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THE IMPACT OF HYDRATION STATUS DURING HEAT ACCLIMINATION ON PHYSIOLOGICAL STRAIN AND EXERCISE PERFORMANCE

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IMPACT OF HYDRATION STATUS DURING HEAT ACCLIMATION ON PHYSIOLOGICAL STRAIN AND EXERCISE PERFORMANCE

By

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Thesis Paper

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The Impact of Hydration Status During Heat Acclimation on Physiological Strain and Aerobic Performance

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Heat acclimation promotes adaptations to attenuate physiological and perceptual strain associated with heat stress, and may be enhanced by promoting dehydration during acclimation. **Purpose:** To determine i) the effect of fluid delivery during acclimation by inducing dehydration (DEH=0.5 ml•kg⁻¹•min⁻¹) vs. euhydration (EUH=2.0 ml•kg⁻¹•15 min⁻¹) following three heat acclimation bouts on heat stress factors, and ii) to determine if fluid delivery (EUH vs. DEH) affects aerobic performance in the heat. **Methods:** Thirteen aerobically fit males completed 90-minute heat stress test (HST) in hot conditions (40°C, 30% RH) walking at 50% VO₂ max prior to and following three days of EUH and DEH acclimation trials. Acclimation trials were in the same environment and intensity as HST, and separated by one day. Participants wore standard Wildland Firefighter (WLFF) Nomex: shirt, pants, and a cotton T-shirt. Following each HST, aerobic performance was assessed by a graded ramp protocol increasing grade 1% until 15%, and then increased 1.6km*h⁻¹ every minute until volitional exhaustion. Peak core temperature (T_c), skin temperature (T_sk), heart rate (HR), rating of perceived exertion (RPE), physiological strain index (PSI), aerobic performance, and sweat rate were collected following HST. Skin blood flow (SBF) was measured via laser doppler flowmetry at 30, 60, and 90 minutes during exercise, and a five-minute recovery period. All data was analyzed by a two-way analysis of variance (ANOVA) 2 (Trt) x 2 (Time). **RESULTS:** Acclimation significantly decreased peak T_c (DEH= 39.5°C± 0.10 to 39.0± 0.12, EUH=39.5°C± 0.12 to 39.9± 0.12, p<0.001), peak T_sk, (DEH=37.8°C± 0.19 to 37.6± 0.12, EUH=37.9°C± 0.20 to 37.5± 0.10, p=0.005), peak HR (DEH=178.1 b*min⁻¹ ± 3.33 to 164.1± 4.43, EUH= 179.3 b*min⁻¹ ± 3.38 to 167.4 ± 3.72 p<0.001), peak RPE (p<0.001), and peak PSI (p<0.001), while significantly increasing aerobic performance (p<0.001) in both DEH & EUH groups. There was no main effect for Time for peak SBF or SBF recovery (p=0.313, p=0.361) respectively. Sweat rate was significantly greater in DEH (1.5 L*h⁻¹± 0.06 to 1.9 L*h⁻¹ ± 0.09) compared to EUH (1.6 L*h⁻¹± 0.06 to 1.8 L*h⁻¹± 0.08) (TrtXTime: p=0.015), and blood plasma percentage increased in the DEH group as a main effect for Time (DEH= 7.1% ± 1.84, EUH= 4.1% ± 2.46, p=0.002). **CONCLUSION:** Short-term heat acclimation effectively attenuates heat stress, and improves aerobic performance in the heat. Fluid delivery strategies during acclimation do not affect thermal strain or performance, but may increase sweat capacity and plasma volume to additionally protect from heat stress.
# Table of Contents

**Chapter One: Introduction**

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>Problem</td>
<td>4</td>
</tr>
<tr>
<td>Purpose</td>
<td>4</td>
</tr>
<tr>
<td>Null Hypothesis</td>
<td>4</td>
</tr>
<tr>
<td>Significance of Study</td>
<td>5</td>
</tr>
<tr>
<td>Limitations</td>
<td>5</td>
</tr>
<tr>
<td>Delimitations</td>
<td>6</td>
</tr>
<tr>
<td>Definition of Terms</td>
<td>6</td>
</tr>
</tbody>
</table>

**Chapter Two: Review of Literature**

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
</tr>
</tbody>
</table>

**Chapter 3: Methodology**

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants</td>
<td>18</td>
</tr>
<tr>
<td>Preliminary Testing</td>
<td>18</td>
</tr>
<tr>
<td>Experimental Trials</td>
<td>20</td>
</tr>
<tr>
<td>Acclimation</td>
<td>21</td>
</tr>
<tr>
<td>Measurements</td>
<td>22</td>
</tr>
<tr>
<td>Statistical Analysis</td>
<td>24</td>
</tr>
</tbody>
</table>

**Chapter 4: Results**

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acclimation Data</td>
<td>25</td>
</tr>
<tr>
<td>Heat Stress Data</td>
<td>27</td>
</tr>
<tr>
<td>Figures</td>
<td>30</td>
</tr>
</tbody>
</table>

**Chapter 5: Discussion**

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>49</td>
</tr>
</tbody>
</table>

**Appendix I: IRB-Approval**

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>58</td>
</tr>
</tbody>
</table>

**Appendix II: PAR-Q**

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>65</td>
</tr>
</tbody>
</table>

**Appendix III: Supplementary Data**

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>66</td>
</tr>
</tbody>
</table>

**References**

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>74</td>
</tr>
</tbody>
</table>
Chapter 1: Introduction

Introduction

Aerobic exercise in the heat challenges both athletes and occupational athletes such as military personnel and Wildland firefighters (WLFF) to sustain arduous tasks for long periods, threatening their ability to maintain cardiovascular and thermoregulatory stability. Heat stress results from the interaction of environmental conditions (temperature, humidity, solar radiation), physical work rate (metabolic heat production), and heavy clothing that obstructs heat loss (1-4). Therefore, maintaining work outputs in the heat impedes proper thermoregulation and increases cardiovascular and thermal strain associated with long duration events (5-8). These effects can be more pronounced in the event of dehydration (11-13) or when wearing personal protective equipment (PPE). Together, these efforts translate to declining exercise performance (14) and increased susceptibility to heat related illness (HRI). The physically demanding environment of WLFF can be hazardous in that two of every 1,000 workers are at risk for HRI (6). Occupations such as those carried out by WLFF are at increased risk due to metabolic demands for long periods (8), dehydration, and use of PPE (3, 10). Human ability to acclimate to hazardous environments may be advantageous in attenuating environmental stresses induced by heat, and decreasing the susceptibility to HRI.

Human ability to acclimate to the heat involves promoting stresses over several days with aims to increase core temperature ($T_c$), skin temperature ($T_{sk}$), and promote sweating (14, 15). Promoting these stresses induces adaptations to attenuate both cardiovascular and thermal strain associated with exercising in the heat (5, 16-20). Additionally, heat acclimation can be applied as an ergogenic aid to improve aerobic performance (5, 21, 24, 25). The physiological adaptations
associated with heat acclimation include an attenuated core temperature ($T_c$), skin temperature ($T_{sk}$) and heart rate (HR) at a given exercise intensity (5, 17-19, 21, 23-25). This effect limits cardiovascular and thermoregulatory strain attributed to improved sweat response (9, 17-19, 25) to enhance evaporative cooling and increase the core-to-skin ($T_c-T_{sk}$) temperature gradient (16, 26, 61) necessary to provide heat dissipation and lower the demand for blood flow in the peripheries. The applied aspects involving heat acclimation include improved aerobic performance by measuring VO$_2$ max (5, 7, 21), mean work output during time-trials (20), increased work rate at lactate threshold (21, 25), and increased time to exhaustion (18). Acclimation to the heat can thus overcome impairments associated with heat stress, allowing the body to maintain cardiac output and oxygen delivery to working muscles (28) necessary to sustain performance.

Blood plasma volume (PV) is an important physiological marker associated with improving cardiovascular and thermal stability in the heat (17-19, 21, 24, 27, 29, 30, 31). By promoting water retention, acclimated individuals have shown to increase total body water (TBW) by 2-3 L (~5-7%) (19, 29). PV expansion in the body acts as a buffer to overcome the inevitable thermal effects of dehydration (11, 13), allowing high sweat responses, but maintaining the blood volume necessary to deliver oxygen to working muscles. PV expansion has been shown to increase cardiac output by increasing the Frank-Starling effect, resulting in increased stroke volume due to higher preloads (18, 21), and lowering cardiovascular strain necessary to maintain cardiac output (11). Performance benefits have been shown to be present due to PV expansion in the absence of heat acclimation (32), and utilizing heat acclimation to induce PV expansion increases oxygen delivery to working muscles for use as an ergogenic aid.

PV expansion is increased in the body by secretion of the fluid regulatory hormones aldosterone and vasopressin (33, 34) to increase fluid retention in the kidney tubules (14). In
combination with heat exposure, dehydration has been shown to increase the release of aldosterone and vasopressin (12, 24, 27, 33-36). Therefore, increased blood osmolality induced by dehydration may increase PV acting as an ergogenic intervention to improve performance in the heat. Therefore, the positive feedback mechanism due to increased osmolality suggests that oversecretion of these hormones can lead to further PV expansion. Dehydration increases the rate of cardiovascular drift when exercising in the heat (5,11, 13) caused by lowering blood volumes due to sweating and lowers aerobic performance in the heat (9). Therefore, dehydration should not be consistently practiced in extreme environments without rehydration, and should be used as an additional training stress in preparation for the heat.

An extensive amount of research has focused on the effect of long-term heat acclimations (LTHA) (5, 9, 16-20) that can create robust adaptations. However, short-term heat acclimation (STHA) requiring ≤ 5 days heat exposure has also shown significant adaptations resulting in improved aerobic performance (22, 24, 27) and attenuations in thermal and cardiovascular strain similar to LTHA (37). If STHA promotes similar adaptations compared to LTHA, a lower amount of exposures may provide a more applicable and cost effective alternative for occupational athletes such as WLFF and soldiers residing in mild climates that have limited time to adapt to the heat. By combing a STHA protocol and promoting dehydration to induce PV expansion, an effective protocol may be designed to limit the cardiovascular and thermoregulatory strain associated with long duration aerobic exercise in the heat, benefiting the performance and safety of individuals.
Problem

WLFFs, military personnel, and competitive athletes are often faced with rapid displacement to hot environments and expected to sustain moderate work outputs without prior acclimatization. Determining an affective STHA protocol can be implemented to benefit this population. By promoting the stress of dehydration, these adaptations can be augmented within a shorter period, providing an ergogenic aid to further promote physiological adaptations associated with acclimation. Application of an effective protocol can be implemented for rapid response groups to enhance safety and work outputs for these individuals prior to departing to hot environments.

Purpose

The purpose of this study was to evaluate the impact of hydration status during STHA by comparing its effects on physiological markers of heat stress \( [T_c, T_sk, \text{physiological strain index (PSI)}, \text{sweat rate, cardiovascular strain, and skin blood flow (SBF)}] \) during a 90-minute heat stress test (HST), aerobic performance, and plasma volume associated with acclimation.

Null Hypothesis

1. No significant difference in \( T_c, T_sk, \text{HR, and PSI after acclimation, and between DEH and EUH groups following acclimation.} \)
2. No significant difference in rate of perceived exertion (RPE) after acclimation, and between DEH and EUH groups following acclimation.
3. No significant difference in SBF after acclimation, and between DEH and EUH groups following acclimation.
4. No significant difference in sweat rate after acclimation, and between DEH and EUH groups following acclimation.
5. No significant difference in plasma volume after acclimation, and between DEH and EUH groups following acclimation.

6. No significant difference in aerobic performance after acclimation, and between DEH and EUH groups following acclimation.

**Significance of Study**

The findings of this research have implications in applying a STHA protocol used to rapidly acclimate WLFF, soldiers, and recreational athletes. If dehydration can influence the heat acclimation process, then a STHA protocol with permissive dehydration can be implemented to enhance these adaptations and decrease the risk of heat related illness, thus increasing work outputs in hot climates.

**Limitations**

- Subjects were not be recruited by random sample, but rather by convenience.
- Subjects and research personnel were aware of the training intervention and cannot produce a double-blind design. Subjects were randomly assigned to training interventions prior to testing.
- In order to minimize confounding changes in physiological adaptations and performance, all subjects were asked to complete a physical activity and dietary log for the day prior to the test and during the acclimation periods. Each subject was required to restrain from alcohol and strenuous exercise the day before HST trials and acclimation trials.
Delimitations

- All subjects were males. This occurs due to fluctuation of core temperature in females due to the menstrual cycle.
- All subjects had a VO₂ max >40 ml•kg⁻¹•min⁻¹ and <65 ml•kg⁻¹•min⁻¹. This was followed in order for subjects to maintain a prolonged aerobic demand, and prevented further aerobic adaptations from the acclimation trials. They were not too fit in order to prevent anaerobic influences due to grade of the treadmill adjusted to relative workload.
- All subjects refrained from traveling to a hot environment (ex. Hawaii) and regular sauna use at least two weeks before the first trial, and during the washout period in order to prevent confounding adaptations to the heat.

Definition of Terms

- **Acclimation:** Adaptations developed following repeated heat exposures in an artificial (laboratory) setting. The aims of acclimation are to induce sweating, increase Tc, and TsK. (15).
- **Acclimatization:** Exposure to natural environments that elicit response to adaptations in the heat. (23).
- **Heat Stress:** Sum of metabolic heat (physical work) plus heat gained from environment (temperature, humidity, solar radiation) minus evaporative heat loss (sweating) (1).

**Physiological Strain Index (PSI):** Method to calculate heat strain by monitoring changes in Tc and HR. The index rates PSI on a 0-10 scale using the following equation.

\[
PSI = 5 \times \left( \frac{Tc - Tco}{39.5 - Tco} \right) + 5 \times \left( \frac{HR - HRo}{180 - HRo} \right)
\]
Where $T_{C0}$ and $HR_0$ are $T_C$ and $HR$ at rest. $T_{C1}$ and $HR_1$ are the core temperature and heart rates collected at any one time during exercise (38).

**Sweat Rate:** Amount of sweat lost by the body over time and calculated by determining the change in weight plus the amount of liquid consumed over the specific amount of time (39).

**Skin Blood Flow (SBF):** The amount of blood flow through vessels of the dermis. SBF increases 15-20 fold when heated due to arterial dilation and dermal capillary recruitment (40).

**Laser Doppler Flowmetry:** A noninvasive way to measure the flow rate of blood and its red cells through the skin. (40).

**Core-to-Skin ($T_C$-$T_{sk}$) temperature gradient:** Difference between core temperature produced from metabolic heat production and skin temperature that measures the effectiveness of heat offloading to the environment (41).

**Frank Starling Mechanism:** The ability of the heart to change its force of contraction and stroke volume in proportion with venous return.

**Fluid Oncotic Pressure:** The effect of proteins in blood plasma (most notably albumin) that tends to displace water into the intravascular space.
Chapter Two: Review of Literature

Exercise Performance in the Heat

It is well-known that hot environments negatively impact sustained exercise and work performance (14, 43) and performance decrements occur in the presence of modest hyperthermia (38.5°C) (42) and in environmental conditions as low as 10°C WBGT (43). Physiological factors limiting exercise performance in the heat include cardiovascular mechanisms to attenuate stroke volume (11, 18) requiring an increased HR to maintain cardiac output to the working muscles.

Nielsen et al. 1990 (44) studied the limitations of exercise in the heat in which subjects (n=7) completed 90 minutes uphill walking in a cool environment (~20°C), then transitioned to a hot environment (40°C) where they exercised for either 60 minutes or until exhaustion at ~65% VO$_2$ max. The investigators measured leg blood flow and obtained muscle biopsies to determine muscle glycogen utilization from the heat. The main finding of this study determined no difference in leg blood flow despite subjects reaching near HR max, and ample glycogen availability in the muscles. Therefore, the researchers concluded there were no cardiovascular or substrate limitations to exercise in the heat, but rather a critical core temperature of ~40°C that reduces the central nervous system’s drive to maintain motor function or perceptual mechanisms that limit performance. This study proposed a “critical core temperature” hypothesis that limits performance in the heat.

It is also accepted that skin temperature influences fatigue in the heat rather than the “critical core temperature”. Sawka et al. (2010) (45) reviewed that as T$_{SK}$ increases, impairments to the cardiovascular system limit the diffusing capacity of heat in blood from the body to the
environment. They found aerobic performance was able to be performed when core temperature exceeds 40°C as long as skin was cool (<35°C) and when subjects were euhydrated. The review also looked at the effect of hypohydration on aerobic performance, and stated that reduced cardiac filling occurs in the presence of hypohydration (decreasing stroke volume), attenuating blood flow requirements associated with exercise in the heat. Therefore, they concluded increased $T_{SK}$ (>35°C) narrows the $T_c$-$T_{SK}$ gradient, and increases blood demand for the skin. High $T_c$-$T_{SK}$ gradients lower oxygen availability to the working muscles (28), and concurrent with hypohydration to exacerbate these effects, straining the cardiovascular system more to impair cardiac output.

Cuddy et al. (2014) (26) studied the effect of the $T_c$-$T_{SK}$ gradient on exercise performance capacity by determining time to fatigue in 60 subjects in 18°C, 26°C, 34°C, or 42°C environments by measuring the relationship between $T_c$, $T_{SK}$, and time to exhaustion. The 18°C and 26°C group were able to run 13% and 22% longer respectively compared to the other groups, despite no difference in core temperature. The critical $T_c$-$T_{sk}$ gradient was found to be 2.1°C, and when skin reached above 35°C, cutaneous blood flow increased in order to promote convective heat loss through the skin, causing a limitation in exercise blood flow to the muscle. Therefore, $T_c$-$T_{sk}$ gradient was more indicative of exercise performance to fatigue in hot environments rather than the “critical core temperature” of 40°C.

Hydration status affects the body’s ability to thermoregulate at a given relative intensity and is amplified based on the magnitude of dehydration compared to when individuals are adequately hydrated. Montain and Coyle (1992) (13) studied the effect of graded dehydration at 4%, 3.4%, 2.3%, and 1.1% dehydration in 8 subjects during 2h cycling at 65% $VO_2$ max. The researchers found $T_c$ and HR increased in proportion to percent dehydration compared to when euhydrated.
Forearm blood flow decreased with the magnitude of dehydration, demonstrating a decreased ability to dissipate heat due to a lower volume displaced to cutaneous circulation. Limiting fluid ingestion affects cardiovascular drift by increasing HR to offset the decline in stroke volume due to lower blood volumes in order to maintain cardiac output. Therefore, a loss of blood volume due to prolonged sweating and inability to rehydrate can be detrimental on the body’s ability to attenuate heat stress.

Although hydration status is critical for thermoregulation, maintaining hydration is not the sole factor to completely protect humans from HRI in hot environments. Cuddy and Ruby (8) published a case study on a WLFF collapsing from heat exhaustion after 7 hours of hard work. Despite aggressive drinking (840 ml*h⁻¹) when the mean ambient temperature was 44.6°C, it was not enough to prevent Tc from reaching 40.1°C. They suggested that incidents of HRI are still possible despite aggressive fluid intake when work output and ambient temperatures are high over extended periods. Therefore, the only way to prevent HRI is the decrease work rates by limiting metabolic heat production, or utilize other cooling mechanisms.

Human thermoregulatory capabilities are an advantage we have as a species by promoting evaporative heat loss to the environment. Montain et al. 1994 (14) studied the effect of exercise intensity, protective clothing, and climate during a heat stress test. Seven previously heat acclimated subjects walked on a treadmill for 180 minutes in a hot-dry (43°C, 20% RH) and tropical (35°C, 50%RH) in which the evaporative cooling alone does not adequately attenuate the rise in core temperature over time. The main finding were that PPE gear worn by soldiers and WLFF augments physiological strain associated with exercise in the heat compared to when partially clothed. This study also demonstrated the rate of rise in Tc can be used as a model to predict the incidence of exhaustion due to heat stress while wearing protective clothing. Therefore,
populations such as WLFF and soldiers put themselves at risk for HRI by sacrificing their ability to thermoregulate for the safety of protecting themselves from solar radiation and hazardous debris associated with tasks.

**Heat Acclimation**

Heat acclimation can be achieved through repeated exposures to heat in combination with exercise that requires $T_C$ and $T_{SK}$ temperature elevations to promote sweating (18, 17, 46). Acclimation and acclimatization are different in that heat acclimation is defined as “producing adaptations to the heat in an artificial setting, while acclimatization is “natural” and achieved in the field. They both produce adaptations that attenuate cardiovascular and thermal strains associated with exercise in the heat (15).

A monumental study to determine physiological responses to chronic heat exposures was published by Senay et al. 1976 (17). Four men completed 10 consecutive days of exercise at ~45% $VO_2$ max in a 45°C environment. The focus of this study was to determine body fluid adjustments over time, in which investigators observed increases in total circulating protein (TCP) that cause expansion of plasma volume (PV), an advantageous adaptation involved with heat tolerance. In this study, PV increased 23% when exercising after the 10 day period, but PV expansion began quickly in that they saw a 9% increase after two sessions. The investigators contributed PV expansion to the amount of TCP within the blood to increase the oncotic pressure within the vascular space to allow greater fluid retention. PV expansion was noted as the key contributor to increasing stroke volume and optimizing cardiac output while performing in the heat. Mitchell et al. (1976) (60) published data from the same study to conclude body temperature change is inversely proportional to sweat rate. By the 4th hour of exercise at the final day of acclimation, $T_C$ decreased 1°C and HR decreased 30 beats•min$^{-1}$. They contributed the reduction in cardiovascular
and thermal strain due to a 10% increase in total sweat evaporation. Increasing evaporative sweat loss allowed skin temperature to remain low by promoting heat dissipation to mitigate thermal strain and discomfort. Our body’s ability to adapt to increase sweat rate is an advantage of the human ability to increase the capacity to thermoregulate.

Nielsen et al. (1993) (13) studied the effect of exercise in the heat at 60% VO$_2$ max for 9-12 consecutive days until exhaustion in a hot, dry environment (40°C, 10% RH, n=8) vs. control (20°C, n=5). Endurance capacity was increased from beginning to end of the acclimation in that average time to exhaustion (TTE) improved from 48 minutes to 80 minutes respectively. Lower HR, T$_C$, increased sweat rate, and 15% increase in SBF occurred from beginning to end of acclimation. Body fluid measurements included PV expansion of 13.1% in the heat acclimated group vs. 2.7% in control. PV expansion was attributed to increased TCP and aldosterone, a fluid regulation hormone responsible for sodium and water retention. Due to PV expansion, the subjects gained a greater capacity to increase stroke volume, and further sustain cardiac output at exhaustion. Cardiac output at exhaustion increased from the first to final exposure, increasing from 19.6 L•min$^{-1}$ to 21.4 L•min$^{-1}$. Finally, the authors concluded that all subjects exhausted when T$_C$ reached 39.7 °C. This result concluded that a critical T$_C$, not circulatory failure is the deciding factor in exhaustion at heat stress. The circulatory contributions to heat acclimation lowered T$_C$ at a given intensity and allowed them to exercise longer before reaching the “critical temperature” which was proposed as the limitation to exercise at the time.

Lorenzo et al. (2010) (21) were the first investigators to conclude heat acclimation increases aerobic performance in both hot and cool environments. This study was a group design in which subjects were placed in a heat acclimation group (n=4) or a control group (n=8). The protocol consisted of the heat acclimation group cycling for 90 minutes at 50% VO$_2$ max for ten days in a
hot environment (40°C, 30%RH), and the control in a cool (13°C, 30%RH) environment performing the same protocol. PRE and POST acclimation measures were a VO$_2$ max test, 60-minute time trial, and a lactate threshold test. The novel findings of this study showed the heat acclimated group had a higher work output at lactate threshold, increased VO$_2$ max, and increased average power output during the time trials in both hot (38°C, 30%RH) and cool conditions (13°C, 30%RH) compared to controls. This study demonstrated the ergogenic effects of heat acclimation improves performance in multiple environments (not just the heat). The thermal effects of heat acclimation during steady state exercise were compared between days 1 and 10 of the acclimation period. After ten days acclimation, the heat acclimation group attenuated HR (165 to 150 b•min$^{-1}$), final T$_C$ decreased following the final 90-minute exercise (39.3 to 38.8°C), and increased total plasma volume increased (3.0 to 3.2 L) between days 1 and 10 of acclimation. The authors compared the benefits of heat acclimation to Levine and Stray-Gendersen’s “live high train low” model to promote adaptations associated with hypoxia. Stating that heat acclimation may be just as effective for improving performance as high altitudes, but in this case would be considered a train high live low scenario. The physiological adaptations attributed to performance benefits from heat acclimation include increased plasma volume (6.5%) that can be attributed to increased maximal cardiac output of 26.9 L•min$^{-1}$ to account for 5% increase in VO$_2$ max. Increased SBF and sweat rates attributed to peripheral adaptations to attenuate T$_{SK}$ and allow for improved heat dissipation. The investigators also performed a novel aspect by infusing the peripheral skin with endothelium-dependent vasodilator acetylcholine (ACH) via the microdialysis method, and found that heat acclimation improves the sensitivity of the endothelium to vasodilate (16) in response to ACH, promoting greater heat dissipation. Therefore, suggesting that increased vascular functions
due to increased vasodilation in the cutaneous system promotes a wider $T_C - T_{sk}$ gradient and allows a greater magnitude of heat dissipation.

**Short-Term Heat Acclimation**

The previous studies mentioned contribute to our understanding towards heat acclimation, but were all long-term heat acclimation (LTHA) of ≥9 days. Short-term heat acclimation (STHA) has gained attention in research due the applicability and cost-effectiveness of less time spent in a laboratory setting. Periard et al. (2015) (15) reviewed the induction and decay of heat acclimation and determined that 75-80% of the total heat acclimation occurs within 4 days (rapid acclimation), and the rest is a slower adaptation that is complete within ~ 9-10 days.

Gibson et al. (2015) (37) compared the effects of varying days of heat exposures by comparing cardiovascular and thermoregulatory measurements from a heat stress test (HST) after 5 days acclimation (STHA) and 10 days (LTHA) during a 90-minute cycling protocol at 50% VO$_2$ max intensity in a hot environment (40°C, 30% RH). Subjects completed an initial HST, followed by five days cycling in the heat, then a HST after the fifth day (determine STHA), followed by five more days of heat acclimation cycling (determine LTHA). By comparing STHA vs. LTHA, investigators concluded that STHA was just as effective as LTHA. Subjects significantly decreased peak $T_{sk}$, $T_C$, HR, and increased sweat rate after 5 and 10 days, but the HST from STHA and LTHA were not statistically different from each other, suggesting the efficacy of STHA protocol for these subjects. Therefore, the first five heat exposures when cycling in the heat for 90-minutes per day will be more important in attaining adaptations compared to the following five days, demonstrating the effectiveness of STHA in preparation for hot environments.
Garrett et al. (2009 (30), 2012 (24), and 2014 (27)) has contributed several studies suggesting that five days heat acclimation in hot and humid conditions (40°C, 60%RH) for 90-minutes per day can significantly improve factors of heat stress and improve exercise performance in the heat.

Garrett et al. (2014) (27) studied the effect of a STHA protocol on nine subjects for five-days heat acclimation (40°C, 60% RH) by having subjects cycle for 90-minutes at fixed thermal (isothermic) temperature (38.5°C) during the acclimation period. Therefore, the intensity was adjusted Accordingly to maintain Tc once 38.5°C was achieved. Prior to the acclimation period, a HST was conducted by having subjects cycle for 60 minutes in a hot environment (35°C, 60% RH) at 50% VO₂ max. Fluid given was 150 ml of a 4% CHO electrolyte beverage every 15 minutes. Following the HST, a graded aerobic test was performed by increasing power output on a cycle ergometer by 2% every 30 seconds. The same HST protocol was repeated after the five consecutive day STHA protocol. The design of the study was a randomized-crossover design, in which a five-week washout period separated whether they acclimated euhydrated or dehydrated. During the acclimation period subjects either drank 250ml of a 4% CHO beverage every fifteen minutes fluid (euhydration), or were supplemented with 100ml of fluid before each acclimation bout (dehydration). Following acclimation, the dehydrated group demonstrated a lower HR: -19 b•min⁻¹ vs. acclimating euhydrated: -10 b•min⁻¹, Tc was attenuated by 0.4 and 0.2 °C in dehydration and euhydration acclimation groups respectively. Resting forearm blood flow increased in both euhydration and dehydration groups. The dehydration group increased their work output at exhaustion after the acclimation period by 19% compared to 14% in the euhydrated group (p=0.06). However, the authors can only suggest that training dehydrated can increase performance, because although work capacity increased in the dehydrated group to a greater magnitude than the euhydrated group, it was not statistically greater than when acclimating
euhydration. The authors proposed PV expansion of 8% in the dehydrated group compared to 4% PV euhydration group allowed subjects to perform at a higher work outputs, sustain higher sweat rates, and maintain cardiac output over extended periods. The mechanisms behind PV expansion were increased aldosterone secretion and total circulating proteins that were negative feedback secretions due to hypovolemia.

Neal et al. (2015) (25) studied the effects of STHA while dehydrated in ten trained cyclists. Each subject completed five days acclimation in hot conditions (40°C, 50% RH), and Tc was fixed at 38.5°C as described above. Therefore, the intensity was manipulate during each acclimation session to maintain constant Tc. Dehydration was implemented by eliminating any fluid ingestion during acclimation, and 30 minutes afterwards. Following each acclimation session and after the subjects were dehydrated, they were then given 1.75L of 3.6% CHO electrolyte beverage. The level of dehydration was about 3.1% body weight loss for every day of acclimation. A 60 minutes HST consisted of cycling at 35% aerobic peak power output in a 40°C, 30% RH environment. Fluid supplementation during the HST was 312ml every 15 minutes of a 3.6 CHO-electrolyte beverage (1.25 L total). However, a control group was not implemented in this study to compare the effect of dehydration. During the HST, subjects decreased Tc (37.95 to 37.77 ºC), HR (141 to 134 b•min⁻¹), and increased sweat rate (1.84 to 1.97 L•hour⁻¹). End HST SBF was increased from 306 AU to 316 AU (arbitrary units, an accepted value to measure red blood cell flux). Plasma volume slightly increased 1.18% from PRE to POST acclimation. The researchers suggested that a lack of PV expansion may be due to the subject population being elite athletes. Highly fit athletes have been shown to have greater PV expansion in the absence of any heat acclimation (12). Aerobic performance measurements included increased work rate at lactate threshold and increased mean power output at the end of a twenty-minute time trial, supporting the use of
dehydration as an ergogenic aid for heat acclimation even in elite athletes. However, a lack of control group cannot suggest dehydration as an ergogenic aid.

**Markers of Acclimatization**

The literature summarized above demonstrates the robust capacities of humans to adapt to the heat. HR, T<sub>sk</sub>, T<sub>C</sub>, sweat rate, and SBF flow are all important factors that produce dynamic changes and have been reviewed extensively (15). These markers are important measurements for tracking adaptations to heat acclimation and determining the impact of hydration status during acclimation on these physiological markers and performance.

**Practical Applications**

Heat acclimation provides physiological adaptations that can attenuate thermal and cardiovascular strain. By optimizing the efficacy of a STHA trial, and by combining the effects of dehydration, the optimal protocol for heat acclimation can provide a cost effective method for WLFF and soldiers in need of rapid departure to a hot climate without the proper acclimation. This research is in effort to prevent heat related illness and sustain performance in these individuals.
Chapter Three: Methodology

Participants

Thirteen recreationally active males were recruited from the University of Montana and surrounding community. Inclusion criteria included an age range between 18 and 40 years old, and VO_2 max > 40 ml•kg^{-1}•min^{-1} and < 65ml•kg^{-1}•min^{-1}. Subjects filled out a Physical Activity Readiness Questionnaire (PAR-Q) to assess cardiovascular disease factors and signed an informed consent approved by the University of Montana Institutional Review Board. Upon completion, they were provided with detailed descriptions of the experimental procedures as well as expectations for participating in this study. They were also informed with discomforts associated with participating in this study from exercising in the heat (40°C, 30%RH). Preliminary testing and acclimation took place in the Human Performance Laboratory at the University of Montana. PRE and POST acclimation HST, and aerobic performance tests (following HST) took place in an environmental chamber (Tescor, Warminster, PA) in the Montana Center for Work Physiology and Exercise Metabolism (WPEM). Participants were informed the study was a random crossover design, and underwent both acclimation sessions under DEH and EUH interventions. Each acclimation session was separated by a five-week washout period to prevent crossover of adaptations from the previous acclimation session.

Preliminary Testing

Physical Activity Readiness Questionnaire (PAR-Q)

All participants were pre-screened for known coronary artery disease risk factors. This prevented potential complications from symptoms the participant might not be aware. Subjects who answered, “yes” to any questions were dropped from the study.
Maximal Aerobic Capacity (VO\textsubscript{2} peak)

VO\textsubscript{2} peak was determined for each subject by an incremental test to exhaustion using the Bruce Protocol (47) on a motorized treadmill (Fullvision, Inc., Newton KS). Subjects arrived a minimum 3 hours fasted prior to the exercise test.

To measure VO\textsubscript{2} peak, expired gases were collected and averaged every 15 seconds via a metabolic cart (Parvomedics, Inc., Sandy, UT). In order to reach VO\textsubscript{2} peak, one of the following criteria was met: 1) plateau in VO\textsubscript{2} despite an increased workload; 2) Respiratory exchange ratio (RER) >1.10, 3) Heart rate within 10 beats per minute of subjects’ predicted HR max (206bpm – AGE • 0.6667); and 4) rate of perceived exertion (RPE) > 17 before exhaustion determined via the 6-20 Borg scale (48).

Hydrodensitometry

Body composition was measured by hydrodensitometry following a three hour fast. Residual lung volume was estimated based on height and weight of the subject (49). Dry weight was collected prior to entering the water tank (Befour Inc., Cedarburg, WI). The hydrostatic weighing tank contained three force transducers using data collection software (Exertech, Dresbach, MN) that measure underwater weight. Subjects submerged and expired as much air as possible. Multiple weights took place until three measures agreed within 100g of each other. Body fat percentage was determined using the Siri equation (50).

\[
\% \text{ Body Fat} = \left( \frac{495}{\text{dry weight}} \right) \left( \frac{(\text{dry weight} - \text{wet weight}) - \text{Residual Lung Volume} - 0.1 - 450}{\text{dry weight}} \right) \times 100
\]
Experimental Trials

<table>
<thead>
<tr>
<th>40°C, 30% RH</th>
<th>Acclimation (DEH or EUH)</th>
<th>HST-POST</th>
<th>Five-week Washout</th>
<th>40°C, 30% RH</th>
<th>Acclimation (DEH or EUH)</th>
<th>HST-POST</th>
</tr>
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<tr>
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<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>HST-PRE</td>
</tr>
</tbody>
</table>

**Table 1**: Schematic of study including EUH and DEH acclimation trials in a randomized, counterbalanced design separated by a five-week washout period.

**Heat Stress Test (HST)**

All HST trials began following a minimum 3-hour fast in the WPEM laboratory. Participants completed a 24-hour diet and activity log prior to the first HST, and were asked to replicate the diet and activity log for all proceeding HST trials. Pre-acclimation HSTs occurred two days prior the first acclimation period and POST acclimation HSTs occurred two days following the third acclimation trial. After voiding, a urine sample was collected followed by nude body weight and a blood draw. Subjects exercised by walking at 50% VO\(_2\) max on a motorized treadmill for 90 minutes in hot conditions (40°C, 30% RH) while wearing personal protective equipment (PPE) Nomex outerwear worn by Wildland Firefighters (WLFF) and a cotton undershirt. Treadmill speed was set at 5.63 km•h\(^{-1}\), and grade set accordingly to relative VO\(_2\) peak. All subjects consumed the same relative amount of fluid (2.0 ml•kg\(^{-1}\)•15 min\(^{-1}\)) during HSTs. HR and RPE were recorded upon fluid delivery every 15 minutes. Core temperature (T\(_{c}\)) and skin temperature (T\(_{sk}\)) were monitored continuously throughout the trial. Laser Doppler Flowmetry (LDF) measured skin blood flow (SBF) at minutes 30, 60, and 90 in a seated position. SBF measurements
were averaged over one minute periods at the 30 and 60-minute point, and a five minutes at the 90-minute point to monitor recovery.

**Aerobic Performance Test**

Following the five-minute break period concluding the HST, a graded aerobic test occurred until exhaustion. The first stage was set at a grade 1% higher than the 50% VO\textsubscript{2} max intensity, and increased grade 1% every minute until 15%. If the subject completed the test to a grade of 15%, then the speed increased by 1.61 km\textbullet{}h\textsuperscript{-1} every minute until exhaustion. Following the aerobic performance test, a blood sample was taken followed by nude body weight and a urine sample. Nomex gear was also weighed before and after every trial.

**Acclimation**

Heat acclimation trials consisted of 90 minutes walking in an environmental heat chamber (40°C, 30%RH) at 50% VO\textsubscript{2} max on a motorized treadmill (Soma Technology Inc., Bloomfield CT) in the Human Performance Laboratory at the University of Montana. Three acclimation sessions were completed within a one-week period, and one day separated each acclimation trial. Before commencement of each trial, participants provided a urine sample, followed by nude body weight. HR and RPE were collected every 15 minutes of the trial along with fluid administration. The order of fluid delivery was randomly chosen, but kept consistent for each acclimation period (three consecutive trials). Fluid consumption for the euhydrated trials (EUH) was 2.0 ml\textbullet{}kg\textsuperscript{-1}•15 min\textsuperscript{-1} and the dehydrated trials (DEH) was 0.5 ml\textbullet{}kg\textsuperscript{-1}•15 min\textsuperscript{-1}. Following each acclimation session, nude body weight was measured followed by a urine sample. Nomex pants, shirts, and cotton undershirts shirt were weighed before and after each acclimation session.
Clothing

Subjects were provided with PPE gear as worn by WLFFs, and a cotton t-shirt was used as a base layer. Subjects were required to supply their own running shoes and socks for all trials.

Measurements

Core Temperature and Skin Temperature

T_C and T_SK was monitored continuously and recorded at 1 Hz on a digital data logger (Physitemp Instruments Inc., Clifton, NJ) during the HST. T_C was measured by a rectal thermistor inserted 12 cm past the anal sphincter. T_SK was monitored by placing a skin temperature sensor on the left pectoral region.

Sweat Rate

Whole body sweat rate was determined by calculating the change in body weight, fluid intake, urine output, and respiratory water loss over time. Body weight measurements occurred following the initial urine sample, and immediately post-exercise. Sweat Rate was calculated as followed:

\[
\text{Sweat Rate (L} \cdot \text{h}^{-1}) = [\text{BW}_{\text{pre}}(\text{kg}) + \text{ingested liquid (kg)})] - [\text{BW}_{\text{post}}(\text{kg}) + \text{urine loss (kg)} + \text{respiratory water loss (kg)}]
\]

• Respiratory water loss was calculated from the equation derived from Mitchell et al. (51).

\[
M_e = 0.019 \cdot \text{VO}_2 \cdot (44 - P_a)
\]

• Where \(M_e\) = rate of evaporative loss (g•min\(^{-1}\)) and \(P_a\) = water vapor pressure (mmHg)

• Water vapor pressure was calculated using the following equation:

\[
P_a = 13.955 - 0.6584 \cdot (T) + 0.0419 \cdot (T^2)
\]
• T = Temperature (40°C)
• VO₂ used was 50% of each subject’s VO₂ max, used as the intensity for HSTs and acclimations, and determined from preliminary testing.

Heart Rate

HR (beats•min⁻¹) was collected using a Polar heart rate monitor (Polar Electro Inc., Lake Success, NY). Each HR measurement occurred at the beginning of each trial, and every 15 minutes until the end of trial.

Physiological Strain Index (PSI)

PSI was calculated from the equation developed by Moran et al. (38) based upon strain in Tₐ and HR over time. Resting values of Tₐ₀ was the Tₐ recorded at initiation of the HST, and HR₀ was standardized at 70 (beats•min⁻¹).

\[
PSI = 5 \times \left( \frac{Tₐ - Tₐ₀}{39.5 - Tₐ₀} \right) + 5 \times \left( \frac{HR - HR₀}{180 - HR₀} \right)
\]

Skin Blood Flow

SBF was measured via LDF (Moor Instruments, Wilmington, DE) during the HST for one minute at 30 and 60 minutes during HST trials, and five minutes following the 90-minute trial. Data from the 90-minute time-point was averaged for the first and fifth minute following the 90 min trial at rest. Measurements were taken by placing a probe over the chest and under the shirt in the pectoral region. Subjects were asked to maintain as still as possible during collection. SBF data was measured based on red blood cell flux and outputs were given as arbitrary units.

Blood Samples

A total of eight blood samples (2 per trial x 4 trials) were collected using a venipuncture technique from the antecubital vein. The site was cleaned prior to the blood draw using an alcohol
pad, and wiped clean of excess blood afterwards. Body position was standardized from the supine position. Samples were collected to measured changes in hemoglobin, hematocrit, and plasma volume (PV) was calculated by the Dill and Costill method (52). Acute changes in PV were compared before and after HST sessions, as well as chronic changes compared resting PV before and after acclimation sessions.

_Urine Specific Gravity_  
Urine was collected before and after both HST and acclimation trials. Urine specific gravity (USG) measured the concentration of solutes in the urine and ranges between 1.000 and 1.030 and is used to determine hydration status. Urine was pipetted onto a refractometer (ATAGO U.S.A., Inc. Bellevue, WA) for analysis and discarded.

**Statistical Analysis**  
Physiological and perceptual markers Tc, Tsk, HR, PSI, and RPE during the HST were monitored, and peak measurements at 90 minutes were analyzed by a 2x2 analysis of variance (ANOVA) with repeated measures (Treatment X Time). Sweat Rate, aerobic performance, PV, Hb, and Hct were measured using a 2x2 ANOVA with repeated measures. Pre-exercise body weights over the course of five acclimation sessions in both DEH and EUH acclimation sessions were analyzed by a 2X5 ANOVA with repeated measures with treatment as the between group (2) and trial number as the within groups measure (5).

Data collected during the acclimation trials included sweat rate, peak HR, peak RPE, and pre-exercise USG. Within-subjects factors included treatment (EUH or DEH) and acclimation trials were analyzed by a 2x3 ANOVA with repeated measures. HR and RPE data was analyzed using area under curve analysis to take into effect the entire trial by a 2x3 ANOVA. All values are presented as mean ± SEM and significance set at p<0.05.
Chapter 4: Results

Subject Descriptive Data

Thirteen recreationally active males completed both DEH and EUH acclimation trials. Age: 23.0 ± 0.9 yrs., weight: 82.1 ± 2.0 kg, height: 184.0 ± 2.5 cm, body fat: 13.8 ± 1.4 %, VO₂ max: 53.2 ± 1.5 ml·kg⁻¹·min⁻¹ (Table 2).

Body Weights

Pre-exercise body weight (Table 3) increased over time in both DEH and EUH groups (p=0.02) as a main effect for Time. Post-hoc analysis demonstrated body weight was significantly greater at Trial 5 compared to 1 (p=0.043) in the DEH group, and significantly greater at Trial 2 compared to Trial 1 (p=0.047) in the EUH group.

Acclimation Data

Percent Dehydration

Percent dehydration (Figure 1, Table 4) was greater in the DEH group for all three acclimation Days compared to EUH as a main effect for Treatment (p<0.001). There was a main effect for Day (p=0.033) at Days 2 and 3 (p=0.007, 0.016) compared to Day 1 in DEH group. The EUH group showed a main effect for Day at Day 3 (p=0.04) compared to Day 1. There was no TrtXDay interaction (p=0.30).

Urine Specific Gravity

Pre-exercise USG (Figure 2, Table 4) showed a main effect for Treatment (p=0.01), in which EUH group was higher than DEH at Day 2 (p=0.007), but not Days 1 (p=0.40) or 3 (p=0.62). There was no TrtXDay interaction (p=0.17).
**Heart Rate**

Peak HR (Table 4) showed no main effect for Day (p=0.53) or TrtXDay interaction (p=0.99).

**RPE**

Peak RPE (Figure 3, Table 4) showed a main effect for Day (p=0.05) in DEH and EUH groups, but no TrtXDay interaction (p=0.57). Post hoc analysis showed RPE significantly decreased in DEH group at Day 2 (p=0.03) and Day 3 (p=0.05) compared to Day 1, and no main effects for Day were found in EUH group.

**Sweat Rate**

Sweat Rate (Figure 4, Table 4) increased as a main effect for Day (p=0.04). Sweat Rate at Days 2 and 3 was significantly greater than Day 1 in the DEH group (p=0.01, 0.008 respectively), and EUH was significantly greater at Day 3 compared to Day 1 (p=0.05). There was a TrialXDay interaction for Nomex weights (p=0.02)

**Nomex and Cotton Undershirt Weight**

Nomex weight (Table 5) significantly increased as a main effect for Trial (p<0.001) following each acclimation trial for DEH and EUH groups (pre vs. post exercise). There was a main effect for Day in the DEH group at Day 2 and 3 (p=0.019, 0.01 respectively) compared to Day 1

Cotton undershirts significantly increased as a main effect for Trial (p<0.001) following each acclimation session (pre vs. post exercise). There was a TrialXDay interaction over acclimation Days in the DEH (p=0.018) and EUH (p=0.013) groups.
Heat Stress Test Data

Urine Specific Gravity (USG)

Pre-exercise USG decreased as a main effect for Time (p=0.03), and a trend for Treatment by Time (TrtXTime) interaction (p=0.08). Post hoc analysis showed a main effect for Time in the DEH group (p=0.02), decreasing prior to the POST-acclimation HST, but not in the EUH group (p=0.55) (Table 10). Post-exercise USG showed a TrtXTime interaction (p=0.004) in which the DEH group was lower compared to EUH post-acclimation.

Core Temperature (Tc)

Peak Tc decreased as a main effect for Time (p<0.001) following acclimation in both DEH and EUH groups (Figure 5, Table 6). There was no TrtXTime interaction (p=0.71).

Skin Temperature (Tsk)

Peak Tsk (Figure 6, Table 6) decreased as a main effect for Time (p=0.006) following acclimation in DEH and EUH groups. There was no TrtXTime interaction (p=0.48).

Heart Rate (HR)

Peak HR decreased following acclimation as a main effect for Time (p<0.001) in both DEH and EUH groups (Figure 7, Table 6). There was no TrtXTime interaction (p=0.62).

Rating of Perceived Exertion (RPE)

Peak RPE decreased following acclimation as a main effect for Time (p<0.001) in both DEH and EUH groups (Figure 8, Table 6). There was no TrtXTime interaction (p=0.55).

Physiological Strain Index (PSI)

Peak PSI decreased following acclimation as a main effect for Time (p<0.001) in both DEH and EUH groups (Figure 9, Table 6). There was no TrtXTime interaction (p=0.99).
Time exercising PSI>7.5 decreased following acclimation as a main effect for Time (p<0.001) in both DEH and EUH groups. There was no TrtXTime interaction (p=0.875).

**Sweat Rate**

There was a significant TrtXTime interaction (p=0.015) for sweat rate (Figure 10, Table 6), increasing at a greater rate for the DEH group compared to EUH.

**Hematocrit (HCT)**

HCT significantly decreased as a main effect for Time (p=0.007) in both DEH and EUH groups (Table 7). There was no TrtXTime interaction (p=0.63).

**Hemoglobin (Hb)**

There was no main effect for Time (p=0.33) or a TrtXTime interaction (p=0.59) (Table 7).

**Plasma Volume (PV)**

PV increased as a main effect for Time (p=0.02) in DEH and EUH groups. Post hoc analysis showed a main effect for Time in the DEH group (p=0.009) but not in the EUH group (p=0.30) (Figure 11, Table 7). There was no significant TrtXTime interaction (p=0.39).

**Skin Blood Flow (SBF)**

SBF measured at minutes 1 and 5 minutes post HST (Figure 13b, Table 9) showed neither a main effect for Time (p=0.36) or TrtXTime interaction (p=0.74).

**Aerobic Performance**

Aerobic performance measured in time to exhaustion (TTE) increased as a main effect for Time (p<0.001) in both DEH and EUH groups following acclimation (Figure 10, Table 6). There was no significant TrtXTime interaction (p=0.73). Total work output (KJ) during the graded aerobic test increased as a main effect for Time (p<0.001), but no TrtXTime (p=0.33) (Table 6).
Physiological Markers at Exhaustion.

Physiological markers measured following the aerobic performance test include $T_C$, HR, and $T_{sk}$ (Table 12). $T_C$ was significantly lower at exhaustion as a main effect for Time ($p=0.002$) in DEH and EUH groups, and there was no TrtXTime interaction ($p=0.63$). HR was not different at exhaustion as a main effect for Time ($p=0.13$) or a TrtXTime interaction ($p=0.742$). There was no difference for $T_{sk}$ as a main effect for Time ($p=0.93$) or a TrtXTime interaction ($p=0.33$).

Nomex and Cotton Undershirt Weight

Nomex (Table 13) significantly increased as a main effect for Treatment ($p<0.001$) following HST trials in DEH and EUH groups (pre vs post exercise). There was a TrtXTrial interaction in DEH ($p=0.02$) and EUH ($p=0.01$) groups POST acclimation.

Cotton Undershirts significantly increased as a main effect for Treatment ($p<0.001$) in DEH and EUH groups following HST trials. There was no TrtXTrial interaction following acclimation in DEH ($p=0.42$), but a trend for a TrtXTrial interaction following acclimation in the EUH group ($p=0.06$).
## Tables and Figures

### Characteristics

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td><em>Age (yrs.)</em></td>
<td>23.0 ± 0.9</td>
</tr>
<tr>
<td><em>Height (cm)</em></td>
<td>184.0 ± 2.5</td>
</tr>
<tr>
<td><em>Weight (kg)</em></td>
<td>82.1 ± 2.1</td>
</tr>
<tr>
<td><em>Body Fat (%)</em></td>
<td>13.8 ± 2.4</td>
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<tr>
<td><em>VO₂ max (ml·kg⁻¹·min⁻¹)</em></td>
<td>53.2 ± 1.5</td>
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**Table 2:** Physical characteristics for 13 male subjects. Data represented as mean ± SEM

### Body Weights

<table>
<thead>
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<th></th>
<th>Body Weight (kg)</th>
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<tbody>
<tr>
<td><strong>TRIAL</strong></td>
<td><strong>1</strong></td>
</tr>
<tr>
<td><em>DEH</em></td>
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<tr>
<td><em>EUH</em></td>
<td>81.6 ± 2.3</td>
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**Table 3:** Mean Body weight (kg) over all trials during each acclimation period. *Main effect for Time (p<0.05, vs. Trial 1)
## Acclimation Data

<table>
<thead>
<tr>
<th></th>
<th>DEH</th>
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<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 2</td>
</tr>
<tr>
<td><em>Peak HR</em> (<em>b</em>min⁻¹)</td>
<td>165 ± 3.6</td>
<td>162 ± 3.6</td>
</tr>
<tr>
<td><em>Peak RPE</em></td>
<td>14.6 ± 0.3</td>
<td>14.0 ± 0.3*</td>
</tr>
<tr>
<td><em>Sweat Rate</em> (<em>L</em>hour⁻¹)</td>
<td>1.3 ± 0.1</td>
<td>1.4 ± 0.07*</td>
</tr>
<tr>
<td><em>Percent Dehydration</em> (%)</td>
<td>2.1 ± 0.1</td>
<td>2.4 ± 0.1*</td>
</tr>
<tr>
<td><em>USG</em> (Pre-exercise)</td>
<td>1.015 ± 0.003</td>
<td>1.013 ± 0.002</td>
</tr>
<tr>
<td></td>
<td>0.003</td>
<td>0.002</td>
</tr>
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</table>

**Table 4**: Data during three acclimation Days presented as mean ± SEM.

# Main effect for Treatment (p<0.05). * p<0.05. Main effect for Day (vs. Day 1).

<table>
<thead>
<tr>
<th></th>
<th>DEH</th>
<th>EUH</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 2</td>
</tr>
<tr>
<td><em>NOMEX Pre-Exercise</em> (kg)</td>
<td>1.09 ± 0.02</td>
<td>1.09 ± 0.01</td>
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<tr>
<td><em>NOMEX Post-Exercise</em> (kg)</td>
<td>1.21</td>
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<td></td>
<td>0.02#</td>
<td>0.10#*</td>
</tr>
<tr>
<td><em>T-Shirt Pre-Exercise</em> (kg)</td>
<td>0.14 ± 0.0</td>
<td>0.14 ± 0.0</td>
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<tr>
<td><em>T-shirt Post-Exercise</em> (kg)</td>
<td>0.28 ± 0.01#</td>
<td>0.31 ± 0.01#*</td>
</tr>
</tbody>
</table>

**Table 5**: Nomex and Cotton Undershirt weights. * p<0.05. Main effect for Day (vs. Day 1).

# p<0.05 Main effect for Trial (Pre-exercise vs. Post-exercise).
Figure 1: Percent Dehydration for three acclimation Days. # p<0.001 main effect for Treatment (DEH vs. EUH). ‡ Main effect for Time at Day 2 (p=0.016) and Day 3 (p=0.007) vs. Day 1 in DEH group. ^ Main effect for Time at Day 3 (p=0.05) vs. Day 1 in EUH group.
Figure 2: Pre-exercise urine specific gravity over three acclimation Days. # p=0.007. Main effect for Treatment.
Figure 3: Peak RPE for three acclimation Days.
‡ Main effect for Time in Day 2 (p=0.03) and Day 3 (p=0.05) vs. Day 1 in DEH group.
Figure 4: Sweat rate response for three acclimation Days.
‡ Main effect for Time compared to Day 1 for DEH group (Day 2: p=0.011, Day 3: p=0.008).
## Heat Stress Test Data

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>DEH</th>
<th>EUH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PRE-Acclimation</td>
<td>POST-Acclimation</td>
</tr>
<tr>
<td>Peak $T_C$ ($^\circ$C)</td>
<td>39.5 ± 0.10</td>
<td>39.0 ± 0.12*</td>
</tr>
<tr>
<td>Peak $T_{sk}$ ($^\circ$C)</td>
<td>37.8 ± 0.19</td>
<td>37.6 ± 0.12</td>
</tr>
<tr>
<td>Peak $T_C$-$T_{sk}$ gradient ($^\circ$C)</td>
<td>1.7 ± 0.18</td>
<td>1.4 ± 0.12</td>
</tr>
<tr>
<td>Peak HR ($b\times min^{-1}$)</td>
<td>178.1 ± 3.33</td>
<td>164.1 ± 4.43*</td>
</tr>
<tr>
<td>Peak RPE</td>
<td>17.0 ± 0.61</td>
<td>14.4 ± 0.54*</td>
</tr>
<tr>
<td>Peak PSI</td>
<td>9.9 ± 0.34</td>
<td>8.1 ± 0.44*</td>
</tr>
<tr>
<td>PSI: Time above 7.5</td>
<td>35.15 ± 3.79</td>
<td>18.27 ± 4.3*</td>
</tr>
<tr>
<td>Sweat Rate ($L\times hour^{-1}$)</td>
<td>1.5 ± 0.06</td>
<td>1.9 ± 0.09*</td>
</tr>
<tr>
<td>Aerobic Performance $TTE$ (sec)</td>
<td>290.3 ± 48.22</td>
<td>464.9 ± 32.67*</td>
</tr>
<tr>
<td>Total Work Output ($KJ \times 10^3$)</td>
<td>27.7 ± 7.21</td>
<td>75.0 ± 6.18*</td>
</tr>
</tbody>
</table>

Table 6: PRE and POST acclimation data for DEH and EUH groups.  
* p<0.05: main effect for Time. † p<0.05: Significantly different from EUH group.
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>DEH</th>
<th>EUH</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PRE-acclimation</td>
<td>POST-acclimation</td>
<td>PRE-acclimation</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>47.1 ± 0.89</td>
<td>45.9 ± 0.68 *</td>
<td>47.5 ± 0.93</td>
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<tr>
<td>Hemoglobin (g*dl⁻¹)</td>
<td>14.6 ± 0.32</td>
<td>14.0 ± 0.43</td>
<td>14.1 ± 0.19</td>
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<tr>
<td>Plasma Volume (%)</td>
<td>52.9 ± 0.95</td>
<td>56.6 ± 1.22 *</td>
<td>52.4 ± 0.92</td>
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<tr>
<td>Plasma Volume ( % change)</td>
<td>7.1 ± 1.84</td>
<td></td>
<td>4.1 ± 2.46</td>
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</table>

**Table 7**: Pre-exercise blood values PRE and POST acclimation. * p<0.05. Main effect for Time.

<table>
<thead>
<tr>
<th>Time</th>
<th>DEH</th>
<th>EUH</th>
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</thead>
<tbody>
<tr>
<td>30 min</td>
<td>187.6 ± 18.2</td>
<td>170.8 ± 14.1</td>
</tr>
<tr>
<td></td>
<td>198.1 ± 16.9</td>
<td>171.8 ± 14.4</td>
</tr>
<tr>
<td>90 min</td>
<td>200.4 ± 18.5</td>
<td>189.2 ± 22.1</td>
</tr>
</tbody>
</table>

**Table 8**: Skin Blood flow at 30, 60, and 90 minutes during HST.

<table>
<thead>
<tr>
<th>Time</th>
<th>DEH</th>
<th>EUH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 min</td>
<td>200.5 ± 18.5</td>
<td>189.3 ± 22.1</td>
</tr>
<tr>
<td>5 min</td>
<td>201.3 ± 23.1</td>
<td>202.1 ± 17.3</td>
</tr>
</tbody>
</table>

**Table 9**: Skin blood flow 1 minute and 5 minutes post HST.
Table 10: Urine Specific Gravity. * p=0.022. Main effect for Time (Pre-exercise).
† p=0.004. TrtXTime interaction (Post-exercise)

<table>
<thead>
<tr>
<th></th>
<th>DEH</th>
<th>EUH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>PRE-acclimation</strong></td>
<td><strong>POST-acclimation</strong></td>
</tr>
<tr>
<td><strong>DEH</strong></td>
<td>1.018 ± 0.003</td>
<td>1.021 ± 0.002</td>
</tr>
<tr>
<td><strong>EUH</strong></td>
<td>1.016 ± 0.003</td>
<td>1.0195 ± 0.002</td>
</tr>
</tbody>
</table>

Table 11: Markers of dehydration following each HST. * p<0.05: Main effect for Time
† p<0.05. TrtXTime interaction. # p<0.05. Main effect for Treatment.

<table>
<thead>
<tr>
<th></th>
<th>DEH</th>
<th>EUH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>PRE-acclimation</strong></td>
<td><strong>POST-acclimation</strong></td>
</tr>
<tr>
<td><strong>PV SHIFT (%)</strong></td>
<td>-12.64 ± 2.73</td>
<td>-11.54 ± 1.74</td>
</tr>
<tr>
<td><strong>Percent Dehydration (%)</strong></td>
<td>1.58 ± .091</td>
<td>2.27 ± 0.11 †*</td>
</tr>
<tr>
<td><strong>POST-exercise USG</strong></td>
<td>1.021 ± 0.002</td>
<td>1.012 ± 0.003 *</td>
</tr>
</tbody>
</table>

Table 12: Physiological markers measured at exhaustion during the aerobic performance test
* p<0.05: main effect for Time.
<table>
<thead>
<tr>
<th></th>
<th>DEH</th>
<th>EUH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-Acclimation</td>
<td>Post-Acclimation</td>
</tr>
<tr>
<td>NOMEX</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-exercise</td>
<td>1.08 ± 0.02</td>
<td>1.05 ± 0.01</td>
</tr>
<tr>
<td>Post-exercise</td>
<td>1.21 ± 0.02 #</td>
<td>1.37 ± 0.08 *#</td>
</tr>
<tr>
<td>COTTON UNDERSHIRT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-exercise</td>
<td>0.14 ± 0</td>
<td>0.14 ± 0</td>
</tr>
<tr>
<td>Post-exercise</td>
<td>0.31 ± 0.03 #</td>
<td>0.33 ± 0.01 #</td>
</tr>
</tbody>
</table>

Table 13: Nomex and Cotton Undershirt weight * p<0.05. Main effect for Time (Pre vs. Post acclimation). # p<0.05 Main effect for Treatment (Pre vs. Post-exercise).
Figure 5: Peak $T_c$ PRE and POST acclimation for EUH and DEH groups. * $p<0.001$ Main effect for Time for peak $T_c$. 
Figure 6: Skin temperature ($T_{sk}$) measured PRE and POST Acclimation in EUH and DEH groups. * $p=0.005$. Main effect for Time for peak $T_{sk}$. 
Figure 7: Heart rate (HR) response measured PRE and POST acclimation in EUH and DEH groups. *p<0.001 Main effect for Time for peak HR.
Figure 8: Rating of Perceived Exertion (RPE) measured PRE and POST acclimation in DEH and EUH groups. * p<0.001. Main effect for Time for peak RPE.
Figure 9: PSI measured PRE and POST acclimation in DEH and EUH groups. * p<0.001. Main effect for Time for peak PSI.
Figure 10: Sweat Rate following 90 minute HST. *p< 0.001. Main effect for Time. † p=0.015. TrtXTime interaction.
Figure 11: Pre-exercise Plasma Volume PRE and POST Acclimation.

* p=0.002. Main effect for Time.
Figure 12: Aerobic performance measured in time to exhaustion following 90 minute HST. * p<0.001 Main effect for Time.
Figure 13a: Skin blood flow at 30, 60, and 90 minutes during HST.

Figure 13b: Skin Blood Flow recorded 1 and 5 minutes post HST.
Chapter 5: Discussion

This study was designed to determine the effects of a protocol promoting dehydration during short-term heat acclimation (STHA) on physiological markers following heat acclimation. Also, to determine how the attenuation of these markers applies to performance in the heat following a 90-minute HST. It was found that a total of 4.5 hours of STHA over 7 days significantly attenuates thermal and cardiovascular strain associated with heat stress and improves thermal tolerance. Attenuation in these markers allowed individuals to increase exercise capacity following the HST regardless of fluid delivery strategy during acclimation. The second key finding was by attenuating fluid delivery to promote dehydration (DEH) during the three acclimation bouts resulted in greater sweat capacity (Figure 10), and resting plasma volume (PV) (Figure 11) compared to optimal fluid deliveries with four-fold higher fluid intake. Despite fluid restriction, adaptations to the heat occur similarly, if not greater, compared to more rigorous attempts to maintain hydration. STHA can be applied to populations such as Wildland firefighters (WLFF), military personnel, and athletes in preparation for departure to hot environments. Enhancing adaptations to the heat may not only attenuate susceptibility to heat related illness (HRI), but also improve work rates over long durations.

In this study, we have shown STHA in a hot environment (40°C, 30% RH) while exercising at 50% VO₂ max attenuates physiological markers associated with heat stress in a one-week period. The majority of research in this field has involved long-term heat acclimation (LTHA) (5, 7, 13-17) of approximately ten or more exposures, doubling heat exposure time. Although LTHA is equally effective for acclimation, STHA may be a more cost-effective and convenient approach for individuals in preparation for hot environments. STHA promotes a “rapid phase” of acclimation that occurs in approximately the first four exposures as described by Periard et al. (15).
Then following “rapid acclimation” in the laboratory, a “complete acclimatization” can occur in
the field within a short period. By promoting these adaptations using STHA, subjects were able to
attenuate total heat stress when wearing PPE gear that impedes their thermoregulatory capabilities.
To our knowledge, this may be the first study to promote adaptations acclimating in PPE gear, and
contradicts prior research by Cheung et al. (9) who found no difference in $T_C$ during a HST
following acclimation when wearing PPE gear. The design implemented by Cheung et al. involved
a 90-minute HST at 35°C, 50% RH. PPE gear worn was nuclear, biological, chemical (NBC) suits
rather than WLFF PPE.

The findings of this study are similar to others involving permissive dehydration over a
short-term periods (24-25). Studies by Neal et al. (24) and Garrett et al. (25) both involved a five-
day STHA period, but clamped $T_C$ at 38.5°C, resulting in altered exercise intensity in order to
maintain a constant $T_C$. Therefore, the intensity of acclimation sessions were lower than the
current study. The current study used a fixed intensity protocol (50% VO$_2$ max), involving a
constant workload over the entire acclimation period. Thermal strain may have been dampened
towards the end of the acclimation trials (but not measured). Similar to the current study, Garrett
et al. observed no significant difference in $T_C$ between EUH and DEH groups, did not measure
whole body sweat rate, and increased aerobic capacity following the HST by 19% in DEH and
14% in EUH groups. This in agreement with the current study which observed an increased work
capacity of 71% in the DEH group and 59% in the EUH group. The magnitude of aerobic
improvement demonstrates how detrimental performance can be under high heat stress while
wearing PPE gear in the non-acclimated individual. Neal et al. found aerobic performance is
improved following acclimation, resulting in increased work rate at lactate threshold.
In the current study, ~2.3% dehydration was accomplished over three acclimation trials by delivering 0.5 ml\(\cdot\)kg\(^{-1}\)\(\cdot\)15 min\(^{-1}\) compared to EUH trials with ~1.4% dehydration who received 2.0 ml\(\cdot\)kg\(^{-1}\)\(\cdot\)15 min\(^{-1}\). The design was a randomized crossover design separated by a five-week washout. All trials occurred during the winter months (Nov-Feb, 46.9°N), preventing adaptation crossover through acclimatization.

Each acclimation session for the DEH group resulted in percent dehydration values above 2% body weight loss (Figure 1), which is considered a threshold for dehydration (2, 9, 39) that is recommended to avoid when exercising in the heat (56). Subjects showed no signs of “chronic dehydration” while participating based on average pre-exercise USG values never exceeding 1.020 in any trials (Figure 2), or any significant difference between EUH and DEH groups for hydration (Table 10) prior to the HST. However, there was a main effect for Treatment in which EUH was significantly higher than DEH (p=0.007) prior to acclimation trial two (Figure 2). Pre-trial body weight did not significantly decrease over the course of the 5 trials compared to Trial 1 (Table 3). An increase in body weight at Trial 5 in the DEH group and Trial 2 in the EUH group compared to Trial 1 suggests weight gain from fluid retention. The ~48 hours between acclimation bouts may benefit individuals by preventing chronic dehydration over multiple days, thus improving recovery and promoting fluid retention. Importantly, subjects were not scripted with a rehydration strategy following any of the acclimation trials, and were therefore reliant on thirst.

The effect of heat acclimation on the cardiovascular system was measured by HR and recorded every 15 minutes during the trial. A significant decrease (p<0.001) in peak HR by 14 and 12 b\(\cdot\)min\(^{-1}\) occurred following the HST POST acclimation in EUH and DEH groups respectively (Figure 7, Table 6). One mechanism responsible for lowering HR at a given intensity involves the adjustment in extracellular fluid compartments to increase stroke volume of each cardiac
contraction. This fluid adjustment occurs by increasing plasma volume (PV), which in turn increases total body water (17) to help defend from the effects of dehydration (11, 13) and maintain blood pressure (1, 14) following sweat loss. Although it may be inevitable to prevent dehydration during aerobic exercise in the heat (13, 56), PV expansion aids to alleviate the effects of dehydration over extended work periods. At constant workloads, cardiac output is directly proportional to exercise intensity and stroke volume. PV expansion following acclimation promotes increased cardiac filling (and end diastolic volume) as well as cardiac contractility, and thus an increase in stroke volume. PV increased by 7.1 and 4.4% in the DEH and EUH group respectively, showing a main effect for Time (p=0.002) following acclimation. We observed a decrease in hematocrit (Table 7), and slight decrease in hemoglobin (Table 7) due to a hemodilution from plasma expansion. These values are consistent with other studies involving LTHA (5, 7, 17-19 21), but requiring half the exposures to achieve similar results. Measuring PV changes over long durations involves large individual variability. In the current study, PV changes ranged between -9 and 19% following acclimation. Other studies with similar designs demonstrated high ranges in PV change from 3 - 27% (17-19, 29). Causes of variability in PV change may stem from factors such as fitness level, response to the heat, free-living human decisions made outside the laboratory, and the effectiveness of the Dill and Costill methods over extended time periods. PV expansion may be attenuated in higher fit individuals, as endurance trained individuals may already exhibit expanded PV (25). Therefore, the practical aspect of PV expansion can especially benefit WLFF in the low to moderate fitness category as a way of training pre-season to prevent HRI.

A benefit of PV expansion allows individuals to sustain high sweat rates for long periods to prevent the physiological implications associated with dehydration (11,13). In this study, we
saw a significant interaction for sweat rate (p=0.015) increasing from 1.5 to 1.9 L\text{\textbullet}\text{hour}^{-1} and 1.6 to 1.8 L\text{\textbullet}\text{hour}^{-1} in DEH and EUH groups respectively. Although we showed that four heat exposures increases PV, it is still unknown whether plasma volume expansion is maintained long-term (multiple weeks to months) or has only short-term effects. Prior research has shown plasma volume decreases back to pre-acclimation levels after 14 days (29) and over the fire season (23). Therefore, it is imperative individuals depart to warm environments within one week following acclimation (27), when PV may be at its peak.

One mechanism responsible for PV expansion is an increase in aldosterone released from the adrenal cortex, and vasopressin released from the posterior pituitary gland (17, 19, 33-36, 60). The current data suggests these hormones may be enhanced by dehydration to induce PV expansion, but were not measured. Garrett et al. (27) observed a positive correlation between dehydration and aldosterone release following acclimation, suggesting a positive feedback mechanism associated with increased plasma osmolality caused by dehydration promotes aldosterone release. The investigators found PV to change by 8% and 4% in their DEH and EUH groups respectively, similar to PV expansion of the current study (Table 4). Fluid delivery administered in the DEH trials of the current study averaged approximately 0.24 L during the 90-minute HST to induce an average of $\sim$2.3% dehydration. This is compared to 1.2 L water administered during the EUH sessions to induce $\sim$1.4% dehydration. Receptors in the hypothalamus sense hypovolemia when blood osmolality increases, thus fluid retention is promoted to maintain blood volume from release of these hormones (12, 24, 27, 35-36). Aldosterone is responsible for retention of sodium in the renal tubules of the kidney, and in turn maintains blood volume by retaining water bound to these ions upon polar attractions. Therefore, conserving water and sodium ions in the extracellular compartment of blood to increase PV (19).
Another physiological effect to increase PV is to increase the protein oncotic pressure in the intravascular space (17). This increase in protein content (specifically albumin) in the blood causes a shift induced by the polarity of proteins from the interstitial to the intravascular space (14-15) to promote water retention.

Two noteworthy adaptations caused by heat acclimation to aid in thermoregulation involve enhancing sweat rates to increase evaporative heat loss, and enhancing SBF to aid in convective heat loss. Not only is sweat rate increased with acclimation, but the onset of sweating occurs at a lower $T_c$ (7, 14, 18) due to increased sensitivity of local muscarinic receptors to acetylcholine in the region of the sweat gland to promote sweating (16). This allows the thermoregulatory process to occur earlier when acclimated. The stress from multiple bouts of dehydration may promote adaptations to thermoregulate due to higher stresses caused by dehydration compared to a maintained hydration status. This did not manifest into improved $T_c$, Tsk, HR or performance in the heat. However, this response may be advantageous over extended periods (12-16 hour shifts) to better maintain $T_c$ as long as blood volume is maintained with fluid ingestion.

Increasing SBF during acclimation involves shunting blood from the core to the peripheries at a given $T_c$ (7, 16, 18) to allow more heat dissipation through the skin. Following acclimation, SBF is increased due to an increased sensitivity in cutaneous vasculature to signal smooth muscle relaxation in the endothelium (16), resulting in vasodilation. Therefore, this sensitivity allows vasodilation to increase blood transport to the peripheries, and allows more blood to reach sweat glands concurrent with greater sweat rate (16). In this study, SBF was measured for one minute at 30, 60, and 90 minutes during the HST, and for five minutes resting following the HST (Table 8, 9, Figure 13a, 13b). Laser Doppler Flowmetry (LDF) was used to measure SBF, and placed in the pectoral region. No significant difference was found for peak SBF, but we found attenuations
in SBF following a five-minute recovery period (Figure 13b) following the HST. This finding may suggest heat acclimation to improve the recovery process due to lower SBF response after five minutes rest. One possible limitation in this measurement can be from placement of the LDF probe in the pectoral region, in which the movement caused by ventilation may add an artifact. It was suggested during collection for subjects to limit heavy breathing during SBF measurements, but could not be prevented. Future studies should consider using an alternative location and/or posture for LDF measurement to prevent variability during data collection caused by movement.

By attenuating strain in $T_C$ and HR following acclimation, individuals maintained PSI levels below a critical marker of 7.5 for longer periods (Table 6), lowering time spent in the “at risk” zone for heat related illness (HRI), as described by Buller et al. (62). By increasing risk for HRI (when PSI>7.5), individuals in this category cannot maintain work output in the current environment, and must cease work to prevent further injury. Therefore, putting themselves at harm for risks associated with firefighting or warfighting. Non-acclimated individuals reached the “at risk” mark 17 and 18 minutes earlier in the DEH and EUH group respectively compared to the acclimated group. The importance of acclimation demonstrates that individuals will be able to sustain heavy work-loads for longer periods, and avoid entering the “at risk” category when work demands increase.

It is well known exercise performance diminishes in hot environments (42, 43). In addition, PPE gear creates microenvironments diminishing $T_C$-$T_{SK}$ temperature gradients to a greater extent compared to clothing which promotes heat dissipation. This causes $T_{SK}$ to increase independent of individual thermoregulatory capacity. Narrow $T_C$-$T_{SK}$ temperature gradients are found following the HST (Table 6) due to PPE gear, and decreases post-acclimation. This data is counterintuitive to prior research that states performance is attenuated in conjunction with lower
Tc-Tsk temperature gradient (26, 61). Therefore, a critical Tc-Tsk gradient should not be used as a predictor for exercise performance involving PPE gear. PPE gear attenuates blood flow to the working muscles caused by greater blood displacement to the peripheries as result of high Tsk, a limitation to exercise in the heat (14, 26, 61). Attenuating Tc should be of greater focus to sustaining aerobic performance when PPE gear is worn, and utilizing acclimation along with internal cooling mechanisms should be considered as an intervention for these individuals. Following the aerobic performance test at exhaustion, individuals were able to achieve a higher maximum HR due to higher work outputs and tolerance, but Tc was significantly lower at exhaustion (Table 12). Therefore, attenuating Tc induced by acclimation allowed accomplishment of higher work rates.

Aerobic performance significantly increased time to exhaustion POST-acclimation in both DEH and EUH groups by approximately 71% and 59% respectively (Figure 12, Table 6), demonstrating more work output occurring during a graded ramp test (Table 6). Increasing work capacity in the heat following acclimation impacts the safety of individuals by allowing them to perform in time of need and escaping hazards. Over the course of the study, it was known a training effect plays a role towards improving performance, but the magnitude of increase in both groups was unexpected. Markers of aerobic performance previously mentioned in the literature to be improved by heat acclimation include improved exercise economy (1, 5), higher work output at lactate threshold (21, 25), and higher cardiac output (17) due to higher cardiac preload. The obvious mechanism for increased aerobic performance in EUH and DEH groups occurred because the graded aerobic test was initiated at lower Tc and HR. This contributed to a higher thermal tolerance, and capacity to perform longer to achieve higher work rates. PV expansion increases aerobic performance by the maintenance of stroke volume (18), and allows for more sweat
dissipation to occur, preventing the onset of thermal stress induced by dehydration (11, 13) in conjunction with high sweat rates.

In summary, STHA effectively reduces both physiological and perceptual strain associated with heat stress, allowing a cost-effective and applicable protocol to promote thermoregulatory adaptations. With four exposures in a controlled setting, the combination of heat stress and exercise attenuates these physiological markers, thus increasing aerobic performance and safety. Therefore, when the optimal fluid delivery is not maintained throughout training, acclimation will be present at the same magnitude. Stresses associated with attenuating fluid delivery may aid in fluid regulatory adjustments associated with PV expansion, and thermoregulatory adjustments associated with sweat capacity. Initiating adaptations responsible for heat acclimation occurs quickly (<1 week), and may be an effective strategy for performance and safety for individuals quickly relocating to hot environments.
SUBJECT INFORMATION AND CONSENT FORM

PROJECT IN BRIEF: Impact of Hydration Status during Heat Acclimation on Exercise Performance

SPONSOR: United States Forest Service (MTDC)

RESEARCHERS: Dr. Charles Dumke (406) 243-6176
               Dr. Brent Ruby

The University of Montana
32 Campus Drive
McGill Hall – HHP
Missoula, MT 59812

Please read the following information carefully and feel free to ask questions. Only sign the final page when you are satisfied procedures and risks have been sufficiently explained to you.

REQUIREMENTS

This research study requires that you meet the following criteria:

- Participants must be males between the ages of 18 and 40.
- Participants must have a VO2 max ≥ 40 ml/kg/min and <85 ml/kg/min
- Participants must refrain from regular sauna/hot tub use or exposure to hot climates during the course of the study (such as travel to Hawaii/Mexico during the washout period)

PURPOSE OF THE STUDY

Heat acclimation has been shown to significantly improve exercise performance in the heat, and may be a useful resource for individuals performing difficult tasks without prior heat exposures. With the addition of dehydration as an added stress during the acclimation process, an individual may improve their adaptations to the heat, increasing performance and reducing the likelihood of heat related illness. The purpose of this study is to evaluate the effect of hydration status during short-term heat acclimation (SSTA), and its effect of exercise performance in the heat. This study will involve a randomized crossover design, where subjects will train either euhydrated (EUH) or dehydrated (DEH) during three days of heat acclimation. Exercise performance and physiologic responses during a heat stress test (HST) will be measured before and after each training intervention.

The University of Montana IRB
Expiration Date, 10-6-2014
Date Approved, 12-5-2015
Chair/Admin
TEST PROCEDURES

11 VISITS TO THE LABORATORY WILL BE REQUIRED, AS SUMMARIZED BELOW

<table>
<thead>
<tr>
<th>Visit Number</th>
<th>Preliminary Screening</th>
<th>PRE Testing</th>
<th>Acclimation (3 Days)</th>
<th>POST Testing</th>
<th>Winter Break Washout Period</th>
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<td>1</td>
<td>2</td>
<td>3, 4, 5</td>
<td>6</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11</td>
</tr>
</tbody>
</table>

PRELIMINARY SCREENING (Visit 1, ~1 hr)

a. A screening assessment which involves a health/exercise questionnaire (Par-Q) and family history questionnaire.
   Prior to any testing, you will complete a physical activity readiness questionnaire (PAR-Q) to screen for known risk factors of coronary heart disease and a family history questionnaire to screen for history of heat related illness or death.
   If you successfully complete the PAR-Q, you will then provide written informed consent following the reading of this document.

b. A measure of percent body fat obtained using underwater weighing
   This test session will require that you do not eat for a minimum of 3 hours prior to the testing. Prior to the test, body weight will be recorded in your bathing suit. You will then be asked to complete between 3 – 6 underwater weighing procedures. The underwater weight requires that you are submersed in our weighing tank (similar to a hot tub) and that you maximally exhale as much air as possible while underwater. The underwater weight will be recorded within 2-4 seconds and then you will be signaled to surface. This procedure will be repeated until three measurements have been obtained that are within 100 grams of each other. This test will take approximately 20 minutes.

c. A maximal treadmill ergometer test to measure aerobic fitness
   This test will consist of walking and running on a laboratory treadmill to volitional fatigue. The speed and/or grade of the treadmill will increase and will progress to fatigue. You will be encouraged to continue to walk/run until volitional fatigue. During this test you will wear a nose clip and headgear that will support a mouthpiece. This will allow us to measure the amount of oxygen that the body uses during this exercise. Heart rate will be measured using an elastic chest strap that is worn on the skin under your shirt around your chest. This test will take approximately 30 minutes. You will fast for approximately 3 hours prior to this test.

PRE AND POST TESTING (VISIT 2, 6, 7, & 11: ~2 HOURS EACH)

Experimental Protocol

a. The day prior to the first experimental testing session you are allowed to eat and drink as normal, but must NOT consume any alcoholic beverages. For the subsequent testing session, you will replicate this eating and drinking situation.

b. PRE and POST acclimations will involve you completing a heat stress test (HST) and aerobic performance test in hot conditions (40°C (104°F), 30%RH). PRE tests will
occur one to two days prior to the acclimation periods, and POST tests will occur one to two days after the three day acclimation period.

**Heat Stress Test**

c. You will arrive to the laboratory following a 3 hour fast. When you arrive, you will void your bladder and provide a urine sample followed by nude body weight recordings in privacy. A blood sample will be taken before commencement of exercise. You will be equipped with skin temperature sensors on the chest and will insert a rectal thermometer while in privacy so that core body temperature can be monitored.

d. You will exercise at a pace of 50% VO₂ max for 90 minutes in the heat and this exercise intensity mimics the intensity used in prior research for heat acclimation studies and similar to the work rate identified for wildland firefighting. Clothing will include personal protective equipment (PPE), and you will be required to bring your own cotton undershirt, athletic shoes, and socks. Skin blood flow will be measured via laser doppler flowmetry at the initiation of exercise (0), 30, 60, and at the end of the trial (90). During the HST, 2 ml/kg water will be administered every 15 minutes. Rating of perceived exertion (RPE) and heart rate (HR) will be recorded every 15 minutes. After the 90 minute HST, you will rest for five minutes before the exercise performance test. A urine sample followed by measurement of nude body weight will occur to determine sweat rate, then a blood sample.

**Maximal Treadmill Ergometer Test to Measure Aerobic Fitness**

e. After a 5 minute rest period, you will then perform an incremental test to exhaustion. This test will consist of walking and running on a laboratory treadmill to volitional fatigue. The speed and/or grade of the treadmill will increase every minute and will progress to fatigue. You will be encouraged to continue to walk/run until volitional fatigue. HR and RPE will be measured as described above.

**HEAT ACCLIMATION TRIALS (VISITS 3-5 & 8-10: ~ 2 HOURS EACH)**

Three acclimation trials will occur within a one week period. You will be required to complete 90 minutes of exercise at 50% VO₂ max in a hot room (40°C (104°F), 30% RH). Upon entering the lab, you will be required to void if necessary and provide a urine sample followed by nude body weight in private. RPE and HR will be recorded every 15 minutes. Fluid delivery will be provided as described below, but each intervention will occur for three straight acclimation days. Water will be room temperature to avoid influences of cold fluid ingestion on core and skin temperature. Following each acclimation trial, a urine sample and nude body weight will be recorded. Clothing will be PPE as described above, and you will be required to bring your own cotton undershirt, athletic shoes, and socks. A dietary recall involving a record of food type and quantity will be collected to determine if consistent dietary habits occur for each trial.

a. Dehydration trials will involve the same exercise protocol as described above. Fluid supplementation will involve 0.5 ml/kg every 15 minutes. After each trial, you will be permitted to drink fluid ad libitum.

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*The University of Montana IRB*

Expiration Date: 10-6-2016
Date Approved: 10-5-2015
Chair/Admin: [Initials]
b. Euhydration trials will involve the same exercise protocol as described above. Fluid supplementation will involve 2 ml/kg water every 15 minutes. After each trial, you will be permitted to drink ad libitum.

MEASUREMENTS

a. Dietary and Activity Recall

For 24-hours before your first exercise trial you will be asked to record the foods and quantity that you consume. You are not allowed to consume any alcohol during this time period, as it is a diuretic and compromises hydration status. For the second trial, you will consume the same foods and quantity of those foods that you consumed for the first trial. Two days before your first trial day you can exercise as you wish, but this must be repeated at the same time of day and the same exercise prior to the second trial. For the 24-hours before each trial you cannot participate in any physical exercise.

b. Body Weight

Nude body weight will be measured in private on a calibrated scale. Weights will be taken before and after the trials.

c. Blood Samples

A total of 8 blood samples (2 per trial x 4 trials) will be collected using a venipuncture technique. The site will be cleaned with alcohol prior to the blood draw, and wiped clean afterwards. These samples will be collected to measure changes in plasma volume, electrolytes, and hormones involved in the heat acclimation process. All of the blood samples will be obtained under the direction of Dr. Charles Dumke, Ph.D. Blood samples will be taken a) prior to the exercise trial, b) post exercise trial, ~5 ml will be drawn each time for a total of ~10 ml (~1/2 tablespoon) per trial.

d. Laser Doppler Flowmetry

Skin blood flow will be recorded using a laser doppler ultrasound. This is collected by holding a wand up to the chest underneath the clothing. This is non-invasive and presents no harm.

e. Skin and Core Temperature

Skin temperature patches will be placed on the pectoralis major (chest) prior to commencing exercise. This involves (potentially) shaving some hair from the specific sensor area and sticking the sensor on. A rectal thermometer thermistor will be used so that your core body temperature can be monitored throughout the exercise period.

f. Urine

You will be asked to void your bladder before the trial. After the initial void, urine will be collected in a disposable plastic container for hydration status indicators (urine specific gravity). The urine will then be discarded.
RISKS AND DISCOMFORTS

1. Mild discomfort may result during and after exercise. These discomforts include shortness of breath, tired or sore legs, nausea and possibility of vomiting.

2. Exercising in the heat chamber will result in profuse sweating and the perception of feeling very hot. Adverse reactions to heat stress can include heat exhaustion, heat stroke, and fainting. However, core body temperature will be monitored during testing session; if core temperature goes above 41°C, the exercise test will be terminated. Fluids will be provided to you as well to mitigate these risks. If you feel too hot to continue exercise, the test will be terminated and you will be removed immediately from the heat chamber.

3. Muscle soreness after the tests may occur as a result of the exercise, but should not persist.

4. Certain changes in body function take place when any person exercises. Some of these changes are normal and others are abnormal. Abnormal changes may occur in blood pressures, heart rate, heart rhythm or extreme shortness of breath. Very rare instances of heart attack have occurred. Every effort will be made to minimize possible problems by the preliminary evaluation and constant surveillance during testing. The laboratory has standard emergency procedures should any potential problems arise.

5. Mild symptoms of dehydration such as headache and general fatigue may result during and after the exercise. To minimize the risk of excessive dehydration, your core temperature will be monitored continuously during exercise. If core temperature goes above 40°C, the exercise test will be terminated.

6. You will be informed of any new findings that may affect your decision to remain in the study.

7. During any of the exercise tests should symptoms, such as chest discomfort, unusual shortness of breath or other abnormal findings develop, the exercise physiologist conducting the research will terminate the test. Guidelines by the American College of Sports Medicine will be followed to determine when a test should be stopped. These symptoms include moderate to severe angina (chest pain), increased dizziness, shortness of breath, fatigue and your desire to stop.

8. When blood samples are collected for this study, participants may feel a slight sting or "pinch" in their arm, they may suffer a small bruise, and there is a very slight possibility of infection. Should participants notice unusual redness, bruising, or swelling at the blood sampling site they should seek medical attention and contact the study director, Dr. Charles Dumke. During the blood draw, precautions (cleaning the site with alcohol, sterile supplies, and wearing a Band-Aid) will be taken to minimize deleterious effects.

9. Certain medications could increase the risk for adverse effects during this heat related study. If you are taking any medications, you must check with your physician before participating in the study.

BENEFITS OF PARTICIPATION

1. The information from these tests will provide you with an accurate assessment of your aerobic fitness and body composition that can be compared with norms for your age and sport but may be of little benefit to your understanding of your personal fitness. There are no other direct benefits to the participants in the study.

2. The scientific benefit includes expanding current understanding of heat stress and heat acclimation. This information may contribute to the reduction of heat related illnesses in at risk populations.

The University of Montana IRB
Expiration Date 10-6-2014
Date Approved 10-7-2015
Chair/Admin
CONFIDENTIALITY

1. Your records will be kept confidential and not be released without consent except as required by law.
2. Only the researcher and his research assistants will have access to the files.
3. Your identity will be kept private.
4. If the results of this study are written in a scientific journal or presented at a scientific meeting, names will not be used.
5. All data, identified only by an ID #, will be stored in our laboratory.
6. The signed consent form and information sheet will be stored in a locked cabinet separate from the data.

COMPENSATION FOR TIME

After completion of all trials, subjects will receive $200.

COMPENSATION FOR INJURY

In the event that you are injured as a result of this research you should individually seek appropriate medical treatment. If the injury is caused by the negligence of the University of Montana or any of its employees, you may be entitled to reimbursement or compensation pursuant to the Comprehensive State Insurance Plan established by the Department of Administration under the authority of M.C.A., Title 2, Chapter 9. In the event of a claim for such injury, further information may be obtained from the University’s Risk Manager (406-243-2700; kathy.krebsbach@umontana.edu) or the Office of Legal Counsel (406-243-4742; legalcounsel@umontana.edu). (Reviewed by University Legal Counsel, May 9, 2013)
VOLUNTARY PARTICIPATION AND WITHDRAWAL

It is important that you realize that you are free to withdraw from the study at any time. If you decide to drop out of the study you will receive compensation for the test sessions you completed.

QUESTIONS

You may wish to discuss this with others before you agree to take part in this study. If you have any questions about the research now or during the study contact Dr. Charles Dumke, PhD at (406) 243-6176 (office). If you have any questions regarding your rights as a subject, you may contact the chair of the IRB through the University of Montana Research Office at (406) 243-6672.

STATEMENT OF CONSENT

I have read the above statements and understand the risks involved with this study. I authorize Dr. Charles Dumke, PhD, and such assistants that he may designate, to administer and conduct the testing as safely as possible with a minimal amount of discomfort. If I have additional questions, I may contact Dr. Charles Dumke, PhD, at (406) 243-6176.

Participant (print) _____________________________________________

Signature _____________________________________________

Date _____________________________________________

STATEMENT OF CONSENT TO BE PHOTOGRAPHED DURING DATA COLLECTION

During the study, I understand that pictures may be taken. I provide my consent to having my picture taken during the course of the research study. I provide my consent that my picture may be used in some presentations related to this study. If pictures are used at any time for presentation, names and/or physiological data will not be associated with them.

Signature _____________________________________________ Date  ________

The University of Montana IRB
Expiration Date 10-6-2016
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Appendix II: PAR-Q

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

1. Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?

YES NO

2. Do you feel pain in your chest when you do physical activity?

YES NO

3. In the past month, have you had chest pain when you were not doing physical activity?

YES NO

4. Do you lose your balance because of dizziness or do you ever lose consciousness?

YES NO

5. Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?

YES NO

6. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?

YES NO

7. Do you know of any other reason why you should not do physical activity?

YES NO

If you answered YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- Start becoming much more physically active — begin slowly and build up gradually. This is the safest and easiest way to go.
- Take part in a fitness appraisal — this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

DELAY BECOMING MUCH MORE ACTIVE:

- If you are not feeling well because of a temporary illness such as a cold or a fever — wait until you feel better; or
- If you are or may be pregnant — talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

Informed Use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction.

NAME ____________________________

SIGNATURE ____________________________

DATE ____________________________

WITNESS ____________________________

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

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Appendix III: Supplementary Data

Area Under Curve Data

Heat Stress Trials

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>DEH PRE-Acclimation</th>
<th>DEH POST-Acclimation</th>
<th>EUH PRE-Acclimation</th>
<th>EUH POST-Acclimation</th>
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<tbody>
<tr>
<td>$T_C$ ($^\circ$C)</td>
<td>133 ± 6.3</td>
<td>103 ± 7.0*</td>
<td>131 ± 7.6</td>
<td>104 ± 7.5*</td>
</tr>
<tr>
<td>$T_{SK}$ ($^\circ$C)</td>
<td>374.7 ± 11.52</td>
<td>365.5 ± 9.0</td>
<td>372.1 ± 11.46</td>
<td>357.4 ± 8.24</td>
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<td>$HR$ ($b*min^{-1}$)</td>
<td>7803 ± 283</td>
<td>6904 ± 302*</td>
<td>7810.96 ± 305</td>
<td>6887 ± 271*</td>
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<tr>
<td>$RPE$</td>
<td>423.5 ± 23.6</td>
<td>310.4 ± 22.46*</td>
<td>420.6 ± 27.0</td>
<td>276.9 ± 24.3*</td>
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<tr>
<td>$PSI$</td>
<td>558.0 ± 21.9</td>
<td>469.2 ± 26.62*</td>
<td>557.0 ± 26.4</td>
<td>480.4 ± 21.9*</td>
</tr>
</tbody>
</table>

*Table 1: Area under curve analysis for HST variables *p<0.05. Main effect for Time.*

*Figure 1: $T_C$ area under curve during HST. * p<0.001. Main effect for Time.*
**Figure 2:** $T_{sk}$ area under curve during HST.

**Figure 3:** HR area under curve during HST. * $p<0.001$ Main effect for Time.
Figure 4: RPE area under curve. * $p<0.001$. Main effect for Time.

Figure 5: PSI area under curve. * $p<0.001$. Main effect for Time.
Acclimation Data

<table>
<thead>
<tr>
<th></th>
<th>DEH</th>
<th></th>
<th>EUH</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 2</td>
<td>Day 3</td>
<td>Day 1</td>
</tr>
<tr>
<td><strong>RPE</strong></td>
<td>529 ± 21</td>
<td>497 ± 21</td>
<td>486 ± 27</td>
<td>515 ± 20</td>
</tr>
<tr>
<td><strong>HR</strong></td>
<td>6675 ± 254</td>
<td>6407 ± 278</td>
<td>6464 ± 337</td>
<td>6928 ± 332</td>
</tr>
</tbody>
</table>

*Table 2: Area under curve data over three acclimation Days.*

*Figure 6: Peak HR during for three acclimation Days.*
Figure 7: HR area under curve during for three acclimation Days.

Figure 8: RPE area under curve for three acclimation Days.
Blood Data

**Figure 9:** Pre-exercise Hemoglobin (Hb) concentration measured PRE and POST acclimation.

**Figure 10:** Pre-exercise hematocrit measured PRE and POST acclimation. * p=0.007. Main effect for Time
Figure 11: Pre-Exercise Plasma percentage PRE and POST acclimation

Figure 12: Post-Exercise Plasma percentage PRE and POST acclimation
Figure 13: Resting Plasma Percentage PRE and POST acclimation.

Figure 14: Change in Time to Exhaustion during performance (POST-PRE) in relation to VO$_2$ max
References


