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### Effects of 5-Day Heat Acclimation on Workers Wearing Personal Protective Clothing

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#### Abstract

**Introduction:** Elevated ambient temperature and personal protective clothing (PPC) induce physiological strain which may be counteracted by heat acclimation. The purpose of this study was to determine if 5-day heat acclimation training (HAT) improves thermal and perceptual responses while wearing chemical, biological, radiological, and nuclear (CBRN) PPC.

**Methods:** Nine healthy men completed a heat stress test (walking for one hour with CBRN PPC) in 35°C and 50% relative humidity (RH) before and after 5-day HAT. The HAT consisted of five consecutive days of two 45-minute cycling sessions (50% VO2max) wearing athletic clothing separated by a 15 min rest in 45°C and 20% RH. Results of the pre- and post-HAT heat stress tests were compared.

**Results:** Heat acclimation was seen through 5-day HAT; however, thermoregulatory responses did not improve while wearing CBRN PPC. Improvement (p<0.05, day 1 vs. day 5 HAT) in skin temperature ( $38.0\pm0.5^{\circ}$ C vs.  $37.6\pm0.5^{\circ}$ C), body temperature ( $38.6\pm0.4^{\circ}$ C vs.  $38.3\pm0.4^{\circ}$ C), sweat rate ( $2.26\pm0.3$ kg vs.  $2.64\pm0.3$ kg), RPE ( $15.8\pm2.4$  vs.  $13.9\pm3.1$ ), and heat perception ( $5.7\pm0.6$  vs.  $4.9\pm1.0$ ) were noted. However, no physiological or perceptual improvements (p>0.05) were found in the post-HAT heat stress test.

**Conclusions:** Heat acclimation adaptations may be blunted by CBRN PPC, thus requiring differing or extended HAT.

#### Keywords

heat stress test; occupational hazards; hyperthermia

#### Introduction

Occupational and military activities are often performed outdoors where the ambient temperature can exceed 30°C. When ambient temperature is above 35°C, the thermal gradient between skin and environment is reduced, and the human body gains heat from the environment. In many occupational activities, workers are required to wear

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full body personal protective clothing (PPC) to protect them from a variety of external physical and chemical hazards. The combination of a hot environment and PPC impairs thermoregulation and exercise performance. Increased core body temperature (Tcore) can result in dehydration and cardiovascular strain, as well as heat-related illnesses that range from heat cramps to heat stroke 1-3.

Heat acclimation (HA) is one of the strategies to reduce the incidence of heat-related illness in hot environments and is conducted in elevated ambient temperatures. The benefits of full HA have been reported within a week (as early as 3-4 days)<sup>4</sup>, while longer duration of HA results in a greater decrease in heart rate (HR) for a given work load <sup>4,5</sup>. Previous studies report that 4-14 days of HA produce cardiovascular and thermoregulatory adaptations, such as lower Tcore and metabolic rate, improved thermal comfort, exercise performance, sweat production, skin blood flow and fluid balance which result in reduced cardiovascular strain and increased thermal tolerance <sup>6,7</sup>. HA enhances thermoregulation through an increase in sweat rate and decrease in Tcore<sup>8</sup>. Also, HA attenuates cardiovascular strain through an increase in blood plasma volume and stroke volume <sup>5,8,9</sup>. Previous studies demonstrated that approximately 5 days of HA decreases Tcore approximately 0.3 – 0.4°C for a given work rate 5,10. It is documented that human thermal homeostasis is a balance between heat production and heat loss <sup>11</sup>. Typically, PPC limits the evaporation of sweat and increases heat storage for a given work rate or metabolic heat production <sup>13,14</sup>. While much is known regarding the benefits of HA in thermoregulatory responses, perceptual responses, and cardiovascular strain in hot environments during treadmill walking, the effects of HA via differing modes of exercise and relatively short periods of training while wearing PPC have been afforded less scientific scrutiny 15-17. Thus, the purpose of this study was to determine if the effects of short-term HA can elicit similar benefits on individuals who are required to wear PPC. We hypothesized that HA would enhance thermoregulation, perceptual response, and attenuate physiological strain.

#### Methods

#### Participants

The study protocol was approved by the National Institute for Occupational Safety and Health (NIOSH) Institutional Review Board, and both oral and written informed consent were obtained from each subject prior to participation. Nine healthy male volunteers (age= $22\pm1$  years, mass= $78.7\pm10.7$  kg, height= $180\pm10.0$  cm, BMI= $23.7\pm2.5$ kg·m<sup>2</sup>, body surface area= $2.0\pm0.2$  m<sup>2</sup>, VO<sub>2</sub>max= $62.1\pm5.0$ ) underwent medical history and physical screening by a licensed physician. An angio-catheter was inserted by a physician intravenously in an upper extremity for blood sampling. Subjects were asked to abstain from strenuous exercise, caffeine, and alcohol for at least 24 hours prior to a test and to have a light meal no later than two hours prior to the trials. Subjects were also asked to refrain from consumption of all over-the-counter medications and any herbal products (ephedrine-based, etc.) that increase HR and/or BP, for at least 48 hours prior to trials. Subjects with a (current or past) history of smoking, cardiovascular disease, or metabolic disease were excluded.

#### Protocol

The current investigation employed a repeated measures, within-subjects design to measure pre- vs. post-HA training (HAT). Prior to testing, subjects underwent a graded exercise test in a thermoneutral laboratory to determine maximal oxygen uptake (VO<sub>2</sub>max) and assess cardiovascular fitness and capacity.

On two separate occasions (pre- and post-HAT), each subject performed a heat stress test consisting of walking (4.8 km/hr and 3.0% grade) on a treadmill for 60 minutes in a hot environment ( $35^{\circ}C$  and 50% relative humidity). During this testing, subjects wore a Chemical/Biological/Radiological/Nuclear (CBRN) ensemble (Model: Extended Response Suit, Lion Apparel, Dayton, OH) consisting of a one-piece coverall of semi-permeable fabric with attached glove liners and booties encapsulating the whole body, except a frontal face area, open for a respiratory facemask. The respiratory protective facemask was replaced by a spirometer mask ((Model K4b<sup>2</sup>, Cosmed, Rome, Italy) that allowed breath-by-breath gas exchange analysis. Prior to the heat stress test, subjects provided a urine sample for assessment of hydration status and then were asked to consume 500 ml of water, over approximately 30 minutes, before entering the environmental chamber. Each heat stress test was separated from the HAT trials by 72 hours.

72 hours after the pre-HAT heat stress test, subjects conducted 5 consecutive days of HAT, during which they cycled on an electronically-braked cycle ergometer (VIAsprint<sup>TM</sup> 150P, CareFusion, Hochberg, Germany) at 50% relative VO<sub>2</sub>max for two 45-minute bouts in hot, dry environmental conditions (45°C, 20% RH) with a 15-minute rest, in the hot environment, in between sessions. Subjects wore t-shirts, shorts, and athletic shoes. Physiological measurements (rectal temperature [Tre], skin temperature [Tsk], oxygen consumption [VO<sub>2</sub>], HR) were recorded continuously. During the 15 minutes of rest, subjects were given a controlled amount of water (5 mL·kg<sup>-1</sup> body weight). HAT was terminated when subjects expressed volitional fatigue or Tre >39°C, however, the subjects remained in the environment chamber for the remainder of the 45-minute training period.

Core body temperature was measured using a rectal probe (REF-4491, YSI Temperature, Dayton, OH) inserted 13 cm beyond the anal sphincter. Tsk was measured with skin thermistors (T-type copper/constantan, Concept Engineering, Old Saybrook, CT) affixed with a transparent dressing film (Tegaderm, 3M, St. Paul, MN) onto four body sites (upper chest, shoulder, anterior thigh, calf) to monitor and calculate weighted mean Tsk<sup>18</sup>. Mean body temperature (Tb) was calculated by the equation previously described by Burton <sup>19</sup>. RPE, using the Borg 6-20 scale <sup>20</sup>, and HP, using a 1 (slightly cool) – 7 (extremely hot) scale <sup>21</sup>, were recorded every 15 minutes during exercise. Whole body sweat rate was calculated by the difference in pre- and post-test nude body weight measured on a calibrated scale (Electronic scale-4450, GSE, Farmington Hills, MI) and divided by time.

Blood samples were taken before and after each exercise session using 10 mL syringes (Becton Dickinson, Franklin Lakes, New Jersey) from the indwelling angio-catheter (REF 381533, Becton Dickenson, Franklin Lakes, New Jersey). Blood samples were immediately transferred into serum separator tubes for the measurement of serum osmolality and electrolytes (sodium [Na<sup>+</sup>], potassium [K<sup>+</sup>], chloride [Cl<sup>-</sup>]), in duplicate (EasyLyte

Plus Analyzer, Medica Corporation, Bedford, MA). Urine specific gravity (USG) was measured from pre- and post-exercise urine samples with a refractometer (PAL-10S Pocket Refractometer, Atago, Tokyo, Japan).

#### **Statistical Analysis**

All statistical analyses were conducted using the Statistical Package for Social Sciences (SPSS) software (v.19.0, IBM, Somers, NY), a two-way repeated measures ANOVA (time by condition) was conducted to determine the effects of HAT on physiological and subjective measurements. The two conditions were defined as before and after HAT. If a significant interaction was found, post-hoc pairwise comparison with Least Significant Differences (LSD) was performed. Paired sample t-tests were conducted to determine the effects of HAT on weight loss and percent change of plasma volume across the two heat stress tests. Statistical significance was set at p 0.05 and all data are presented as mean  $\pm$  SD.

#### Results

All subjects completed the 5-days of HAT. Time had a significant effect (p<0.001) on the studied variables, and absolute values increased over the course of each day of HAT (Table I). Significantly lower values (p<0.05) were seen at the end of exercise on Day 5 compared with Day 1 for Tsk (Day 1: 38.0±0.5, Day 5: 37.6±0.5), Tb (Day 1: 38.6±0.4, Day 5: 38.3±0.4), RPE (Day 1: 15.8±2.4, Day 5: 13.9±3.1), and HP (Day 1: 5.7±0.6, Day 5: 4.9±1.0) (Table I). A significant difference in Tre (p=0.044) was found between Day 1 and Day 5 (Table II). No significant condition by time interaction was noted (Table II). A significant increase in sweat rate was seen from the increase in weight loss from Day 1 (2.26±0.3 kg) to Day 5 (2.64±0.4 kg) (p=0.02).

All subjects completed both pre- and post-HAT heat stress tests. The significance of condition, time, and condition by time interaction upon study variables are reported in Table II. All study variables increased significantly throughout each test, save for USG and serum osmolality, though the latter approached statistical significance (Table II and III). Condition had no significant effect, save for HP (Table II and III). Condition by time interaction was significant only for Tsk (Table II and III). A paired sample t-test revealed that total weight loss during heat stress testing significantly increased in the post-HAT heat stress test (pre:  $1.45\pm0.3$  vs post:  $1.75\pm0.4$  kg, p=0.016). The impact of HAT upon study variables during heat stress testing is found in Table III. All values increased over the course of the test. Percent change of blood plasma volume did not differ between pre- and post-HAT heat stress tests (pre:  $-6.95\pm2.2$  vs post:  $-7.00\pm3.4\%$ , p=0.953).

#### Discussion

The present study examined the effect of 5-day HAT in a hot environment (45°C, 20%RH) on physiological and subjective perceptions of men wearing a CBRN ensemble. It can be concluded that 5-day HAT resulted in subject heat acclimation as noted by the physiological and perceptual changes from Day 1 to Day 5 of HAT (Tsk, Tb, RPE, HP, Weight Loss, and

Tre). However, no physiological or perceptual benefit was seen in the post-HAT heat stress test compared to the pre-HAT heat stress test while wearing CBRN PPC.

While previously described work has explored the effect of heat acclimation on individuals wearing  $PPC^{16,22}$ , this investigation asked if a different HAT protocol may show different results. First, the current study utilized a short term HAT, specifically using cycling, that has not been previously tested with PPC. Additionally, introducing two exercise sessions per day with a rest interval between provided the ability for increased asymptomatic exercise time (1.5 hours compared to the previously describe sessions of 1 hour), potentially effecting the acclimation outcomes while wearing  $PPC^{16,22}$ . The current study was limited in its ability to measure physiological effects of HA across various workloads, modalities, and duration of training sessions as only one protocol was used. Also, the exercise modality used did not closely represent expected occupational physical activity while wearing CBRN PPC. Finally, the study population of men limits the generalizability of these results to women.

HAT showed no decrease in RPE or HP in the post-HAT heat stress test. While HP was significantly different, pairwise comparisons revealed that the differences were found only at the 15-minute and 45-minute time points. This finding, while statistically significant, may not be clinically meaningful as the end exercise HP was unchanged after HAT. This is in agreement with a previous study that showed 6- and 12-day HA significantly decreased RPE and thermal discomfort while wearing normal clothing but remained unchanged while wearing PPC <sup>22</sup>. Our results are however in conflict with a previous investigation showing that 5 days of permissive dehydration HA decreased RPE and thermal comfort without PPC<sup>23</sup>. Elevated RPE limits the workers' ability to sustain continuous work for extended periods of time in a hot environment. Our findings may be of significance in that, while short-term HA has previously been thought to attenuate the RPE response <sup>23</sup>, this may not be the case while wearing PPC <sup>22</sup>. It may be that the effects of short term heat acclimation on RPE and HP are counteracted by PPC due to the increased thermal stress and increased evaporative resistance.

The findings of the present study indicate that 5-day HAT did not improve thermoregulatory, metabolic, and electrolyte responses during the post-HAT heat stress test. This is in agreement with previous investigations, showing chemical protective clothing may limit the benefits of HAT with short (6 days) or long (2 weeks) treadmill exercise protocols <sup>15,16</sup>. While thermoregulatory acclimation was seen from Day 1 to Day 5 of HAT, the current study noted no enhancement of thermoregulatory response or HR. Previous studies have reported that 9 to 12 days of HAT lowered resting Tcore <sup>24</sup>, and even short-term HAT (5 days) reduced Tre and HR in highly-trained subjects <sup>6</sup>. It can be hypothesized that the addition of PPC during the pre-and post-HAT heat stress tests counteracted the effects of heat acclimation shown. While increased sweat rate was shown in pre- vs. post-HAT heat stress tests, the evaporation of that sweat was blunted from the PPC which limited its thermoregulatory potential to slow the rise in core body temperature.

Serum electrolytes (Na<sup>+</sup>, K<sup>+</sup>, CL<sup>-</sup>), serum osmolality and USG responses did not change in pre- to post-HAT heat stress tests. These findings align with a previous study where serum electrolyte concentration remained the same from pre- to post-HAT heat stress tests<sup>26</sup>.

Differing from these results, another study reported that serum osmolality significantly increased during exercise after 16 days of HAT <sup>29</sup>. It may be that the increases in serum electrolytes in the current study were not of practical magnitude to impact serum osmolality significantly.

The current study found no significant evidence of physiological, thermoregulatory, or psychological changes during heat stress testing while wearing PPC following a HAT except for an increase in sweat rate. It has previously been suggested that sweat rate is the final physiological variable to adapt during heat acclimation training and therefore if an increased sweat rate is seen, standard heat acclimation adaptations have occurred <sup>17</sup>. This notion that the subjects were adequately acclimated to the heat was supported by the physiological adaptations seen from Day 1 to Day 5 of HAT. The increased evaporative resistance of the PPC limited the ability for the sweat to be evaporative which limited the potential of the sweat adaptation to regulate body temperature. Thus, no attenuation of the Tre, Tsk, Tb, HR, VO<sub>2</sub>, RPE, or HP was seen. These results are significant as they suggest that short term HAT (5 days) may not work to provide meaningful physiological adaptations for individuals working in hot or humid environments while wearing semi-permeable PPC.

The current study concludes that physiological responses to exercise in workers wearing CBRN PPC remain unchanged following novel HAT of 5 consecutive days. While heat acclimation was achieved, the normal physiological adaptations to heat acclimation training may be counteracted by the use of semi-permeable PPC.

#### **Media-Friendly Summary**

5-day heat acclimation training achieves thermoregulatory and perceptual adaptations to the heat similar to longer acclimation periods. The effects of short-term heat acclimation are counteracted by the use of semi-permeable personal protective clothing. Short-term heat acclimation training may not be suitable to achieve protective effects to the heat in workers wearing semi-permeable personal protective clothing.

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#### Disclaimer:

The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention.

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#### Table I.

Effect of HAT upon physiological and subjective variables.

	Day	1 HAT	Day 5 HAT	
Variables	0 min	End-Exercise	0 min	End-Exercise
Tre (°C)	36.9±0.2	38.9±0.6*	36.8±0.2	38.6±0.5*
Tsk (°C)	32.6±1.4	38.0±0.5*	33.0±1.5	37.6±0.5 <sup>*,†</sup>
Tb (°C)	35.3±0.5	38.6±0.4*	35.4±0.6	38.3±0.4 *,†
HR (beats/min)	92.8±14.3	173.9±11*	92.6±20.6	166.9±16.7*
USG (g·mL <sup>-1</sup> )	1.019±0.009	1.014±0.012*	$1.019 \pm 0.010$	1.022±0.008*
RPE	6.0±0.0	15.8±2.4*	6.0±0.0	13.9±3.1 *,†
НР	1.6±0.8	5.7±0.6*	1.6±0.5	4.9±1.0*,†
Weight Loss (kg)		2.26±0.3		2.64±0.4 <sup>†</sup>

\* significantly different from 0 min, p<0.05

 $\dot{\tau}_{significantly}$  different from Day

#### Table II.

Statistical analysis of main effect for time, condition and interaction.

Heat Acclimat	tion Traini	ng	
Variables	Time	Condition	Interaction
variables	p-value	p-value	p-value
Tre (°C)	< 0.001	NS	NS
Tsk (°C)	< 0.001	NS	NS
Tb (°C)	< 0.001	NS	NS
HR (bpm)	< 0.001	NS	NS
Heat Stress Test with Pers	onal Prote	ctive Clothin	g
Tre (°C)	< 0.001	NS	NS
Tsk (°C)	< 0.001	NS	< 0.05
Tb (°C)	< 0.001	NS	NS
HR (bpm)	< 0.001	NS	NS
VO <sub>2</sub> (ml/kg/min)	< 0.001	NS	NS
RPE	< 0.001	NS	NS
HP	< 0.001	< 0.01	NS
USG (g·mL <sup>-1</sup> )	NS	NS	NS
Serum Na+ (mmol/L)	< 0.01	NS	NS
Serum K+ (mmol/L)	< 0.01	NS	NS
Serum CL-(mmol/L)	< 0.001	NS	NS
Serum Osmolality (mOsmol/kgH <sub>2</sub> O)	NS	NS	NS

NS: p>0.05

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# Table III.

Physiological and subjective variables measured during heat stress tests with personal protective clothing.

			Pre- HAT					Post- HAT		
Variables	0 min	15 min	30 min	45 min	60 min	0 min	15 min	30 min	45 min	60 min
Tre (°C)	$37.0 \pm 0.2$	$37.1 \pm 0.3$	$37.4{\pm}0.5$ *	$38.1{\pm}0.3$	$38.6{\pm}0.5$ *	36.8±0.2	$36.9{\pm}0.1$	$37.3{\pm}0.2$	$37.9{\pm}0.3$ *	$38.4\pm0.4$ $^{*}$
Tsk (°C)	32.5±0.7	$34.6\pm0.7$ *	$35.7{\pm}0.4$ *	$36.4{\pm}0.4^{*}$	$37.1 \pm 0.4$	32.7±0.7	$34.6{\pm}0.7$ *	$35.6{\pm}0.4$ $^{*}$	$36.3{\pm}0.4$	$37.0{\pm}0.4$ *
Tb (°C)	$35.4\pm0.3$	$36.2\pm0.4$ *	$37.0{\pm}0.0^{*}$	$37.4{\pm}0.5$ *	$38.1{\pm}0.3$	35.3±0.3	$36.1\pm0.3$	$36.7{\pm}0.2$	37.3±0.3*	$37.9{\pm}0.4$ *
HR (bpm)	76.7±9.2	$122.1\pm16.7$ *	$135.7\pm15.6^{*}$	$150.4{\pm}14.5$ *	164.6±12.7 *	73.8±8.6	$111.7\pm 19.7$ *	124.3±21.3*	$140.0{\pm}22.0$ *	$158.0{\pm}20.4$ *
VO2 (ml/kg/min)	$5.6 {\pm} 0.7$	$18.9{\pm}1.7$	$20.5{\pm}1.6^{*}$	$22.0\pm 2.2$	$22.5\pm 2.6$ *	$5.6 \pm 0.6$	$16.9{\pm}4.6^{\ast}$	$18.9{\pm}2.2$ $^{*}$	$20.5\pm 2.1$	$20.7{\pm}1.6$
RPE	$6.0{\pm}0.0$	$8.9{\pm}1.2$	$10.4{\pm}1.3$ *	$12.1{\pm}1.8^{*}$	$13.8\pm 2.0^{*}$	$6.0{\pm}0.0$	$8.8{\pm}1.1^{*}$	$10.4{\pm}1.4$ $^{*}$	$12.0{\pm}1.0^{*}$	$13.8{\pm}2.4$ $^{*}$
dH	$2.2 \pm 0.7$	$3.9\pm0.9$	$4.7{\pm}0.7$ $^{*}$	$5.4{\pm}0.7$ *	$5.7{\pm}1.0^{*}$	$1.9 \pm 0.6$	$3.4{\pm}0.7$ * $^{+}$	$4.3\pm0.5$ $^{*}$	$4.9{\pm}0.9^{*}^{\acute{\tau}}$	$5.3{\pm}1.0^*$
USG (g·mL-1)	$1.016 \pm 0.010$				$1.012 \pm 0.012$	$1.021{\pm}0.007$				$1.012 \pm 0.009$
Na <sup>+</sup> (mmol/L)	$141.8 \pm 1.4$				$143.1\pm 2.1 ^{*}$	$141.9\pm0.9$				$143.7{\pm}1.7$ *
K <sup>+</sup> (mmol/L)	$4.2 \pm 0.3$				$4.6{\pm}0.3^{*}$	$4.2 \pm 0.3$				$4.5{\pm}0.2$
Cl <sup>-</sup> (mmol/L)	$102.4{\pm}3.0$				$104.9\pm 2.9^{*}$	$102.4\pm 1.2$				$105.1{\pm}1.8^{*}$
Osmolality (mOsmol/kgH2O)	296.3±4.8				$299.6 \pm 4.1$	298.2±5.8				$300.1\pm3.3$
Weight Loss (kg)					$1.45 \pm 0.3$					$1.75{\pm}0.4^{\circ}$
* significantly different from 0 min, p<0.05	t, p<0.05									

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 $\dot{\tau}^{}$  significantly different from Pre-Heat Acclimation Test