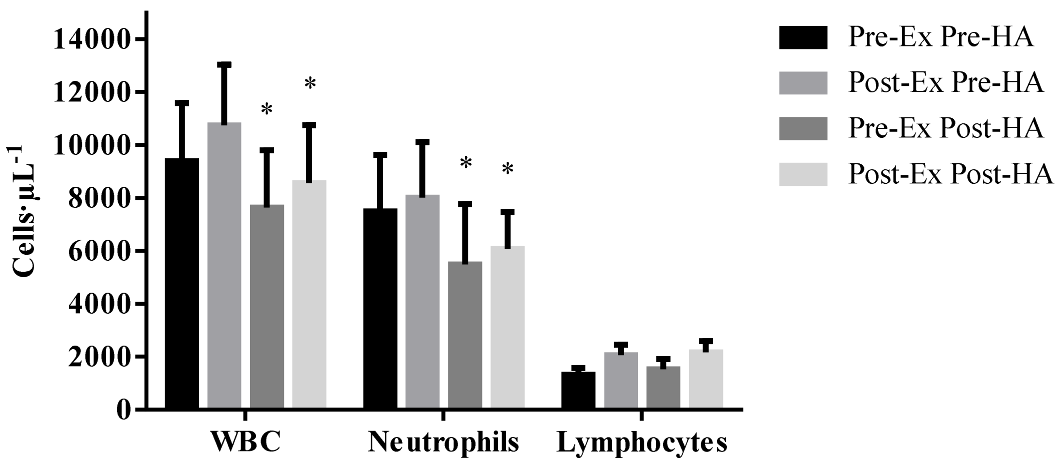

Figure 3.2. Blood cell counts during Bout 2 Pre- and Post-Heat Acclimation. WBC = White Blood Cells. HA = heat acclimation. * indicates different from corresponding Pre-HA value.

Subsequent Exercise Day

Thirteen subjects completed the full 115 min exercise Pre- (113 ± 12 min) and Post-HA (111 ± 15 min, p = 0.726). Of the participants who ended exercise early Pre-HA, 3/5 stopped due to T_{re} reaching the laboratory cut-off, 1/5 stopped due to symptoms of heat illness, and 1/5 stopped due to fatigue. Post-HA 4/5 stopped due to T_{re} reaching the laboratory cut-off and 1/5 stopped due to symptoms of heat illness.

Subjects reported a similar change in environmental symptoms Pre- (9 ± 7 units) and Post-HA (7 ± 7 units, $p = 0.284$).

Similar levels of DOMS were reported at rest on Day 2 ($p = 0.862$) but subjects felt slightly more recovered Post-HA (16 ± 2 units) than Pre-HA (15 ± 2 units, $p = 0.035$). Thirst, HR, and T_{re} were similar ($p > 0.05$) before and after exercise on both days. Thermal sensation and fatigue were similar before exercise ($p > 0.05$), but slightly lower IPE Post-HA (6.5 ± 1.0 units, 5 ± 3 units) compared to Pre-HA (7 ± 0.5 units, 6 ± 2 units; $p = 0.049$, $p = 0.007$, respectively). Sweat rate was higher Post-HA (1.80 ± 0.41 $L \cdot hr^{-1}$) than Pre-HA (1.49 ± 0.33 $L \cdot hr^{-1}$, $p < 0.001$). Cortisol, BUN, Creatinine, BUN:Creatinine, AST, ALT, WBC, Lymphocytes, and Neutrophils were similar IPE Day 2 Pre- and Post-STHA ($p > 0.05$).

Discussion

The purpose of this investigation was to determine if five days of heat acclimation mitigates thermal strain during multi-bout exercise and subsequent day activity in the heat. The results suggest that STHA mitigates exercising T_{re}, HR, perceived exertion, thirst, fatigue, and environmental symptoms during a two hour exercise session. During a second two hour bout of activity on the same day, thermal sensation, T_{re}, HR, and perceived exertion were mitigated following STHA, but environmental symptoms, thirst, and fatigue were similar. On the second day of exercise after STHA, only thermal sensation and fatigue were lower than Pre-HA.

Similar to previous studies with 4-6 day STHA protocols, this study demonstrated lower exercising HR (7, 9, 11, 12, 21-23), exercising internal temperature (7, 11, 12, 21-23), and thermal sensation (11). Sweat rate was increased within 6 days of HA, similar to previous findings (7, 11). Unlike the current results, lower resting HR (11, 12, 23) and resting T_{re} (11, 12, 23) were previously reported within six days of HA. These differences may be due to population (obese and lean boys (23)), environment in which resting measures were taken (thermoneutral environment (11)), or temperature measurement site (esophageal temperature (12)).

Fewer participants (3 out of 17) completed the entire double-session protocol Post-HA due to physiological strain and symptoms of heat illness compared to 5 out of 17 participants Pre-HA. This may indicate that the current heat acclimation guidelines for preseason sports may not fully protect athletes from EHI during multi-bout exercise, although it should be noted that the exact suggested HA protocol was not followed in the current study (2 instead of 3 hours rest between exercise bouts) (14). It is unlikely, however, that one additional hour of rest between activity would have allowed all 14 individuals to complete the second exercise bout. McLellen et al. demonstrated that approximately six hours of rest between less intense exercise bouts in slightly cooler conditions resulted in early cessation of exercise during a second exercise bout, although this was Pre-HA and on the second day of exercise (24). Similar reasons for early exercise cessation were reported (i.e., fatigue, difficulty breathing, nausea, dizziness). Alternatively, it may have been the cumulative physiological strain and resultant fatigue that facilitated the reduced exercise-heat tolerance Post-HA. Regardless, greater periods of inter- and intra-day rest remain prudent recommendations for preventing EHI and preserving physical performance.

Extreme importance should be placed on the attire worn during the laboratory trials. Only shorts and a T-shirt were worn during the experiment. This drastically differs from the full protective gear and uniform suggested by HA guidelines on the first double session day after STHA (14). Similarly, a helmet is allowed on the first day of practice which was not included in the current study. This additional equipment and clothing is known to create uncompensable conditions and would likely exacerbate observed increases in physiological and perceptual variables during exercise, decreasing the efficacy of STHA (25, 26).

To most accurately assess current best practice guidelines, the exact STHA protocol should be tested to determine the level of protection against EHI during preseason athletic practices. Field studies investigating actual sport preseason practices may yield different results due to additional uniforms and equipment, and possible exercise intensity self-modulation instead of a structured exercise intensity, depending on personality (i.e., overzealousness) and drive (motivation to make the team, please the

coach, or attain a starting position). Additionally, both laboratory and field studies are warranted in warm, hot, dry, and humid environmental conditions.

Five days of HA effectively reduced thermal strain during a single session of intermittent exercise in hot, humid conditions. During multi-bout exercise on the sixth day of HA, almost all participants were unable to complete the full exercise trial, stopping prematurely commonly due to attainment of high T_{re} or symptoms of heat illness. If athletes are not allowed or unable to self-determine exercise intensity during multi-bout exercise in heat, they may be at risk of EHI despite undergoing five days of HA. This warrants a review of current heat acclimatization policies to determine if additional HA days or extended rest periods are needed to reduce thermal load, resulting in lower EHI risk.

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APPENDICES

Appendix A

UCONN
UNIVERSITY OF CONNECTICUT
Department of Kinesiology
Human Performance Laboratory

Consent Form for Participation in a Research Study

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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

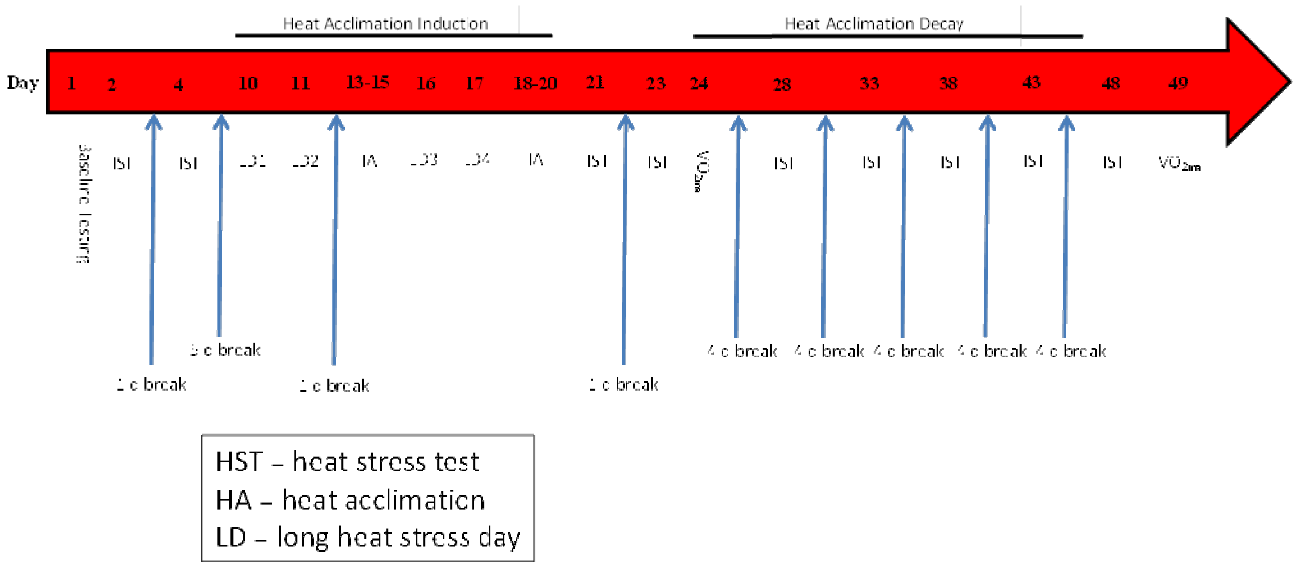
You are invited to participate in a research study evaluating the effectiveness of acclimating to the heat on physical activity in the heat, hand cooling efficacy, and the decay heat acclimation adaptations. You are being asked to participate because you are recreationally active, healthy, male, and 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 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°F, 40% relative humidity) while the exercise only group will undergo the exercise in a cool room (72°F, 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°F, 40% relative humidity) while the no heat exposure group will undergo the same exercise but a cool room (72°F, 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°C, 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, 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°C, 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)

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°C, 40% relative humidity). During the first 30 minutes of the protocol, the goal will be to increase rectal temperature to 38.5°C. The goal of the remaining 60 minutes is to maintain rectal temperature between 38.5-39.99°C. 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°F, 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°F, 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.

What are the risks or inconveniences of the study?
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	Commit-ment Hours
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.

<u>How will my personal information be protected?</u>
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.

<u>Can I stop being in the study and what are my rights?</u>
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.

<u>Whom do I contact if I have questions about the study?</u>
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.

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:
____________________	____________________	__________
Signature of Person Obtaining Consent (Research Assistant)	Print Name:	Date:

Appendix B

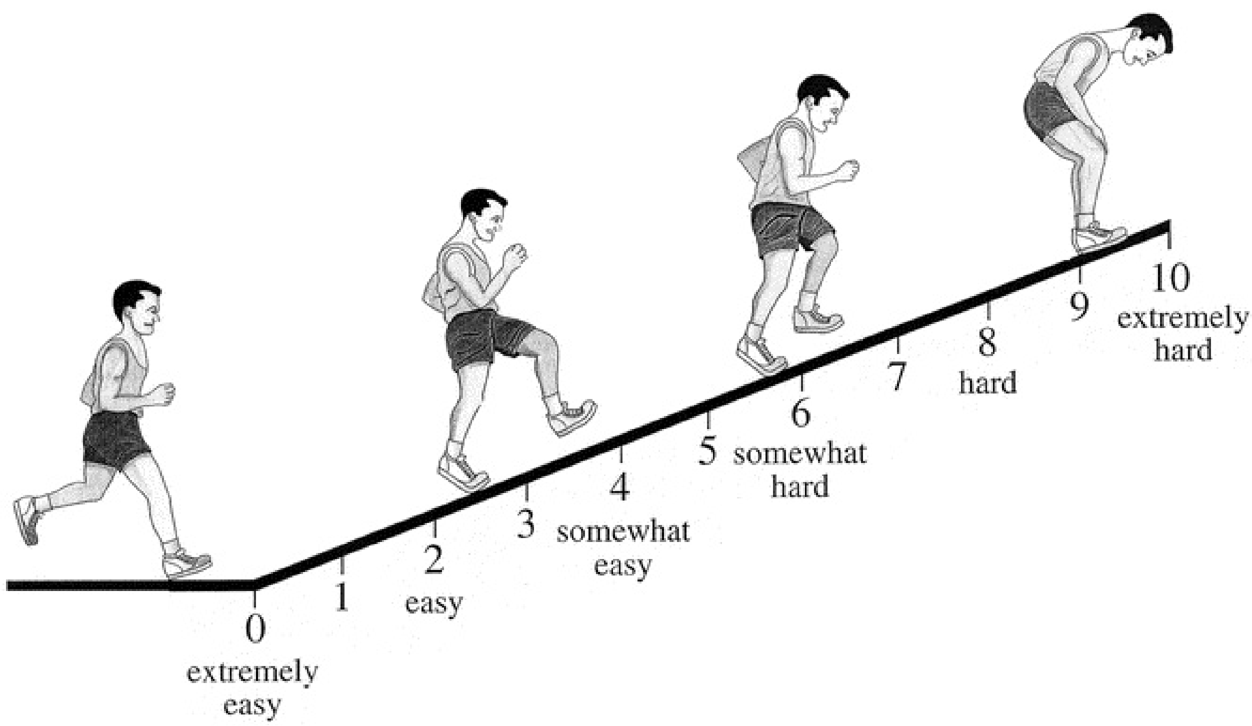
How Do You Feel Questionnaire

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 play 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						

Appendix C

OMNI Scale of Perceived Exertion



Appendix D

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

Appendix E

Thermal Sensation Scale

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

Appendix F

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

Appendix G

Delayed Onset of Muscle Soreness

No Soreness | Unbearable Soreness

Appendix H

Total Quality Recovery

6	No recovery at all
7	Extremely poor recovery
8	
9	Very poor recovery
10	
11	Poor recovery
12	
13	Reasonable recovery
14	
15	Good recovery
16	
17	Very good recovery
18	
19	Extremely good recovery
20	Maximum recovery