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Heat Exposure and Hypohydration Exacerbate Physiological Strain During Load Carrying

Running head: Heat Exposure and Hypohydration During Load Carrying

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Abstract
Heat exposure and hypohydration induce physiological and psychological strain during exercise; however, it is unknown if the separate effects of heat exposure and hypohydration are synergistic when co-occurring during loaded exercise. This study compared separate and combined effects of heat exposure and hypohydration on physiological strain, mood state, and visual vigilance during loaded exercise. Twelve males (mean±SD; age, 20±2 years; body mass, 74.0±8.2 kg; maximal oxygen uptake, 57.0±6.0 mLkg-1min-1) completed 4 trials under the following conditions: euhydrated temperate (EUT), hypohydrated temperate (HYT), euhydrated hot (EUH), and hypohydrated hot (HYH). Exercise was 90 min of treadmill walking (~50% VO2 max, 5% grade) while carrying a 45 lb rucksack. Profile of Mood States and the Scanning Visual Vigilance Test were completed pre and post exercise. The separate effects of heat exposure (EUH) and hypohydration (HYT) on post-exercise Tre were similar (38.25±0.63°C vs. 38.22±0.29°C, respectively, p>0.05), while in combination (HYH), post-exercise Tre was far greater (39.32±0.43°C). Increase in Tre per 1% body mass loss (BML) for HYH (vs. EUH) was greater than HYT (vs. EUT) (0.32°C vs. 0.04°C, respectively, p=0.02); HR increase per 1% BML for HYH (vs. EUH) was 7 bpm compared to HYT (vs. EUT) at 3 bpm (p=0.30). HYH induced greater mood disturbance (post-pre exercise) (35±21 units) compared to other conditions (EUT=3±9 units; HYT=3±16 units; EUH=16±26 units; p<0.001). No differences occurred in visual vigilance (p>0.05). Independently, heat exposure and hypohydration induced similar physiological strain during loaded exercise; when combined, heat exposure with hypohydration, synergistically exacerbated physiological strain and mood disturbance.

Keywords: heat illness; rectal temperature; mood disturbance; visual vigilance; military rucksack
INTRODUCTION

Over recent decades, the prevalence of exertional heat illnesses (EHI) in military and occupational personnel has risen, making this a global challenge (10,34). In 2014 alone, 2,027 United States military members were diagnosed with an EHI, with 344 of these cases classified as exertional heat stroke (EHS) (3). In the same year, 2,630 occupational workers suffered from EHI with 18 deaths from EHS attributed to on-the-job causes (8). Exertional heat illnesses compromise the health, safety, and productivity of our military and occupational workers. To reduce the occurrences of these potentially life-threatening conditions, comprehensive knowledge on the risk factors of EHI must be obtained in situations specific to these populations. In particular, military and occupational workers, as well as athletes such as hikers, often require load carrying during prolonged exercise; however, there remains a lack of knowledge on the physiological and psychological effects of common stressors, such as heat exposure and hypohydration, while in the presence of load carrying.

The weight of the required load warfighters carry during long distance marches is ever increasing. Prior to the 18th century, military rucksacks rarely exceeded 15kg [33 lbs], whereas today, soldiers carry upwards of 55 kg [121 lbs] (22). Load carriage increases metabolic cost while reducing exercise tolerance and work capacity (35). Similarly, hypohydration induces physiological strain, defined as an increased core body temperature and heart rate (HR), while reducing sweat evaporation potential (31). The physiological effects of heat exposure and hypohydration have been studied independently and in combination, with classic studies showing a proportional relationship between the percent dehydration achieved during exercise and the cardiovascular strain and elevated core body temperature experienced (31,27,19). Heat exposure,
hypohydration, and the addition of load carrying, however, have not been studied in full combination. It is important to the future improvement of military, occupational, and athletic safety guidelines to explore the possible synergistic effects of these risk factors.

In addition to physiological strain, psychological decrements can compromise work productivity and accuracy. Both hypohydration and dehydration disrupt homeostatic function and lead to psychological impairments. Mild dehydration, defined as ~2% body mass loss (BML), has been shown to impair attention and psychomotor skills (2), as well as negatively impact mood, perception of task difficulty, and concentration in women (4). Moreover, mild dehydration has been shown to adversely affect vigilance, working memory, and tension/anxiety in men (17). The psychological effects of hyperthermia alone and hyperthermia and dehydration combined remain varied (9,14,33), while little is known about the psychological effects of loaded exercise.

Heat exposure, hypohydration, and load carrying during exercise significantly increase an individual’s risk for EHI; however, there remains a lack of knowledge on the possible additive effects of heat exposure and hypohydration when co-occurring during loaded exercise. Therefore, the purpose of this study was to investigate the separate and combined effects of hypohydration and heat exposure on physiological and psychological measures while load carrying. We hypothesized the combined effects of hypohydration and heat exposure would synergistically impair psychological function and exacerbate physiological strain compared to heat exposure or hypohydration alone.
METHODS

Experimental Approach to the Problem: Subjects completed 2 baseline sessions to become familiar with the study procedures. Following this, a randomized, crossover design was utilized to test 4 experimental conditions: euhydrated temperate (EUT), hypohydrated temperate (HYT), euhydrated hot (EUH), and hypohydrated hot (HYH). Testing occurred in an environmental chamber (4284-2L-W, Minus-Eleven, Weymouth, MA) set to either temperate (18.0±3.5°C, 50.0±3.5%RH) or hot (34.0±0.3°C, 45.0±4.5%RH), and subjects arrived at the start of each trial either euhydrated or hypohydrated, defined by hydration indices described below. Each trial occurred at the same time of day (±1 h) separated by a minimum of 3 days to ensure adequate rest. Sneakers and lightweight exercise clothing (shorts and T-shirt) were worn during exercise trials and were kept consistent within subjects and between all conditions.

Subjects: Twelve moderately trained, young men (mean±SD; age, 20±2 years; height, 182±8 cm; body mass, 74.0±8.2 kg; body fat, 8.8±2.8%; maximal oxygen uptake [VO$_2$ max], 57.0±6.0 mL·kg$^{-1}$·min$^{-1}$) participated in this study. Inclusionary criteria were defined as: (a) 18-39 years of age, (b) VO$_2$ max $\geq$ 50 mL·kg$^{-1}$·min$^{-1}$, (c) regular participation in moderately intense exercise (above walking pace, $\geq$ 6 hr·wk$^{-1}$), (d) no history of heat illness, cardiovascular, metabolic, or respiratory disease, (e) no creatine use in the past year, (f) no contraindications to strenuous exercise in the heat, and (g) no tobacco use. Subjects were not heat acclimatized, and testing took place during the winter months in the northeast region of the United States to ensure passive heat exposure was not obtained outside of the testing conditions (3.2±5.6°C average environmental temperature during testing months) (29). All subjects completed a physician approved, medical history questionnaire and were informed of the benefits/risks to this
investigation before signing an informed consent approved by the University’s Institutional Review Board.

**Procedures:** Upon arrival for both baseline testing sessions, subjects provided a urine sample to assure euhydration with the following urinary measures: specific gravity ($U_{sg}$) (refractometry, model A300CL; Atago Inc., Spartan, Tokyo, Japan [5]), osmolality ($U_{Osm}$) (freezing point depression, model 3320; Advanced Instruments, Norwood, MA[5]), and color ($U_{col}$) (urine color chart [5]); euhydration was defined as $U_{sg} \leq 1.020$, $U_{col} \leq 4$, and $U_{Osm} \leq 800$. Thereafter, nude body mass was measured to the nearest 0.1 kg using a digital scale (model BWB-800A, Tanita Corporation, Tokyo, Japan). Subject’s baseline body mass was defined as the average body mass from both baseline days.

During the first baseline session only, height was measured and subjects were familiarized to 3 perceptual scales: Rating of Perceived Exertion (RPE) (7), Thirst sensation (15), and Thermal sensation (37). Thirst sensation was rated on a 9-point scale ranging from 0 (not thirsty at all) to 9 (very, very thirsty) in 1.0 unit increments. Similarly, thermal sensation was rated on a 17-point scale ranging from 0 (unbearably cold) to 8 (unbearably hot) in 0.5 unit increments. Further, profile of mood states (POMS) was utilized to assess general wellbeing, and scanning visual vigilance test (VVT) was utilized to assess awareness. Finally, expired gases were collected and analyzed to measure aerobic fitness using a graded treadmill VO$_2$ max test (PARVO metabolic cart, model CPX/D, PARVO Medics, St. Paul, MN) (6).
During the second familiarization visit, body fat percentage was obtained via a three-site skinfold analysis (chest, abdomen, thigh) (30) and a pre-exercise nude body mass was obtained. Subjects entered the environmental chamber set to 35°C; 45-55%RH and were fitted with a 20 kg [45 lb] military grade rucksack. The rucksack weight was uniform for all subjects, mimicking the expectations for “forced marches” or “humps” as suggested by the United States Army Recruiting Command pamphlet (USAREC Pam 601-25). Subjects were seated for 15 minutes to allow for the stabilization of physiological variables, followed by 30 minutes of treadmill walking at 50% of VO2 max (1.34-1.78 m·s⁻¹; 5% grade). The speed each subject used during his familiarization trial was the same speed to be used during his experimental trials. Thus, the speed was adjusted slightly during the familiarization exercise, if needed, to ensure subjects would be able to complete the 60 minutes of exercise during the subsequent experimental trials. Following exercise, the rucksack was removed and the POMS and VVT were completed. Subjects exited the environmental chamber where post-exercise nude body mass was obtained to calculate sweat rate for the purposes of determining fluid replacement necessary for subsequent euhydrated trials.

**Hydration Protocol:** Dietary logs were recorded for 24 hours prior to each testing session to ensure the same meals and fluids were being consumed across trials. For euhydrated trials (EUH and EUT) subjects were instructed to drink *ad libitum* plus an extra 500 mL of fluid the night prior and the morning of testing. For hypohydrated trials (HYH and HYT) subjects were fluid restricted for 20-22 hours and instructed to perform 60 minutes of exercise (elliptical, bike, or treadmill walking) the afternoon prior to testing. The mode and intensity of this exercise was kept consistent within individuals across all experimental trials. This procedure was used to ensure subjects were hypohydrated at 1-2% BML at the start of exercise the next day.
Experimental Trials: Pre-exercise measures included urinary indices (U_{col}, U_{sg}, U_{Osm}), body mass, and HR (Polar Electro, Port Washington, NY). Experimental measurements of body mass were subtracted from the subject’s baseline body mass to calculate %BML at the time of arrival. Hydration status was assessed in all subjects prior to participation to assure euhydration utilizing the criteria mentioned above. Subjects then inserted a rectal thermistor (model 401, Yellow Springs Instruments, Yellow Springs, OH) 10 cm beyond the anal sphincter for rectal temperature (T_{re}) measures.

Subjects then entered the environmental chamber and sat for 15 minutes to allow physiological variables to stabilize, and performed the POMS and VVT. The 20 kg [45 lb] military rucksack was secured and subjects performed 90 min of treadmill exercise (~50% of VO_{2 max}; 5% grade). The treadmill speeds for each participant during the first trial were recorded and replicated across all 4 trials. This ensured all subjects completed consistent exercise intensity across trials. To confirm this, oxygen uptake was measured for a 2-minute interval during the 75th and 85th minute of exercise using indirect calorimetry. Prior to, every 15 minutes during, and post-exercise, T_{re}, HR, and perceptual scales were recorded. Exercise was terminated if subjects met one of the following criteria: (a) HR > 190 bpm for 5 consecutive minutes, (b) T_{re} >40°C, or (c) signs and symptoms of heat exhaustion or heatstroke. During HYH and HYT trials, no fluid was given; however, during EUH and EUT trials, subjects were given equal boluses of room temperature water every 15 minutes according to their personal sweat rate. Immediately post-exercise, POMS and VVT were administered and subjects exited the environmental chamber where a post-exercise nude body mass and a urine sample were obtained.
Psychological Measures: During this VVT task, a faint stimulus appeared randomly on a laptop computer screen and remained visible for 2 seconds. Subjects pressed the keyboard space bar as quickly as possible upon detection of the stimulus. Correct responses, false alarms, and reaction time were recorded. This test is extremely sensitive to a wide variety of environmental conditions (25). During the POMS questionnaire, subjects rated 5 mood-related adjectives on a 5-point Likert scale in response to the question “How are you feeling right now?” These adjectives factor into 6 mood sub-scales (tension, depression, anger, vigor, fatigue, and confusion). This is a widely used, standardized inventory of subjective mood state (36).

Statistical Analysis: All data were analyzed using a 2x2x2 (environmental [2 levels, hot and temperate] x hydration [2 levels, hypohydrated and euhydrated] x time [2 levels, pre and post exercise]) repeated-measures ANOVA with planned comparisons post hoc to further explore sources of significant differences. Rectal temperature and HR variables were further analyzed to quantify their increase per 1% BML. Data related to RPE, thermal and thirst sensation, and $U_{col}$ were assumed to possess interval properties to permit identification of interactions between independent variables in data analysis. Analyses were performed in SPSS (V 20.0, IBM Corporation, Champaign, IL, USA) with an $a$ priori alpha level of 0.05. Values are presented as mean±SD with mean differences and 95% confidence intervals showing magnitude of differences where appropriate.
RESULTS

All subjects achieved necessary levels of euhydration and hypohydration prior to all experimental trials (Table 1). Upon exercise completion, both hypohydrated conditions (HYT and HYH) resulted in urinary measures indicating greater hypohydration compared to euhydrated conditions, with HYH resulting in the greatest dehydrated state (p≤0.01, Table 1).

There were no differences in exercise intensity across trials (p=0.73) as indicated by similar measures of percent VO$_2$ max (EUT: 46.66±3.92%, HYT: 47.69±3.31%, EUH: 49.35±4.21%, HYH: 49.54±4.5% of VO$_2$ max) and relative oxygen uptake (EUT: 26.42±2.70 mL·kg$^{-1}$·min$^{-1}$, HYT: 27.92±2.55 mL·kg$^{-1}$·min$^{-1}$, EUH: 27.03±2.28 mL·kg$^{-1}$·min$^{-1}$, HYH: 27.93±4.15 mL·kg$^{-1}$·min$^{-1}$) measured during exercise.

Prior to the commencement of exercise, a higher T$_{re}$ was observed when subjects were hypohydrated (37.37±0.31°C, average of HYH and HYT) compared to when euhydrated (37.10±0.37°C, average of EUH and EUT) (mean difference [95%CI]; 0.27°C [0.16,0.38], p<0.001). Post-exercise, HYH resulted in a higher T$_{re}$ compared to HYT, EUH, and EUT (39.32±0.43°C vs. 38.22±0.29°C, 38.25±0.63°C, and 37.86±0.33°C, respectively, p≤0.002; Figure 1). Compared to a euhydrated and temperate environment (EUT), the addition of heat exposure or hypohydration alone (EUH and HYT) increased post-exercise T$_{re}$ to a similar degree (0.36±0.32°C and 0.39±0.67°C, respectively). With heat exposure and hypohydration combined (HYH), post-exercise T$_{re}$ was 1.11±0.37°C and 1.08±0.92°C greater than heat exposure or hypohydration alone, respectively.
For each condition, $T_{re}$ rate of rise was calculated as the increase in $T_{re}$ (post exercise – pre exercise $T_{re}$), divided by exercise duration. An environment x hydration interaction for $T_{re}$ rate of rise ($p=0.02$) indicated that when subjects exercised in a temperate environment, $T_{re}$ rate of rise was similar despite hydration status (EUT: $0.008\pm0.002{^\circ}C\cdotmin^{-1}$ vs. HYT: $0.009\pm0.003{^\circ}C\cdotmin^{-1}$, $p=0.12$); however, when subjects exercised in a hot environment, $T_{re}$ rate of rise was greater when hypohydrated compared to when euhydrated (EUH: $0.013\pm0.007{^\circ}C\cdotmin^{-1}$ vs. HYH: $0.022\pm0.005{^\circ}C\cdotmin^{-1}$, $p=0.01$). Strikingly, the increase in $T_{re}$ per 1% BML for HYH (compared to EUH) was far greater than HYT (compared to EUT) ($0.32{^\circ}C$ vs. $0.04{^\circ}C$ respectively, $p=0.02$; Figure 2). Similar patterns were observed for the change in HR per 1% BML (HYH: 7 bpm vs. HYT: 3 bpm, $p=0.30$); however, this result did not reach statistical significance. Heart rate during exercise was consistently greater during HYH compared to all other conditions ($p=0.001$, Figure 1).

Profile of mood states revealed a greater total mood disturbance following exercise in the HYH condition (35±21 units) compared to all other conditions (EUT: 3±9 units; HYT: 3±16 units; EUH: 16±26 units, $p<0.001$; Table 2). Additionally for the profile of moods subscales, fatigue and confusion-bewilderment were greater for HYH compared to both EUT and HYT and depression-dejection was greater in HYH compared to HYT (Table 2). Mean differences (post – pre exercise) for VVT indicated no differences occurred in correct responses (EUT: -2±3; HYT:
-2±3; EUH: -1±2; HYH: 1±3), false alarms (EUT: 3±4; HYT: 2±7; EUH: 1±3; HYH: 2±4), or
reaction time (EUT: 0.026±0.034 s; HYT: 0.017±0.035 s; EUH: 0.009±0.013 s; HYH:
0.017±0.032 s) across all conditions (p>0.05).

Perceptual responses included a greater post-exercise RPE for HYT and HYH conditions
compared to their respective euhydrated conditions (p<0.001, Figure 3). As expected, thirst
sensation was greater pre-exercise and post-exercise for HYT and HYH conditions compared to
their respective euhydrated conditions (p<0.001; Figure 3). Thermal sensation was greater post-
exercise for HYT and HYH conditions compared their respective euhydrated conditions
(p<0.001; Figure 3).

**DISCUSSION**

This study examined the separate and combined effects of hypohydration and heat
exposure on physiological and psychological measures during loaded exercise. Previous
investigations have explored the physiological strain experienced during exercise in
combinations of hot, temperate, euhydrated, and hypohydrated conditions. This investigation
sought to relate those findings in the presence of load carrying, a stressor commonly experienced
by military and occupational personnel, as well as athletes such as hikers. Our findings indicate
that individually, heat exposure and hypohydration induce similar, moderate physiological strain.
However, when in combination, heat exposure and hypohydration synergistically exacerbate both physiological strain and mood disturbance.

The most significant discovery within this study demonstrated that hypohydration increased $T_{re}$ $0.32^\circ$C per 1% BML in hot conditions (HYH compared to EUH), while in temperate conditions, hypohydration increased $T_{re}$ only $0.04^\circ$C per 1% BML (HYT compared to EUT). The rather large rate of $0.32^\circ$C per 1% BML is far greater than reported values from other non-load carrying laboratory and field studies ($0.15$-$0.25^\circ$C per 1% BML) (20,11,18) utilizing similar protocols. Interestingly so, a meta-analysis of 20 studies showed that exercise in the heat with no external load increased core body temperature an average of $0.22^\circ$C per 1% BML (21). Comparatively our results show an almost 50% increase above this average, thus suggesting that load carrying contributes to far greater increases in $T_{re}$ per 1% BML during exercise in the heat.

Hypohydration increased HR 7 bpm per 1% BML while in hot conditions (HYH compared to EUH). Across similar studies with no load carried, HR increased an average 3 bpm per 1% BML (32). Our results demonstrate a more than double increase in this rate. These findings relate to military load carrying guidelines, as well as provide implications for occupational, athletic, or exercise activity that may require load carrying of a similar weight. Given the large increase in the rise in core body temperature when hypohydrated during exercise in the heat, additional safety precautions, such pre-determined adjusted work to rest ratios and hydration protocols, may be needed specifically for situations in which load carrying is necessary.
Our results demonstrated that heat exposure and hypohydration combined, synergistically exacerbated a number of physiological measures. First, compared to a euhydrated, temperate condition, the addition of heat exposure increased post-exercise $T_{re}$ by $0.39\pm0.67^\circ C$ (EUT vs. EUH), while the addition of hypohydration increased post-exercise $T_{re}$ by a similar degree of $0.36\pm0.32^\circ C$ (EUT vs. HYT). The addition of both heat exposure and hypohydration increased post-exercise $T_{re}$ by $1.47\pm0.45^\circ C$ (EUT vs. HYH). Therefore, under conditions of hypohydration and heat exposure, post-exercise $T_{re}$ was more than 3.5 times greater than in conditions of just heat exposure or hypohydration alone. Second, the environment x hydration interaction on $T_{re}$ rate of rise indicated that in temperate environments, $T_{re}$ rate of rise was similar for euhydrated and hypohydrated conditions; however, in hot environments, $T_{re}$ rate of rise was greater when hypohydrated than when euhydrated. Third, under hypohydrated, hot conditions, $T_{re}$ did not plateau thus suggesting a state of uncompensable heat stress (23), while HR did plateau thus suggesting cardiovascular drift was not observed during our exercise protocol. Original findings by Sawka et al (32) as well as replicated results (28), found core temperature to be the single best predictor for exhaustion from heat strain and the addition of hypohydration lowered the core temperature able to be tolerated during uncompensable heat stress. As such, our findings can be furthered by exploring the extent to which uncompensable heat stress during exercise with load carrying in hypohydrated, hot conditions increases one’s risk for exertional heat illnesses, as well as how longer exercise duration under these conditions may influence cardiovascular drift. With this, a greater understanding on the physiological limits during exercise under multiple stressors may be obtained and applied to military and athletic personnel for optimized training.
Taken together, our results indicate that the combined effects of hypohydration and heat exposure far exceed the effects of hypohydration or heat exposure independently. While this finding is expected, extra caution and modifications are not always employed under these conditions. By quantifying the extent to which heat exposure and hypohydration compound physiological strain, under conditions of load carrying, we raise awareness on the need for medical professionals, skilled at the recognition, prevention, and treatment of exertional heat illnesses to enforce the compliance of prevention strategies (i.e. modified work-to-rest ratios, proper hydration protocols) in military, occupational, and athletic settings, specifically when multiple stressors may be present.

Increased mood disturbance when hypohydrated (4,17), hypohydrated and hyperthermic (14), and in highly stressful situations (18) have been well documented. In this study, POMS revealed greater mood disturbance in hypohydrated, hot conditions compared to all other conditions, and specifically greater feelings of fatigue, depression, and confusion following exercise compared to both temperate conditions. These findings become concerning during occupational and athletic scenarios, and even more so during military life-or-death scenarios that cause adverse changes to mood and cognitive performance (16). Additionally, our results indicate no changes in visual vigilance measures across all conditions and time points. Similar findings by Ely et al (11) showed hypohydration increased mood disturbance, yet hypohydration and acute thermal stress did not impact psychomotor vigilance. Meta-analyses (12,24,26) indicate heterogeneous, small effects of acute, moderate exercise on cognitive performance, accuracy, and reaction time. Given these mixed findings, further investigations are needed to disentangle how methodological differences between studies, for example, different exercise
protocols (e.g. longer duration), cognitive assessments used, timing of cognitive assessment, (e.g. pre/post versus during exercise), and the type/severity of stressors (e.g. sleep loss, malnutrition, hypoxia, heat exposure) influence these psychological outcome measures. Perceptual results indicated greater post-exercise RPE, thermal sensation, and thirst sensation following hypohydrated trials (HYH and HYT) compared to their respective euhydrated trials (EUH and EUT). These adverse perceptual effects are in line with the current literature (13), and further confirm the need for strict enforcement of proper hydration strategies prior to and during intense exercise, both in hot and temperate conditions.

This investigation was part of a larger study and is not without limitations. Subjects wore lightweight clothing (shorts and a T-shirt) rather than military, occupational, or athletic uniforms that cover a large amount of skin surface area. This lightweight clothing limits the external validity of our findings, yet simplifies the interpretation of our results given that a uniform is not a confounding variable. We hypothesize our findings underestimate the physiological and psychological strain experienced, in comparison to a situation in which individuals wear full uniforms limiting the surface area available for sweat evaporation. Future research should explore the effects of uniforms and the possible interaction effects between uniforms and load carrying on physiological and psychological strain. Second, load carrying was included in all conditions of this study. Our results are compared to similar, past studies examining core body temperature and heart rate responses to exercise in combinations of temperate, hot, euhydrated, and hypohydrated conditions without the addition of load carrying; however, to further understand the direct impact of an added load, future investigations should include a within-person analysis of load and no load carrying trials conducted under the same exercise protocol.
Mechanisms as to how load carrying increases physiological strain should be explored. For example, the increased skin surface area covered by the rucksack may have resulted in greater heat storage and reduced sweat evaporation potential.

**PRACTICAL APPLICATIONS**

This is the first study to quantify both the individual and combined, physiological and psychological effects of heat exposure and hypohydration during exercise while load carrying. Overall, this study demonstrated that individually, heat exposure and hypohydration had similar physiological effects during loaded exercise; however when combined, heat exposure and hypohydration, synergistically exacerbated physiological strain and mood disturbance. Our findings have implications on military, occupational, and athletic settings where load carrying in the heat occurs and varying hydration statuses are likely present. In these scenarios, it is imperative for command leadership, risk managers, coaches, strength and conditioning professionals, and medical professionals to consider the co-occurrence of multiple risk factors and ensure the utilization of appropriate proactive measures (e.g. hydration protocols, heat acclimatization, and adjusted work-to-rest ratios) to prevent heat illnesses. It is vital to the future improvement of safety guidelines and the dissemination of prevention strategies that advocates for military, occupational, and athletic safety raise awareness on the impact that load carrying, heat exposure, and hypohydration combined, can have on risk for exertional heat illnesses.
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Figure 1. A. Rectal temperature during exercise. B. Heart rate during exercise. Data presented as mean±SD. All significance levels set at p<0.05. *=EUT<HYT, EUH<HYH, EUT<EUH, and HYT<HYH. #=EUT<HYT, EUH<HYH, and HYT<HYH. δ=EUT<HYT and EUH<HYH. ϕ=EUH<HYH, EUT<EUH, and HYT<HYH. EUT, euhydrated temperate; HYT, hypohydrated temperate; EUH, euhydrated hot; HYH, hypohydrated hot

Figure 2. A. Rectal temperature increase per 1% BML. B. Heart rate increase per 1% BML. Hypohydrated conditions were compared to their respective euhydrated conditions (Temperate = HYT to EUT; Hot = EUH to HYH). Data presented as mean±SD. *Significantly greater than temperate conditions, p<0.05. BML, body mass loss

Figure 3. Perceptual data displayed as mean±SD. *Significantly greater than respective euhydrated conditions at the identical time point, p<0.05. EUT, euhydrated temperate; HYT, hypohydrated temperate; EUH, euhydrated hot; HYH, hypohydrated hot
TABLE 1. Hydration Indices Across Conditions

<table>
<thead>
<tr>
<th></th>
<th>Body mass loss (%)</th>
<th>Urine specific gravity</th>
<th>Urine osmolality (mOsm·kg⁻¹)</th>
<th>Urine color</th>
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<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td><strong>EUT</strong></td>
<td>-0.1±0.9</td>
<td>-0.5±0.9</td>
<td>1.013±0.007</td>
<td>1.009±0.005</td>
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<td>-2.6±1.3*</td>
<td>-3.8±1.2*</td>
<td>1.026±0.003*</td>
<td>1.029±0.002*</td>
</tr>
<tr>
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<td>-0.3±0.6</td>
<td>-1.3±0.9</td>
<td>1.014±0.008</td>
<td>1.014±0.008</td>
</tr>
<tr>
<td><strong>HYH</strong></td>
<td>-2.7±1.5*</td>
<td>-5.7±1.6*</td>
<td>1.026±0.003*</td>
<td>1.029±0.002*</td>
</tr>
</tbody>
</table>

EUT, euhydrated, temperate; HYT, hypohydrated, temperate; EUH, euhydrated, hot; HYH, hypohydrated, hot
Body mass loss calculated as Pre: (pre exercise – baseline) and Post: (post exercise – baseline)
Data shown as mean±SD. *Statistically higher than EUT and EUH, p<0.05. δStatistically higher than HYT, p<0.05.
TABLE 2. Profile of Mood States

<table>
<thead>
<tr>
<th></th>
<th>Tension-anxiety</th>
<th>Depression-dejection</th>
<th>Anger-hostility</th>
<th>Vigor-activity</th>
<th>Fatigue</th>
<th>Confusion-bewilderment</th>
<th>Total mood disturbance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EUT</strong></td>
<td>1±3</td>
<td>-1±2</td>
<td>1±1</td>
<td>0±4</td>
<td>3±3*</td>
<td>0±2*</td>
<td>3±10*</td>
</tr>
<tr>
<td><strong>HYT</strong></td>
<td>-1±4</td>
<td>1±3*</td>
<td>0±3</td>
<td>0±5*</td>
<td>4±5*</td>
<td>0±3*</td>
<td>3±16*</td>
</tr>
<tr>
<td><strong>EUH</strong></td>
<td>2±5</td>
<td>3±7</td>
<td>2±6</td>
<td>4±10</td>
<td>3±13</td>
<td>1±4</td>
<td>16±26*</td>
</tr>
<tr>
<td><strong>HYH</strong></td>
<td>2±5</td>
<td>6±7</td>
<td>3±4</td>
<td>5±6</td>
<td>13±4</td>
<td>5±5</td>
<td>35±21</td>
</tr>
</tbody>
</table>

EUT, euhydrated, temperate; HYT, hypohydrated, temperate; EUH, euhydrated, hot; HYH, hypohydrated, hot
Data shown as mean±SD. * Statistically lower than HYH condition, p<0.05
A. $\Delta$ Rectal Temperature per 1% BML

B. $\Delta$ Heart Rate per 1% BML