6-21-2016

Rehydration on Subsequent Performance and Recovery Following Exercise-Induced Dehydration: Ad Libitum Versus Prescribed Fluid Replacement

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Maintaining an appropriate level of hydration has been shown to optimize performance and enhance safety during exercise in the heat. However, limited research exists examining the influence of exercise-induced dehydration on quality of sleep and measures of aerobic capacity and neuromuscular control. The purpose of this investigation sought to examine quality of sleep, aerobic capacity and neuromuscular control following prolonged exercise in the heat. Eleven participants (mean±SD; age, 22±3y; height, 178±6cm; VO\textsubscript{2max}, 54.3±5.4ml•kg\(^{-1}\)•min\(^{-1}\); body fat, 11.6±3.9%) completed three exercise sessions in a climate-controlled chamber (ambient temperature, 35.3±0.6°C, relative humidity, 31.3±2.0%). Exercise consisted of 3 hours of exercise on a motorized treadmill followed by 60 minutes of passive rest in a climate-controlled chamber. Neuromuscular control and hematologic hydration measures were assessed before and after each exercise session. Objective and subjective sleep measures along with 24-hour urine were collected the night before and after the exercise session. Participants also arrived to the laboratory 24-30 hours the following day for assessment of hydration status via urinary and hematologic measures and measurements of aerobic capacity and neuromuscular control. Trials consisted of: 1) Participants arrived euhydrated and minimized their fluid losses during exercise and recovery (EUR), 2) Participants arrived euhydrated and progressively dehydrated during exercise and recovery (EUD), and 3) Participants arrived hypohydrated and progressively dehydrated during exercise and recovery (HYD). At the end of exercise, the level of hypohydration was 0.4±1.0%, 3.8±1.2% and 5.6±0.6% for EUR, EUD and HYD respectively. There were no differences in hydration status as
measured by changes in body mass, urinary and hematologic markers between trials or rehydration groups the day following the exercise session of each trial. There were no differences between trial or rehydration group on objective or subjective measures of sleep; however, urine volume was predictive of improved subjective ratings of sleep. Additionally, there were no differences in measures of aerobic capacity or neuromuscular control between trials or rehydration groups the day following each exercise session. Moderate intensity exercise in the heat eliciting graded levels of hypohydration does not seem to adversely affect quality of sleep or neuromuscular control when assessed 24 hours following exercise.
Rehydration on Subsequent Performance and Recovery Following Exercise-Induced Dehydration: Ad Libitum Versus Prescribed Fluid Replacement

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B.S., University of Wisconsin-Madison, 2009
M.S., University of Connecticut, 2012

A Dissertation
Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy at the University of Connecticut

2016
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APPROVAL PAGE

Doctor of Philosophy Dissertation

Rehydration on Subsequent Performance and Recovery Following Exercise-Induced Dehydration: Ad Libitum Versus Prescribed Fluid Replacement

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2016
ACKNOWLEDGEMENTS

Dr. Casa: Dr. Casa, I want to give you my most sincere thanks and appreciation for your mentorship and your contributions to my success over the past 4 years. Your passion for the profession of Athletic Training and establishing ways to keep the athlete, soldier, and laborer safe during their every day lives is truly inspiring. Your selfless dedication to your students and helping them succeed is what makes you the leader that you are. I want to thank you for all the opportunities that you have provided me throughout these past 4 years and I cannot wait to see where the current work we are doing together leads.

Dr. Armstrong: Dr. Armstrong, the opportunity to have you as a professor during my 6-year tenure at UCONN has been a tremendous experience. I am honored to have had the opportunity to have you as an educator and a member on my dissertation committee. Your expertise on thermal physiology and hydration has not only made me a better scholar and researcher, but you have had allowed me to have a greater appreciation on the careful and thoughtful process needed to excel in research and academia.

Dr. DiStefano: Dr. DiStefano, your guidance and mentorship over these past four years at UCONN is something that I am very thankful for. Your passion for teaching and research has been truly inspiring for me and I am grateful for having had the opportunity to learn from you on ways to become a better educator and research. Your ability to structure your classes around the needs of the students to enhance their learning has provided me with ways to become a better educator myself. I look forward to continuing to work together in the future.
Dr. Lee: Dr. Lee, your knowledge and passion for research and teaching are something that I aspire to have as my career continues. Your willingness to educate students, whether it is a new skill in the lab or a new concept is something that I will always be appreciative of. Taking the time out of your day to answer my questions or to provide me advice during my time at UCONN is something that I am truly grateful for. Thank you for helping me become a better researcher and I look forward to being able work with you on future projects.

Classmates and Colleagues: To my fellow classmates, colleagues and friends, especially Yuri, Rob, Lesley, and Becca. I want to thank all of you for everything that you have done for me. You have always been there, through the ups and the downs and that is something that I will always hold dear to me. Your willingness to lend a helping hand, provide another set of eyes and thoughts, and challenging me to become a better has made me what I am today. I am looking forward to seeing where our friendships and future collaborations will go.

Family: To my parents and sister, your continued love and support has been amazing and I cannot thank you enough for everything that you have done for me. Being able to get my PhD is a testament to your love and support for me through everything. I cannot thank you enough for being the parents and sister that you are.

My Wife: To my wife Ami, your unwavering and unconditional love and support throughout the past 5 years has been something that I cannot put into words. You did everything in your power to help and support me in any way that you could and I would not be here today if it were not for you. I thank you with all that I am and I cannot wait to see where our future lies.
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Chapter 1: Review of the Literature

Optimizing athletic performance and reducing risk of injury is important for individuals competing in all sport, especially when they are confronted with external stresses (i.e., exercising in hot environmental conditions) that cause performance deficits or increase the risk of injuries such as exertional heat stroke. Hydration plays a vital role in maintaining normal physiologic function and is essential for sustaining life. During exercise, hydration plays a large role in optimizing performance and safety, especially when exercise occurs in hot environmental conditions. While there is an abundance of literature explaining the role of hydration during and after exercise in the heat and its effects on performance and safety risks, less is known on how hydration may affect other factors such as sleep and neuromuscular control. Therefore, the purpose of this review is to provide an overview of hydration strategies during exercise in the heat to optimize physiologic function, performance, and enhancing safety. It will also discuss the limited literature surrounding the effects of hydration on neuromuscular control and factors that may confound results related to this topic. Lastly, this review will provide an overview on sleep with a discussion of the effects of reduced sleep on exercise performance and physiologic strain. It will then transition to discuss potential areas of future research to examine how physiologic stress may enhance or degrade sleep quality.

Hydration on Thermoregulatory and Cardiovascular Function

In an average human, total body water content makes up 50‐70% of total body weight, where the variability in total body water is attributed to variation in body composition. Furthermore, lean body mass and fat body mass is made up of 73% and ~10% of water. Within the body, total body water is compartmentalized within the intracellular space (65%) and
extracellular space (35%), with the extracellular space being comprised of both plasma and interstitial spaces (174).

During exercise, the increase metabolically produced heat must be dissipated to avoid an uncompensable rise in body temperature. Heat dissipation relies heavily on the environmental conditions in which one is exercising, with a greater reliance on evaporation of sweat from the skin as environmental conditions become more extreme (increase in ambient temperature and relative humidity) (Figure 1.1) (149, 207). As the onset of sweat occurs, there is a loss of total body water, with overall sweat rates dependent upon environmental conditions, clothing, fitness status and acclimatization status of exercising individuals (4, 18, 23, 89, 95, 138, 146, 152, 190, 194).

Figure 1.1. Approximation of hourly sweat rates for runners in varying environmental conditions (174).
As total body water decreases as a result of dehydration, there are losses of body water within both the intra- and extracellular spaces, exacerbating physiologic strain on the body (45, 48, 80, 82, 132, 136, 139, 168, 172–175). As plasma volume decreases as a result of body water losses, there is an increased strain on the cardiovascular system; heart rate increases as stroke volume decreases to maintain cardiac output. As exercise continues and body water losses increase, the body is unable to maintain cardiac output, and thus cardiac output is decreased, having implications on exercise performance (Figure 1.2) (45, 136, 139).

Furthermore, during exercise, the various systems in the body compete for blood flow; the cardiovascular system to maintain cardiac output, the working muscles for power production needed to sustain exercise, and the skin for the dissipation of metabolically produced heat. As levels of dehydration increase, reduction of plasma and blood volume exerts additional strain on the body during exercise, which has adverse effects from a performance and safety standpoint.

Evidence has found that for every 1% loss of body mass from water loss, heart rate increases 3-5 beats•min\(^{-1}\) (2, 33, 115, 136, 172). Although this small increase in heart rate for every 1% dehydration seems minute, the implications on performance are large. For example, exercising heart rate coincides with a set intensity during exercise. If someone is 3% hypohydrated during competition, their heart rate could be 10-15 beats•min\(^{-1}\) higher. Depending on the duration of exercise, the individual would either have to reduce their intensity to maintain normal exercising heart rate or they risk being unable to complete exercise due to fatigue/volitional exhaustion from being unable to maintain their normal exercise intensity with an increased heart rate. Evidence has supported this notion in that during variable intensity
exercise such as a time trial, overall completion time is slower when hypohydrated versus euhydrated, despite similar heart rate responses (33, 76, 184, 211).

![Cardiovascular responses to dehydration during prolonged endurance exercise](image)

Figure 1.2. Cardiovascular responses to dehydration during prolonged endurance exercise (45).

In addition to cardiovascular strain resulting from dehydration, there is a subsequent increase in thermoregulatory strain, which inflates the rise in body temperature during exercise, especially in hot environmental conditions (28, 90, 136, 172, 214). As plasma volume decreases there is a corresponding decrease in sweat rate. As sweat rate decreases, the evaporative capacity for dissipating heat decreases, thus increasing heat storage within the body (138, 207).

Previous findings in a controlled laboratory have found that body temperature increases during exercise as the magnitude of dehydration is increased, despite the same exercise intensity (80, 136, 172). Similar increases in rectal temperature have also been found in field-based studies, with Casa et al., showing that during a maximal and submaximal intensity trail running race, rectal temperature was significantly elevated in the hypohydrated group compared to the euhydrated group (33).

Similar to the inflation of heart rate for every 1% loss in body mass from dehydration (2), body temperature has been shown to increase \(\sim 0.22^\circ\text{C}\) for every 1% dehydration. To put this into
context, for an athlete exercising at a level of 3% hypohydration, body temperature is roughly 0.66°C at a given intensity, which could increase the risk of exertional heat illness depending on exercising intensity.

**Hydration on Exercise Performance**

Dehydration during exercise, especially in the heat, dehydration decreases exercise performance; in particular, endurance performance, aerobic performance, strength, power, cognition, and mood are all adversely affected. Table 1.1 depicts the physiologic responses to exercise in the heat and the corresponding response to overall performance. While performance during exercise in the heat can be approved through mechanisms such as heat acclimatization, any performance benefits are negated in competing in the heat while hypohydrated. In conjunction, hypohydration can also cause performance deficits in cool/neutral environments, however, these deficits are exacerbated when environmental conditions become more extreme (Table 1.2) (46).

Table 1.1 Physiological and performance related responses to exercise in the heat.

<table>
<thead>
<tr>
<th>physiological response</th>
<th>Exercise in the Heat</th>
<th>Exercise in the Heat (Heat Acclimatized)</th>
<th>Exercise in the Heat (Hypohydrated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exercising Heart Rate</td>
<td>↑</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>Sweat Rate</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Rise of Core Temperature</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Skin Blood Flow</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Plasma Volume</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Cardiac Output</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Overall Exercise Performance</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
</tr>
</tbody>
</table>


Table 1.2. Response to hypohydration in both cool and neutral environments (46)
Examining the influence of hypohydration on exercise performance, the most adverse effects occur during prolonged aerobic exercise. Evidence has shown that endurance performance can be impaired between 7% (21) and 29% (60) when people are dehydrated to levels of ~2% of their body mass. Contributing factors of this performance decrement are increased cardiovascular strain, increased thermoregulatory strain, altered central nervous system dysfunction and altered mental function (38).

Anaerobic performance has been shown to be impaired during both single and repeated bouts of anaerobic exercise with moderate levels of dehydration (87). However, during anaerobic exercise lasting less than 30 seconds, there seems to be no effect of hypohydration on performance (107). It is difficult to interpret the results of the effects of hydration on anaerobic performance due to both exacerbating (caloric restriction, increased muscle temperature and fatigue) and masking (endurance training, test type, and menstrual status) factors within the methodological protocols, making it challenging to isolate the effects of dehydration on anaerobic performance alone (101). Despite these factors, the studies that have accurately assess anaerobic muscular endurance(25, 26, 34, 87, 195), dehydration on a magnitude of 3-4% of body mass reduces anaerobic muscular endurance by an estimate of 10%.

In addition, to aerobic and anaerobic performance, muscle strength and muscle power have also exhibit decrements in force production with increasing levels of hypohydration. Confounded by similar exacerbating and masking factors as anaerobic performance, it is difficult
to fully isolate the effects of just dehydration on these measures of performance. However, dehydration on a magnitude of 3-4% of body mass losses can reduce muscular strength (25–27, 87, 176, 201) and muscular power (72, 101, 140, 148, 216) by 2% and 3% respectively.

Cognition and mood are also adversely affected by increasing levels of dehydration. Ganio et al. (74) and Armstrong et al. (13) both found that mild levels of hypohydration (1.5-2%) of body mass loss elicit a disturbance in overall mood in both males and females respectively. Hypohydration can also play an additive factor for increasing total mood disturbance when other factors such as environmental stress, sleep loss, increased workload and impaired nutritional status are involved (134, 143). Furthermore, cognitive function has been shown to become impaired with hypohydration (22, 64, 110, 112, 206). Evidence suggests that dehydration on a magnitude of 1-2% can cause cognitive impairments (49, 84, 110). Aspects of cognitive function such as concentration, alertness, short-term memory, perceptual discrimination, visuomotor and psychomotor skills have been shown to be altered with increasing levels of dehydration in adults as well as in children (42, 43, 51, 61, 62, 67, 83, 165, 185).

**Hydration on Neuromuscular Control**

Maintenance of neuromuscular control relies upon the input of the visual, somatosensory and vestibular systems. Alterations in any of these systems can alter postural stability and controlled movement. Fatigue has been shown to impair neuromuscular control by altering the proprioceptive and kinesthetic properties of the joints in the body (19, 119, 135, 215). Alterations in neuromuscular control such as poor movement technique (94) and poor balance (129) have been associated as primary risk factors for lower extremity injuries which are common in sport and physical activity. While fatigue has been associated with alterations in movement technique
which may increase the risk of lower extremity injury, other secondary factors such as hypohydration may play a contributing role.

Hypohydration has been associated as a contributing factor to both occupational (189, 205) and military (103) related accidents. It is postulated that hypohydration may play a role in these work site related accidents by altering postural stability through mechanisms such as degradation of orthostatic tolerance (5, 20, 30) alteration of baroreceptor responsiveness (36) and reduction of brain blood flow (30). Prior research (52, 55, 64, 77, 155, 177) examining the influence of hypohydration on postural stability and movement technique have conflicting results. Derave et al. (52) and Gauchard et al. (77) both found impairments in postural stability following hypohydration and exercise while Patel et al. (155) did not. Confounding these results is the fact that these authors did not control for factors such as hyperthermia and fatigue, which could play a role in impairing neuromuscular control.

DiStefano et al.,(55) was the first to isolate the effects of hyperthermia, hypohydration and fatigue on movement technique and postural stability. Their findings found that hypohydration alone had not effect on movement technique or postural control, however, a combination of hyperthermia and hypohydration impaired neuromuscular control. Furthermore, Seay et al. (177), also found no effect of hypohydration following 12 hours of recovery after exercise and diuretic induced hypohydration.

**Hydration Strategies for Exercise in the Heat**

Maintaining an appropriate level of hydration during exercise can not only attenuate physiologic strain, especially during exercise in the heat, but also optimize performance. General consensus(31, 124, 164, 169, 181, 192) recommends that prior to exercise, particularly, exercise in the heat, individuals should start exercise in a state of euhydration. Additionally, during
exercise, exercising persons should attempt to minimize fluid losses to prevent losses exceeding 2% body mass losses, the level in which performance deficits being to arise. Following exercise, it is essential to replace remaining fluid and electrolyte losses to return to a state of euhydration prior to the next bout of exercise.

Fluid needs are highly individualized and rely on one’s sweat rate, exercise intensity, body mass, environmental conditions, among others (29, 31, 46, 144, 169, 182, 212). During exercise, it is not uncommon for individuals to become progressively dehydrated, despite consumption of fluid. This process, termed “involuntary dehydration,” is related to the diminished drive to consume fluids during exercise based on perceptual feelings of thirst (70, 85, 130). In addition, in those with high sweat rates, the inability to replace fluid at the same rate at which it is lost is related to rate of gut absorption, which is a rate ~1.2 liters per hour.

During exercise, establishing individualized hydration strategies based on one’s sweat rate may mitigate physiologic strain and optimize performance. Dugas et al. (58) found that ad libitum (free living) consumption of fluid is approximately 66% of actual needs, a level in which they found impaired performance when examining pace. They did not find any differences in thermoregulatory capacity, but that might be attributed to the reduction exercise intensity. Other research (17, 24) has also shown that ad libitum fluid replacement is insufficient to replace fluid losses during exercise as well as decrease performance.

Following exercise it in essential to replace 100% of remaining fluid losses following exercise to prevent progressive dehydration over time. It is recommended that individuals follow a prescribed rehydration plan by replacing 150% of post exercise fluid losses to return back to baseline (pre-exercise) total body water levels (169). This is to account for the diuresis that occurs with fluid consumption following exercise. Evidence also suggests that following
exercise, especially prolonged exercise in the heat, rehydration with fluids that contain macro and micronutrients may enhance fluid retention and maximize the ability for a person to fully rehydrate (66, 125, 128, 181, 182).

It has been shown that ad libitum replacement is insufficient to replace fluid losses following exercise and when tracked for a number of consecutive days, progressive dehydration occurs which can adversely affect performance and increase risk (79, 200). Furthermore, it is postulated that the drive to consume fluid losses is minimized despite dehydration, thus causing the insufficient fluid replacement following exercise (65, 133). While existing literature has shown that using a prescribed rehydration plan is sufficient for replacing fluid losses, little evidence exists to show the ability for one to rehydrate ad libitum over the course of a 24-hour period, which may be the time before the next bout of exercise, prompting further investigation into this perspective. Further, it is also unknown as to how ad libitum fluid replacement replaces all fluid losses when the magnitude of hypohydration increases.

**Implications of Sleep Deprivation: Acute and Long Term Effects**

Sleep is essential for restoration of daily stressors within the body. Loss of sleep or sleep deprivation has both acute and long term consequences on both health and performance (8, 9, 69, 100, 104, 109, 112, 117, 121, 159, 193, 204, 208). In terms of sleep and exercise, it has been shown that adequate sleep optimizes and enhances performance (56, 121, 199) and likewise, exercise has direct benefits on sleep quality (56, 108, 217). However, the benefits of exercise on sleep and sleep quality depend on the intensity of exercise, especially when exercise is performed later in the day; exercise of moderate intensity has shown to benefit sleep (71, 150, 202, 217), whereas exhaustive, high-intensity exercise may disrupt sleep by disrupting the rapid eye movement stage of sleep (57, 104).
While there is evidence to support the effects of exercise on sleep and vice versa, there is little evidence showing the full extent on the intensity of exercise or exercise training on sleep (68, 104). Furthermore, very little evidence has examined the influence of exercise-induced hypohydration and hyperthermia on sleep. It can be postulated that hypohydration or hyperthermia may adversely affect sleep by the subsequent rises of cortisol during these stressors and the role of increased cortisol on disruptive sleep (16, 41, 99, 102, 188, 203). Further research is needed to identify the role of exercise-induced hyperthermia and hypohydration on sleep disruption and sleep quality.

References


51. Flausino NH, Da Silva Prado JM, de Queiroz SS, Tufik S, de Mello MT. Physical exercise performed before bedtime improves the sleep pattern of healthy young good sleepers. *Psychophysiology* 2012;49(2):186–92.


89. Mears SA, Watson P, Shirreffs SM. Thirst responses following high intensity intermittent exercise when access to ad libitum water intake was permitted, not permitted or delayed. *Physiol Behav* 2016;157:47–54.
90. Meyerhoff J, Hebert M, Huhman K, Mougey E, Oleshansky M, Potegal M. Operational stress and combat stress reaction: Neurobiological approaches toward improving assessment of


96. Moore HG, Galloway JL. *We Were Soldiers Once...and Young: Ia Drang - the Battle That Changed the War in Vietnam*. Random House Digital, Inc.; 2004.487


Chapter 2: Influence of prescribed versus ad libitum fluid replacement on subjective and objective measures of sleep quality following exercise-induced dehydration
ABSTRACT

**Purpose:** The purpose of this study was to compare ad libitum versus prescribed fluid replacement on quality of sleep following exercise-induced dehydration. We also sought to identify variables predictive of subjective sleep quality.

**Methods:** Eleven healthy, recreationally active males (mean±SD; age, 22±3y; height, 178±6cm; VO\(_{2}\text{max}\), 54.3±5.4ml•kg\(^{-1}\)•min\(^{-1}\); body fat, 11.6±3.9%) completed three randomized exercise sessions: euhydrated + fluid replacement (EUR), euhydrated + no fluid (EUD) and hypohydrated + no fluid (HYD) in hot conditions (ambient temperature, 35.3±0.6°C and relative humidity, 31.3±2.0%). Participants donned a wrist-worn activity tracker the night prior (PRE) and following (FOLLOW) each session to quantify objective measures of sleep. Subjective assessment of sleep using the Karolinska Sleep Diary (KSD) was measured at PRE and FOLLOW. Exercise consisted of 6-cycles of treadmill exercise (8min at 40% VO\(_{2}\text{max}\), 8min at 60% VO\(_{2}\text{max}\), 8min at 40% VO\(_{2}\text{max}\) and 6min of passive rest) for up to 3h. Following exercise, participants were randomly assigned to either a prescribed or ad libitum rehydration group and returned to the laboratory 24-30h following each exercise session for follow up sleep measures. A mixed design (condition x trial x time) repeated measures ANOVAs with a Tukey post hoc test was used to evaluate differences in continuous dependent variables. Stepwise linear regression was used to identify variables predictive of subjective sleep quality.

**Result:** Sleep quality was not influenced by the magnitude of hypohydration or mode of fluid replacement following exercise-induced dehydration (p>0.05). A greater 24h urine volume the night prior to exercise (odds ratio: 6.07, 95%CI [1.06, 34.65], p=0.04) significantly predicted subjective sleep quality to be greater than median baseline values. Increasing volumes of 24-hour
urine was a significant predictor of greater ratings for overall subjective quality of sleep (KSD_{TOTAL}) at PRE ($r^2 0.27$, $p=0.002$) and FOLLOW ($r^2 0.22$, $p=0.007$).

**Conclusions:** The magnitude of hypohydration or mode of rehydration follow prolonged exercise in the heat did not influence subjective or objective measures of sleep quality. These findings suggest that moderate intensity exercise in the heat eliciting hypohydration up to 5% body mass loss does not adversely affect the subsequent night’s sleep.
INTRODUCTION

Sleep is essential for maintaining normal physiologic function and is vital for maintaining homeostasis related to energy metabolism (105), neural plasticity, hormonal responses (16), and immune function (161). Furthermore, sleep loss and/or deprivation has been shown to have adverse effects on cognitive function (113, 204, 209), thermoregulation (53, 171, 193), and exercise performance (120–122, 151, 159). Exercise has been shown to have positive contributions to overall sleep quality (57, 198, 202, 217), but depending on time of day (198) or intensity (57, 104) overall quality could be disturbed. While exercise may promote improved quality of sleep, anecdotal reports suggest that exercise-induced hypohydration may disrupt sleep quality; however, no known literature provides support or rationale to justify the degree of body water deficit as a contributing factor to perceived sleep quality.

Maintaining an appropriate level of hydration during exercise is essential for optimizing safety (11, 32, 96, 137, 167) and performance (12, 33, 37, 39, 116, 184), especially during exercise in hot environmental conditions. Increasing levels of dehydration exacerbates both thermoregulatory (97, 137, 172) and cardiovascular strain (2, 45, 47, 136, 137), which markedly impairs performance and increases the risk of exertional heat illness. Furthermore, evidence supports that increasing body water losses cause both cognitive deficits (22, 111) and increased mood disturbance (14, 64, 75), even when levels of hypohydration are mild (1.5-2% body mass loss). Establishing individualized hydration strategies is important to mitigate performance deficits or safety concerns.

Current consensus recommends that individuals begin physical activity in a state of euhydration, minimize fluid losses during activity to keep fluid losses from exceeding 2% of body mass, and replace the remaining losses following exercise (125–127). Outside of exercise
and physical activity, fluid homeostasis is normally maintained and is controlled day-to-day by ad libitum consumption of foods and fluids via endocrine and renal responses which respond to both volume and tonicity changes within the body (39, 40). In stressful situations, such as exercise, the drive to consume fluids (ad libitum consumption) to match the rate in which fluid losses occur is insufficient (involuntary dehydration) and has been well established within the literature (4, 6, 15, 17, 24, 59, 85, 86). Furthermore, voluntary consumption of fluid following exercise-induced dehydration has been shown to be insufficient in replacing all fluid losses (78, 79), prompting the recommendation to have a prescribed plan based on individual fluid losses to restore fluid balance (164, 170, 213).

While evidence shows that sleep deprivation can have adverse effects on physiological function and performance during exercise, there is no known evidence that has examined how quality of sleep is affected by graded exercise-induced dehydration. Furthermore, little is known surrounding how rehydration strategies affect sleep following prolonged exercise. Therefore, the purpose of this study was to compare ad libitum versus prescribed fluid replacement on quality of sleep following exercise-induced dehydration. It is also the purpose of this study to determine if physiologic and hydration status markers were predictive of subjective quality of sleep prior to and following prolonged exercise in the heat. It is hypothesized that individuals replacing fluids ad libitum or those presenting in a state of hypohydration will present with poor quality of sleep compared to those given a prescribed rehydration plan.

METHODS

Design

This study utilized a counter-balanced, crossover design in which participants were randomly assigned to each exercise session. The exercise sessions varied in respect to the
participant’s hydration status upon arrival and during exercise and included: 1). Arrive euhydrated, minimize fluid losses during exercise and post exercise recovery (EUR), 2). Arrive euhydrated, progressively dehydrated during exercise and post exercise recovery (EUD), and 3). Arrive hypohydrated, progressively dehydrate during exercise and post exercise recovery (HYD). Following the exercise session participants were randomly allocated to either a prescribed or ad libitum rehydration group. The prescribed rehydration group received instruction to consume fluids matching 150% of their fluid losses (170) whereas the ad libitum rehydration group was permitted to consume fluids as those chose. All sessions were separated by at least 5 d to allow for participants to fully recovery before the next testing session. All sessions took place in a climate-controlled chamber (Model 2000; Minus-Eleven Inc., Malden, MA) with conditions set ambient temperature, 35.3±0.6°C and relative humidity, 31.3±2.0%.

Participants

Eleven healthy, recreationally active males (mean±SD; age, 22±3y; height, 178±6cm; VO2max, 54.3±5.4ml•kg\(^{-1}\)•min\(^{-1}\); body fat, 11.6±3.9%) volunteered to participate in this study. All participants reported participating in exercise at least 4-5 days per week for a minimum of 30 minutes each day. Participants were excluded if they were observed to have one of the following: 1) Fever or illness at the time of testing, 2) History of cardiovascular, metabolic, or respiratory disease, 3) Current musculoskeletal injury that limits their level of physical activity, and 4) Consumption of >3 alcoholic beverages per day (>21 per week). Participants provided written and informed consent to participate in the study, which was approved by the University’s Institutional Review Board, prior to beginning any aspect of the study.

PROCEDURES

Familiarization Sessions
Prior to the three exercise sessions, participants reported to the laboratory for a familiarization session that contained two stages. The first stage was a single visit to the laboratory to allow participants to become acquainted with the testing session procedures (rectal temperature ($T_{REC}$), heart rate (HR), objective and subjective sleep assessments and exercise protocol), assessment of cardiovascular fitness through a VO$_{2\text{max}}$ test, sweat rate and sweat electrolyte concentration assessment. The second stage required participants to arrive to the laboratory for three consecutive days to obtain an accurate baseline hydration status.

Upon arrival to the laboratory for the first stage of the familiarization session, participants provided a urine sample to assess hydration status and then performed a VO$_{2\text{max}}$ test on a motorized treadmill (NordicTrack, Icon Health & Fitness, Logan, UT) to ensure they meet the eligibility criteria (>45mL•kg$^{-1}$•min$^{-1}$) for inclusion into the study. Oxygen uptake (VO$_2$) and related gas exchange was measured using open circuit spirometry using a PARVO Medics TrueOne 2400 Metabolic Measurement System (Parvo Medics, Inc., Salt Lake City, UT). A finger stick was performed after the VO$_{2\text{max}}$ test to assess baseline levels of lactate (Lactate Plus, Nova Biomedical, Waltham, MA) at this time point. To ensure euhydration prior to the familiarization session, participants were asked to consume 500 mL of water prior to going to bed and again upon waking the next day prior to their arrival to the laboratory.

Following the VO$_{2\text{max}}$ test, participants provided a nude body mass (NBM), and then completed 30 minutes of exercise on a motorized treadmill (8 min, 40% VO$_{2\text{max}}$; 8 min, 60% VO$_{2\text{max}}$; 8 min, 40% VO$_{2\text{max}}$; 6 min, rest) in the climate-controlled chamber (ambient temperature, 35.3±0.6°C; relative humidity, 31.3±2.0%) that mimicked the exercise intensity of the exercise sessions to obtain an accurate sweat rate. After exercise, participant’s sweat electrolyte concentration was assessed using previously published methods(10). A final nude
body mass was taken to calculate percent body mass loss (%BML) during exercise to assess fluid needs during the testing sessions. Also, during this session, participants were instructed on the use of the environmental symptoms questionnaire (ESQ), fatigue, and the Karolinska Sleep Diary (KSD) questionnaires. They were also instructed on the proper method of filling out a 24-hour diet record that was utilized for the day before and following the exercise sessions. Last, they were instructed on the proper use of an activity tracker (Jawbone UP3, Jawbone, Inc., San Francisco, CA) that was used to assess the quality of sleep during the three-day baseline hydration days as well as the days surrounding each testing session.

The second stage of the familiarization session involved the participants to arrive to the laboratory for three consecutive mornings in a fasted state. They were instructed to collect all urine in a clean jug provided to them for a period of 24 hours prior to their arrival to the laboratory. Participants also recorded their 24-hour diet for each of the three days to gather an assessment of their normal nutritional intake. They were also instructed to wear the activity tracker the night before each session to measure quality of sleep. Upon arrival to the laboratory, participants returned the urine jug containing a 24-hour collection, the diet log containing a record of all foods and fluids consumed for the previous 24-hours and provided a nude body mass to the nearest 0.1 kg (Defender 5000, OHAUS, Parsippany, NY). Euhydration was defined as a urine specific gravity (USG) <1.020 (Model TS400; Reichert Inc., Depew, NY), urine osmolality (U_{OSM}) <500 mOsm•L^{-1} (freezing point depression, model 3320; Advanced Instruments, Norwood, MA) and a change in nude body mass <2% from day to day.

**Testing Sessions**

For the testing sessions where participants were to arrive euhydrated, they were asked to consume an additional 500 mL of water before going to sleep and upon waking the next morning
to ensure euhydration. For testing sessions with participants arriving hypohydrated, they were restricted from consuming fluids or water heavy foods for a period of 22 hours prior to the start of the trial. This was to ensure a level of hypohydration of ~1-2% of baseline body mass. To account for any potential confounding effects of time, participants were tested at the same time of day ± 1 hour.

Before each testing session, all participants collected their urine over a 24-hour period prior (24h Pre) to testing for subsequent urinalysis. Participants donned the activity tracker the night before each testing session to assess quality of sleep leading up to the session. Upon arrival to the laboratory, the following pre-exercise (PRE) measures were collected: NBM, USG, $U_{\text{OSM}}$, urine volume ($U_{\text{vol}}$).

Participants entered the climate-controlled chamber and took a seated position for 15 minutes to equilibrate to the environmental conditions. Prior to the start of exercise, participants provided a blood sample (PRE), completed the perceptual scales (KSD, ESQ, and Fatigue), and baseline measure of $T_{\text{REC}}$ and HR were measured. Participants began exercise, performing six 30-minute cycles of exercise on a motorized treadmill (8 min, 40% $V_{\text{O2max}}$; 8 min, 60% $V_{\text{O2max}}$; 8 min, 40% $V_{\text{O2max}}$; 6 min, rest). During exercise, measures of $T_{\text{REC}}$, HR, body mass with minimal clothing (BM) and Fatigue were measured. After completion of the exercise protocol, post exercise (POST) post measures of $T_{\text{REC}}$, HR, Fatigue and ESQ were taken.

During the post-exercise (RECOV) recovery period, participants rested for 60 minutes inside the climate-controlled chamber. Fluid replacement for EUR was a volume equal to up to 1% of their body mass losses based on the difference between pre and post BM. For EUD and HYD trial, participants were not provided fluid during the 60-minute recovery period. During post-exercise recovery, Fatigue was measured every 3 minutes while $T_{\text{rec}}$, HR, and ESQ were
measured every 10 minutes. Participants provided a third blood sample at the completion of the post-exercise recovery period while remaining inside the climate-controlled chamber.

Upon leaving the laboratory, participants were given a clean urine jug for 24 h urine collection, a diet record and the activity tracker to monitor their sleep. They were also given an instruction sheet to follow prior to the follow-up (FU) testing session based on the randomly assigned rehydration group: the ad libitum group received instructions on what to bring for the follow up session, while the prescribed group received specific instruction on how much fluid they need to consume based on the difference between PRE and RECOV NBM.

For EUR, EUD, and HYD, participants returned to the laboratory 24-30 hours after completion of the exercise session to complete follow-up (FOLLOW) testing. Upon arrival to the laboratory the following day, USG, U_{OSM}, NBM, Fatigue and KSD was measured. Following these measures participants provide a blood sample for analysis.

**Sleep Quality**

Sleep quality was assessed both objectively and subjectively. The activity tracker provided objective measures of resting heart rate (RHR), total time in bed, total time sleeping, and time spent in light, deep or REM sleep respectively. Sleep efficiency was calculated using the following equation:

\[
\text{Sleep Efficiency} \% = \left( \frac{\text{Time Asleep}}{\text{Time in Bed}} \right) \times 100
\]

To normalize the time spent in each stage of sleep, the time in minutes was converted to percentage of total sleeping time for all participants.

Subjective assessment of sleep quality was assessed using the KSD. The KSD is comprised of 7 five-point Likert scale questions anchored at either end (1 and 5) that asked the questions: “How did you sleep?” (Very Poorly—Very Well), “Feeling refreshed after
wakening?” (Not at all—Completely), “Calm sleep?” (Very restless—very calm), “Slept throughout the allotted time?” (Woke up much too early—Yes), “Ease of waking up?” (Very difficult—very easy), “Ease of falling asleep?” (Very difficult—very easy), and “Amount of Dreaming?” (None—Much). The KSD also asked the participant to input the total number of awakenings throughout the night. To gather an overall assessment of sleep quality from the KSD in addition to the individual Likert scale questions, a total sleep quality score was derived (KSD\text{TOTAL}) by assigning a point corresponding to each interval on the Likert scale (i.e., if the participant rated that it was very difficult falling asleep [Likert score of 1] they received 1 point for that question). Points were added up for each of the 7 questions (a total score of 35).

**Perceptual Measures**

Fatigue was measured using an 11-point likert scale that asked participants were asked “How fatigued do you feel right now?” and where then prompted to rate their perceived level of fatigue at that respective time point. The likert scale was anchored from 0 to 10 with the following word anchors associated with specific numbers: 0—no fatigue at all, 1—very small amount of fatigue, 2—small amount of fatigue, 3—moderately fatigued, 4—somewhat fatigued, 5—fatigued, 7—very fatigued, 9—extremely fatigued, 10—completely fatigued.

The environmental symptoms questionnaire (ESQ) is a validated scale assessing symptoms emanating from environmental stress\(^{(106, 166)}\). We utilized a modified version of the ESQ that has been previously validated to assess symptomology related to heat stress\(^{(183)}\). It consists of a 14-question assessment of symptoms related to environmental stress.

**Blood Collection Measures**

For each blood sampling time point (PRE, RECOV and FU), participants provided 10 mL of blood taken from an antecubital vein using a butterfly needle equipped with an extension tube
and hub (BD Vacutainer Safety-Lok Blood Collection Set, Becton Dickinson, Franklin Lakes, NJ). Blood samples were drawn into tubes pre-treated with lithium-heparin, and a serum tube (Vacutainer, Becton Dickinson, Franklin Lakes, NJ). The serum tube was placed in an upright position and allowed to clot prior to placing in the centrifuge. They were then centrifuged for 15 min at 3000 RPM at room temperature with the resulting plasma and serum aliquotted and stored at -80°C until analysis.

Plasma osmolality (\(P_{\text{OSMO}}\)) was measured in duplicate using freezing point depression (model 3320; Advanced Instruments, Norwood, MA). Percent changes in plasma volume (PV) were derived from measures of hematocrit (HCT) via the microhematocrit technique and hemoglobin (Hb) via a B-Hemoglobin photometer (HemoCue, Inc., Lake Forest, CA) using previously established calculations (54). Creatine kinase (CK) (Creatine-SL Assay, Sekisui Diagnostics, Lexington, MA) concentration was analyzed in duplicate using a plate reader (VersaMax, Molecular Devices, Sunnyville, CA) with absorbance being measured at 340 nm. The intra- and inter-assay coefficients of variation (CVs) were <5% for all analyses.

**Statistical Analysis**

All statistical analyses were performed using SPSS Statistical Software (v.21. IBM Corporation, Armonk, NY). All values are presented as mean ± standard deviation unless otherwise noted. A mixed design (condition x trial x time) repeated-measures ANOVA was used to measure differences in dependent variables (hydration and sleep measures) between rehydration conditions (Ad libitum and prescribed) and all trials (EUD, EUR, HYD) (Figure 2.1). Separate (trial x time) repeated-measures ANOVAs were used to evaluate hydration measures and sleep measures independent of the mode of rehydration. For statistically significant findings, Tukey’s post-hoc analysis was used to determine where differences lied between
factors. Data were also compared using mean differences (MD), 95% confidence intervals (95%CI) and effect size (ES) using Cohen’s d were used to determine small (0.2-0.49), medium (0.5-0.79) and large (>0.8) effects. Stepwise linear regression was used to determine if any hydration measure or physiologic measure (TREC, HR, CK) was predictive of overall subjective ratings of sleep (KSD\textsubscript{TOTAL}) before and following each exercise session. Pearson product correlations were used to assess the relationship between objective and subjective sleep measures to physiologic measures collected during the exercise session and follow up session. Significance was set \textit{a priori} at p<0.05.

**RESULTS**

There were no statistically significant differences in measures of hydration status or measures of sleep quality between ad libitum and prescribed rehydration groups at any time point (p>0.05); therefore, the data were pooled and only the trial x time data are reported below.

**Hydration Indices**

Table 2.1 depicts changes in body mass over the course of each exercise session and Table 2.2 depicts changes in physiologic measure of hydration status over the course of each exercise session. There were no differences between 3-day baseline body mass and 24h pre body mass for EUD (0.1±1.0%), EUR (-0.1±1.0%) and HYD (0.3±0.7%) (p>0.05). There was a significant trial x time interaction assessing %BML between trials across time (p<0.001). HYD exhibited significantly greater changes in %BML than EUR at PRE (MD±SD [95%CI]; 2.4±0.4% [1.4, 3.9], ES=2.8, p<0.001) and RECOV (MD±SD [95%CI]; 5.2±0.4% [4.1, 6.2], d=6.3 p<0.001). Likewise, HYD had significantly greater changes in %BML then EUD at PRE (MD±SD [95%CI]; 2.6±0.4% [1.6,3.7], d=2.8, p<0.001) and RECOV (MD±SD [95%CI]; 1.8±0.4% [0.8, 2.8], d=1.9, p<0.001). %BML in EUD was significantly greater than EUR at
RECOV (MD±SD [95%CI]; 3.4±0.4% [2.3, 4.4], d=3.1, p<0.001). There were no differences in %BML at FOLLOW between any of the trials (p>0.05).

There were no differences in $U_{\text{vols}}$, $U_{\text{osmo}}$, USG or $U_{\text{col}}$ at pre exercise and follow up time points between any trials (p>0.05). $P_{\text{osmo}}$ was significantly higher in HYD than EUR at PRE (MD±SD [95%CI]; 9±3 mOsm•kg$^{-1}$ [1, 16], ES=1.24, p=0.02) and RECOV (MD±SD [95%CI]; 22±3 mOsm•kg$^{-1}$ [15, 28], ES=3.22, p<0.001) time points (p<0.05). $P_{\text{osmo}}$ was significantly higher in HYD than EUD at PRE (MD±SD [95%CI]; 9±3 mOsm•kg$^{-1}$ [1, 16], ES=1.17, p=0.02). $P_{\text{osmo}}$ was significantly higher in EUD than EUR at RECOV (MD±SD [95%CI]; 17±3 mOsm•kg$^{-1}$ [10,23], ES=2.66, p<0.001)

**Fluid Consumption**

Fluid consumption prior to the start of the exercise session of EUR (4.4±2.3L) was significantly greater than HYD (1.6±2.2L) (p=0.01). There were no differences in fluid consumed between EUD and EUR (-1.2±0.8L [-3.3, 0.8], p=0.32) or EUD and HYD (1.6±0.8L [-0.4, 3.7], p=0.15) the 24 hours prior to each exercise session. There were no differences in fluid consumption the 24 hours following the exercise session, despite the differences in overall %BML at RECOV between conditions (p>0.05) (Figure 2.2).

**Sleep**

*Objective Measures.* There were no significant differences in sleep efficiency (Figure 2.3) (p=0.36), time spent in deep (Figure 2.4) (p=0.57), light (Figure 2.5) (p=0.95) or REM (Figure 2.6) (p=0.927) sleep between EUD, EUR or HYD at either PRE or FOLLOW. On average, for the night following the exercise session, sleep efficiency HYD was lower following HYD than EUR (MD±SD [95%CI]; -3.3±2.9% [-11.1, 4.35], ES=0.7, p=0.51) and EUD
However, these differences were not statistically significant.

**Subjective Measures.** There were no differences in perceived quality of sleep as measured by the individual components of the KSD between trials or time points \((p>0.05)\) (Table 2.3). Increasing volumes of 24-hour urine was a significant predictor of greater ratings for overall subjective quality of sleep \((\text{KSD}_{\text{TOTAL}})\) at PRE \((r^2=0.27, p=0.002)\) and FOLLOW \((r^2=0.22, p=0.007)\). There was an association between PRE KSD\(_{\text{TOTAL}}\) and PRE U\(_{\text{VOL}}\) \((r=0.521, r^2=0.27, p=0.002)\) (Figure 2.7). There was a significant relationship between FOLLOW KSD\(_{\text{TOTAL}}\) and the following variables: FOLLOW U\(_{\text{VOL}}\) \((r=0.469, r^2=0.22, p=0.007)\) (Figure 2.8), FOLLOW U\(_{\text{OSMO}}\) \((r=-0.371, r^2=0.14, p=0.037)\) (Figure 2.9), FOLLOW USG \((r=-0.395, r^2=0.16, p=0.025)\) (Figure 2.10) and FOLLOW U\(_{\text{COL}}\) \((r=-0.403, r^2=0.16, p=0.022)\) (Figure 2.11).

**Fatigue and Environmental Symptoms**

Beginning at minute 120 of exercise, perceptual ratings of fatigue were significantly greater in HYD than EUR \((p<0.05)\) (Figure 2.12). However, at FOLLOW, there were no significant differences in perceived fatigue between trials \((p>0.05)\). Environmental symptomology as measured by ESQ was significantly greater in HYD than EUR at \((\text{MD±SD [95%CI]}; 9.2±2.4 [3.2, 15.6], p=0.002)\) and RECOV \((\text{MD±SD [95%CI]}; 10.4±2.7 [3.7, 17.1], p=0.002)\). However, there were no differences in ESQ between HYD and EUD \((p>0.05)\) or EUD and EUR \((p>0.05)\) at any time point (Figure 2.13).

**Blood Measures**

There were no significant changes in levels of circulating CK between trials across time or between trials \((p>0.05)\) (Figure 2.14). There were no differences in levels of circulating lactate at PRE, RECOV, or FOLLOW between any trials \((p>0.05)\).
DISCUSSION

The purpose of this study was to compare ad libitum versus prescribed fluid replacement on quality of sleep following exercise-induced dehydration. To our knowledge, this is the first study to compare how hydration status following graded dehydration via prolonged exercise in hot conditions affects the following night’s sleep. Participants completed the exercise trials in a body water deficit of 0.4±1.0, 3.8±1.2 and 5.6±0.6% for EUR, EUD and HYD trials respectively. Upon assessment, during the FOLLOW session, body water returned to 0.2±1.0%, 0.6±1.0% and -0.4±1.2% of baseline for EUR, EUD and HYD respectively, with no differences in hydration status between the ad libitum or prescribed rehydration groups. Our findings surrounding sleep revealed that overall quality of sleep, as measured by both objective and subjective measures, was unaffected by the extent of exercise-induced dehydration and subsequent rehydration. It was observed that subjective sleep quality (KSD\text{TOTAL}) was associated with urinary hydration makers; subjective ratings of sleep quality improved as hydration status improved as assessed by 24-hour urine volume.

Adam (1) has proposed that sleep plays a restorative role on the body; specifically, increasing amount of time spent in slow-wave (deep) sleep during times of stress have been suggested to improved sleep quality and overall restoration of the body. It was observed in our study, that the time spent in deep sleep was not statistically different between PRE (17.7%, 22.8% and 24.3%) and FOLLOW (16.5%, 17.6% and 16.7%) for EUD, EUR and HYD respectively. This supports prior literature, which found that exercise does not influence the length of time spent in deep sleep, however, it is the level of fitness status of the individual (88, 141, 142, 145, 180, 196). Our participants were all fit individuals (VO\text{2max}, 54.3±5.4ml•kg\textsuperscript{-1}•min\textsuperscript{-1}) which may explain why no differences in time spent in deep sleep were observed despite
prolonged exercise. Similarly, the intensity of exercise (40-60% VO2max) may have been not enough to elicit changes in the time spent in deep sleep as previously shown (57, 179).

Conversely, other evidence shows that intense exercise or increased stress leads to an increase in disruptive sleep as observed by a decrease in sleep efficiency (68, 93, 104, 191). Our results showed that moderate intensity exercise inducing graded levels of hypohydration did not influence sleep efficiency during the subsequent night’s sleep, which provides evidence that the physiologic strain induced by hypohydration may not affect sleep. Our results are similar to other literature examining female swimmers over the course of the season; there were no differences in sleep quality when measured at various points in their training despite changes in intensity and volume of training (191).

Lastly, our study found significant relationships between overall subjective ratings of sleep and markers of hydration status. Subjective ratings of improved sleep quality were associated with more optimal levels of hydration status as measured by U_VOL, U_OSMO, USG and U_COL. More specifically, the relationship of improved ratings of sleep corresponded with increased 24-hour U_VOL and decreasing measures of U_OSMO, USG and U_COL. Our study was the first to look specifically at hydration status in comparison to quality of sleep. Other research (7, 91, 92, 104, 158) has examined nutritional interventions on sleep quality, and while the results are unclear, there appears to be an influence of caloric intake on ratings of sleep quality. While our study attempted to identify the role that hydration status plays on sleep quality, we did not control our participants diet over the course of the study, making it difficult to make any inferences into the true role of hydration on quality of sleep.

Developing a hydration strategy based on individualized fluid needs allows persons to minimize the extent of fluid losses during exercise and attenuate risk of performance deficits or
safety implications. During exercise, ad libitum replacement has been shown to be insufficient in minimizing fluid losses and lead to large increases in body mass loss, exacerbating physiologic strain (17, 59, 186). Furthermore, evidence from our laboratory (unpublished data, Adams, 2016 and Vandermark, 2016) and other published data (78, 79, 133) suggests that following exercise, ad libitum fluid replacement can be insufficient in maintaining returning to baseline levels of euhydration when compared to a prescribed rehydration place (66, 123, 125, 126).

The current study found no differences in the ability to return to baseline euhydration levels despite the various grades of hypohydration (0.4±1.0, 3.8±1.2 and 5.6±0.6%) achieved during the exercise session. A possible explanation as to why the participants in our study were able to successfully return to baseline levels of euhydration despite magnitude of hypohydration and mode of fluid replacement was their normal daily intake of fluid. On average, our participants consumed >3 liters of fluid daily based on their baseline measures, which exceed (63) or meet (98) current recommendations for daily fluid intake. Furthermore, some of our participants consumed >5 liters of fluid per day, which may have affected our results when examining ad libitum versus prescribed fluid replacement.

This study was not without limitations. The inability to measure electroencephalographic brain frequency, the gold standard for assessing objective sleep, may have limited our ability to observe if hypohydration adversely affected sleep (178). Although the activity tracker (Jawbone UP3) has been found to be a reliable measure of sleep, evidence has shown that wrist actigraphy overestimate values associated with sleep (sleep latency, time spent in sleep and time spent in sleep stages) (160, 197). This may be associated with the fact that these devices rely on the integrated accelerometers, bioimpedance and galvanic skin temperature changes to dictate sleep and wakefulness. In addition, on multiple occasions, the activity tracker was unable to record
data specific to our participant’s sleep; the participant either reported the device falling off the wrist during the night, the sensors lost contact with the skin (the device was too loose around the wrist), or non-compliance.

The limited sample size may be attributed to the large variability observed in some of the dependent variables. Furthermore, not controlling for exercise the day prior to the exercise session, the timing of exercise or the rate in which fluid was consumed during the prescribed fluid replacement group may have masked any differences that hydration played in overall quality of sleep. Future research should examine the timing of exercise-induced dehydration in relation to sleep quality to identify if hydration has adverse effects on subsequent sleep.

It can be concluded that following exercise-induced dehydration up to ~5% of body mass loss, individuals are able to return to baseline levels of hydration status using both an ad libitum or prescribed rehydration plan within 24 hours. In addition, individuals are more likely to rate their subjective quality of sleep as higher as hydration improved based on 24-hour urinary hydration markers following exercise-induced dehydration. Prior to exercise prolonged exercise, 24-hour urine volume and circulating levels of Creatine Kinase were predictive of improved subjective ratings of sleep quality when compared to baseline values.

REFERENCES


12. Arnaoutis G, Kavouras SA, Kotsis YP, Tsekouras YE, Makrillos M, Bardis CN. Ad libitum fluid intake does not prevent dehydration in suboptimally hydrated young soccer


26. EFSA. Scientific Opinion on dietary references values for water. *EFSA J* [date unknown];8:1459–506.


41. IOM. Dietary reference intakes for water, potassium, sodium, chloride, and sulfate. Washington, DC: [date unknown].


55. Mears SA, Watson P, Shirreffs SM. Thirst responses following high intensity intermittent exercise when access to ad libitum water intake was permitted, not permitted or delayed. Physiol Behav 2016;157:47–54.


Figure 2.1. Flow chart depicting random allocation to Ad Libitum and Prescribed rehydration groups following exercise.
Table 2.1. Changes in body mass between trials

<table>
<thead>
<tr>
<th>Trial</th>
<th>24h Pre</th>
<th>Pre-Exercise</th>
<th>Recovery</th>
<th>Follow Up</th>
</tr>
</thead>
<tbody>
<tr>
<td>EUD</td>
<td>73.4±9.9</td>
<td>74.1±10.4</td>
<td>70.7±9.9</td>
<td>73.9±10.1</td>
</tr>
<tr>
<td>%Change</td>
<td>-0.837±1.2</td>
<td>3.8±1.2Φ</td>
<td>-0.6±1.0</td>
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</tr>
<tr>
<td>EUR</td>
<td>73.4±10.3</td>
<td>73.9±10.4</td>
<td>73.1±10.4</td>
<td>73.5±10.2</td>
</tr>
<tr>
<td>%Change</td>
<td>-0.654±1.1</td>
<td>0.4±1.0</td>
<td>-0.2±0.9</td>
<td></td>
</tr>
<tr>
<td>HYD</td>
<td>73.6±9.8</td>
<td>72.3±10.1</td>
<td>69.6±10.9</td>
<td>73.3±10.1</td>
</tr>
<tr>
<td>%Change</td>
<td>1.8±0.6*</td>
<td>5.6±0.6*</td>
<td>0.4±1.2</td>
<td></td>
</tr>
</tbody>
</table>

The absolute and relative change (% change) in body mass as a result of fluid loss across time points. Negative values associated with % change correspond to a body water gain compared to the 24-hour pre-exercise measure. Positive values associated with % change correspond to a body water deficit compared to the 24-hour pre-exercise measure. EUR=Euhydrated arrival, fluid intake matches sweat losses during exercise, and fluid replacement during recovery up to 1% of body mass loss; EUD=Euhydrated arrival, progressive dehydration during exercise and recovery; HYD=Hypohydrated arrival, progressive dehydration during exercise and recovery. *=Significant difference when compared to EUD and EUR trials (p<0.05). Φ=Significant difference when compared to only EUR (p<0.05).
Table 2.2. Physiological variables measuring hydration status

<table>
<thead>
<tr>
<th>Variable</th>
<th>Condition</th>
<th>Pre Exercise</th>
<th>Recovery</th>
<th>Follow Up</th>
</tr>
</thead>
<tbody>
<tr>
<td>24h Urine Volume (L)</td>
<td>EUD</td>
<td>3.1±1.6</td>
<td>2.0±1.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EUR</td>
<td>3.0±1.5</td>
<td>2.2±1.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HYD</td>
<td>2.0±1.5</td>
<td>1.6±1.5</td>
<td></td>
</tr>
<tr>
<td>24h Urine Osmolality (mOsm•kg⁻¹)</td>
<td>EUD</td>
<td>404±176</td>
<td>634±270</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EUR</td>
<td>395±219</td>
<td>480±171</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HYD</td>
<td>544±226</td>
<td>684±247</td>
<td></td>
</tr>
<tr>
<td>24h Urine Specific Gravity</td>
<td>EUD</td>
<td>1.011±0.004</td>
<td>1.018±0.008</td>
<td></td>
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<tr>
<td></td>
<td>EUR</td>
<td>1.011±0.006</td>
<td>1.014±0.005</td>
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<tr>
<td></td>
<td>HYD</td>
<td>1.015±0.006</td>
<td>1.020±0.006</td>
<td></td>
</tr>
<tr>
<td>24h Urine Color</td>
<td>EUD</td>
<td>3±1</td>
<td>4±1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EUR</td>
<td>3±1</td>
<td>4±1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HYD</td>
<td>4±1</td>
<td>4±1</td>
<td></td>
</tr>
<tr>
<td>Plasma Osmolality (mOsm•kg⁻¹)</td>
<td>EUD</td>
<td>294±6</td>
<td>305±6Φ</td>
<td>297±4</td>
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<tr>
<td></td>
<td>EUR</td>
<td>294±5</td>
<td>289±6</td>
<td>294±6</td>
</tr>
<tr>
<td></td>
<td>HYD</td>
<td>303±9*</td>
<td>310±7Φ</td>
<td>294±6</td>
</tr>
<tr>
<td>Plasma Volume Change (%)</td>
<td>EUD</td>
<td>-10.8±4.1Φ</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>EUR</td>
<td>-4.7±3.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HYD</td>
<td>-9.8±4.3Φ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluid Consumed (L)</td>
<td>EUD</td>
<td>3.2±2.1</td>
<td>3.2±1.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EUR</td>
<td>4.5±2.4</td>
<td>3.2±2.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HYD</td>
<td>1.6±1.3*</td>
<td>3.3±1.8</td>
<td></td>
</tr>
</tbody>
</table>

Urinary and hematologic measures of hydration status across time. Negative values associated with plasma volume change correspond to a loss of plasma volume compared to the pre-exercise measure. EUR=Euhydrated arrival, fluid intake matches sweat losses during exercise, and fluid replacement during recovery up to 1% of body mass loss; EUD=Euhydrated arrival, progressive dehydration during exercise and recovery; HYD=Hypohydrated arrival, progressive dehydration during exercise and recovery. *=Significant difference when compared to EUD and EUR trials (p<0.05). Φ=Significant difference when compared to only EUR (p<0.05).
Figure 2.2. Fluid consumption 24 hours prior and 24 hours following exercise sessions. * Significantly different than EUD and EUR trials (p<0.05). EUD=Euhydrated arrival, progressive dehydration during exercise and recovery; EUR=Euhydrated arrival, replace fluid losses during exercise and recovery. HYD=Hypohydrated arrival, progressive dehydration during exercise and recovery. Pre=the night prior to the exercise session. Follow=the night following the exercise session.

Figure 2.3. Sleep efficiency (ratio of time asleep to total time in bed) between rehydration groups (ad libitum, prescribed, pooled data) and between trials. EUD=Euhydrated arrival, progressive dehydration during exercise and recovery; HYD=Hypohydrated arrival, progressive dehydration during exercise and recovery. Pre=the night prior to the exercise session. Follow=the night following the exercise session.
Figure 2.4. Time spent in deep sleep the night prior and night following the exercise session. EUD=Euhydrated arrival, progressive dehydration during exercise and recovery; HYD=Hypohydrated arrival, progressive dehydration during exercise and recovery. Pre=the night prior to the exercise session. Follow=the night following the exercise session.

Figure 2.5. Time spent in light sleep the night prior and night following each exercise session. EUR=Euhydrated arrival, fluid intake matches sweat losses during exercise, and fluid replacement during recovery up to 1% of body mass loss; EUD=Euhydrated arrival, progressive dehydration during exercise and recovery; HYD=Hypohydrated arrival, progressive dehydration during exercise and recovery.
arrival, progressive dehydration during exercise and recovery. Pre=the night prior to the exercise session. Follow=the night following the exercise session.

Figure 2.6. Percent time spent in REM sleep the night prior and night following each exercise session. EUR=Euhydrated arrival, fluid intake matches sweat losses during exercise, and fluid replacement during recovery up to 1% of body mass loss; EUD=Euhydrated arrival, progressive dehydration during exercise and recovery; HYD=Hypohydrated arrival, progressive dehydration during exercise and recovery. Pre=the night prior to the exercise session. Follow=the night following the exercise session.

Figure 2.7. Relationship between pre-exercise 24-hour urine volume following the exercise session and PRE KSD\text{TOTAL}. PRE KSD\text{TOTAL}=aggregate score on Karolinska Sleep Diary the night before the exercise session.
Figure 2.8. Relationship between 24-hour urine volume following the exercise session and FU KSD_{TOTAL} at the follow up time point. FU KSD_{TOTAL}=aggregate score on Karolinska Sleep Diary the day following the exercise session.

\[ y = 1.3816x + 21.632 \]
\[ R^2 = 0.21989 \]

Figure 2.9. Relationship between 24-hour urine osmolality following the exercise session and FU KSD_{TOTAL} at the follow up time point. FU KSD_{TOTAL}=aggregate score on Karolinska Sleep Diary at the follow up time point.

\[ y = -0.0067x + 28.338 \]
\[ R^2 = 0.13774 \]
Figure 2.10. Relationship between 24-hour urine specific gravity following the exercise session and FU KSD_{TOTAL}. FU KSD_{TOTAL} = aggregate score on Karolinska Sleep Diary the night following the exercise session.

\[ y = -252.33x + 281.12 \]
\[ R^2 = 0.15609 \]

Figure 2.11. Relationship between 24-hour urine color following the exercise session and FU KSD_{TOTAL}. FU KSD_{TOTAL} = aggregate score on Karolinska Sleep Diary the night following the exercise session.

\[ y = -1.5581x + 30.806 \]
\[ R^2 = 0.16202 \]
**Table 2.3. Subjective ratings of sleep quality assessed by the Karolinska Sleep Diary**

<table>
<thead>
<tr>
<th>Trial</th>
<th>How did you sleep</th>
<th>Feeling Refreshed</th>
<th>Calm Sleep</th>
<th>Slept Through Allotted Time</th>
<th>Ease of Waking</th>
<th>Ease of Sleeping</th>
<th>Amount of Dreaming</th>
</tr>
</thead>
<tbody>
<tr>
<td>EUD</td>
<td>4 (3.5, 4)</td>
<td>3 (3, 4)</td>
<td>3 (3, 4)</td>
<td>4 (2.5, 4.5)</td>
<td>3 (2.5, 4)</td>
<td>4 (3, 4)</td>
<td>3 (2, 3)</td>
</tr>
<tr>
<td>EUR</td>
<td>4 (3.5, 4.5)</td>
<td>4 (3.5, 4)</td>
<td>4 (3.5, 4.5)</td>
<td>4 (3.5, 4.5)</td>
<td>4 (3, 4)</td>
<td>4 (3, 5)</td>
<td>2 (1.5, 3)</td>
</tr>
<tr>
<td>HYD</td>
<td>4 (3, 4)</td>
<td>3 (3, 4)</td>
<td>3 (2, 4)</td>
<td>3 (2, 4)</td>
<td>3 (3, 4)</td>
<td>3 (3, 4)</td>
<td>3 (1.5, 3)</td>
</tr>
</tbody>
</table>

Individual components of the Karolinska Sleep Diary between trials. EUR=Euhydrated arrival, fluid intake matches sweat losses during exercise, and fluid replacement during recovery up to 1% of body mass loss; EUD=Euhydrated arrival, progressive dehydration during exercise and recovery; HYD=Hypohydrated arrival, progressive dehydration during exercise and recovery. Data is presented as median (25%, 75% interquartile range).

**Figure 2.12.** Perceptual feelings of fatigue over the course of exercise, recovery, and the following day. *=Significant difference between HYD and EUR trials (p<0.05). IPE=Immediately post exercise. Follow=Follow up trial the day following the exercise session EUD=Euhydrated arrival, progressive dehydration during exercise and recovery; EUR=Euhydrated arrival, minimize fluid losses by matching sweat rate during exercise and recovery; HYD=Hypohydrated arrival, progressive dehydration during exercise and recovery.
Figure 3.3. Total score on the Environmental Symptoms Questionnaire over at the Pre-exercise, Post-exercise, and Recovery time point. *=Significant difference between HYD and EUR trials (p<0.05). ESQ=Environmental Symptoms Questionnaire. EUD=Euhydrated arrival, progressive dehydration during exercise and recovery; EUR=Euhydrated arrival, minimize fluid losses by matching sweat rate during exercise and recovery; HYD=Hypohydrated arrival, progressive dehydration during exercise and recovery.

Figure 2.14. Concentration of CK at PRE, RECOV and FU timepoints. PRE=Pre-exercise session time point; RECOV=Post-exercise session; FU=Follow up time point (24-hours following exercise session).
Chapter 3. No differences in neuromuscular control 24-hours following exercise-induced hypohydration
ABSTRACT

Purpose: The purpose of this study was to examine how ad libitum rehydration affects neuromuscular control 24 hours following exercise-induced hypohydration compared to prescribed rehydration. We also sought to identify variables predictive of increased risk of lower extremity injury.

Methods: Eleven healthy, recreationally active males (mean±SD; age, 22±3y; height, 178±6cm; VO$_{2max}$, 54.7±5.5ml•kg$^{-1}$•min$^{-1}$; body fat, 11.6±3.9%) completed three randomized exercise sessions: euhydrated + fluid replacement (EUR), euhydrated + no fluid (EUD) and hypohydrated + no fluid (HYD) in hot conditions (ambient temperature, 35.3±0.6°C and relative humidity, 31.3±2.0%). Exercise consisted of 3h of exercise (8min at 40% VO$_{2max}$, 8min at 60% VO$_{2max}$, 8min at 40% VO$_{2max}$ and 6min of passive rest) followed by 60 min passive rest and a 24h follow up. Movement technique and postural control was measured pre-exercise (PRE), post passive rest (RECOV) and 24h following exercise (FOLLOW) using the Landing Error Scoring System (LESS) and Balance Error Scoring System (BESS) respectively. A mixed design (condition x trial x time) repeated measures ANOVAs with a Tukey post hoc test was used to evaluate differences in continuous dependent variables. Logistic regression was used to identify variables predictive of increased risk of lower extremity injury (LESS score ≥5).

Result: There were no differences in LESS scores at RECOV (4.55±1.9, 4.84±3.2, 3.1±1.9) or FOLLOW (3.45±2.2, 2.93±1.9, 4.81±2.7) between EUD, EUR, and HYD respectively (p>0.05). Balance was unaffected by exercise-induced hypohydration at RECOV and FOLLOW (p>0.05). Larger degrees of hypohydration following exercise in the heat was 2.1 times more likely to have a LESS score ≥5 at FOLLOW (p<0.05).
Conclusions: Exercise-induced hypohydration did not adversely affect movement technique or standing balance after an acute (1-hour) or prolonged (24-hours) bout of recovery. However, prolonged exercise in the heat resulting in large body water losses may be associated with increased risk of lower extremity injury. These findings suggest that implementing individualize hydration plans during and following exercise may mitigate risk of injury.

INTRODUCTION
The risk of lower extremity musculoskeletal injury has been associated with deficits in neuromuscular control, with alterations in balance or movement technique being primary risk factors of lower extremity injuries (94, 129, 153). Identifying physiologic mechanisms responsible for contributing to impairment of neuromuscular control may assist in reducing risk of lower extremity injury. Factors including exercise-induced fatigue (44, 118), hypohydration (52, 77), and a combination of hypohydration and exercise-induced hyperthermia (55) have been shown to alter either movement technique or balance, and consequently may increase the risk of injury. Mitigating risk by modifying behaviors, such as improving hydration strategies during exercise in the heat could optimize safety and performance during physical activity.

Hypohydration, defined by a state of body water deficit has been shown to have adverse effects on physiologic function (47, 81, 137, 172), exercise performance (12, 33, 115, 184), and cognitive function (3, 42, 50). Furthermore, hypohydration increases the risk of exertional heat illness by exacerbating thermoregulatory strain during exercise (11, 32, 97, 162). Hypohydration is also implicated in degrading neuromuscular control as measured by postural stability, however, these results are unclear with fatigue or hyperthermia being potential confounding factors association with postural control impairments (52, 55, 77, 155, 177). Other evidence suggests that hypohydration alone does not impair neuromuscular control measured by a dynamic jump landing task or a standing balance task; rather, a combination of hypohydration and hyperthermia elicit these deficits (55).

Establishing individualized hydration plans consisting of starting exercise in a euhydrated state, minimizing fluid losses as measured by individual sweat rate, and replacing remaining losses following exercise provides an optimal environment to maximize performance and
enhance safety during exercise, especially during exercise in the heat(164, 170). During exercise in the heat, it is not uncommon for fluid losses to exceed 3-4% of body mass and this can be attributed to either the constraints of physical activity or fluid availability, or a reduced drive to consume fluids at a rate at which they are lost (involuntary dehydration)(85). Following exercise, the goal is to replace remaining losses in order to begin the next bout of exercise in a state of euhydration. Progressive dehydration over the course of multiple days has been observed in athletes who consume fluid ad libitum during and following exercise(79, 200), however little is known about the ability to return to a euhydrated state within 24 hours of exercise-induced hypohydration of various magnitudes.

Prior research has examined the role of hypohydration, hyperthermia and fatigue on neuromuscular control immediately following exercise and after an acute recovery period. However, little is known on how exercise-induced hypohydration and hyperthermia affects neuromuscular control after a prolonged bout of recovery. Therefore, the purpose of this study is to examine the effects of exercise-induced hypohydration on neuromuscular control following a 24-hour recovery period from exercise. Furthermore, we aim to investigate the effects on the mode of rehydration (ad libitum versus prescribed) following exercise-induced hypohydration on neuromuscular control after prolonged recovery. It is also the purpose of this study to determine if physiologic and hydration status markers were predictive of decreased neuromuscular control and following prolonged exercise in the heat. It is hypothesized that individuals replacing fluids ad libitum or those presenting in a state of hypohydration will present with poor neuromuscular control compared to those given a prescribed rehydration plan.

METHODS
**Design**

This study utilized a counter-balanced, crossover design in which participants were randomly assigned to each exercise session. The exercise sessions varied in respect to the participant’s hydration status upon arrival and during exercise and included: 1) Arrive euhydrated, minimize fluid losses during exercise and post exercise recovery (EUR), 2) Arrive euhydrated, progressively dehydrated during exercise and post exercise recovery (EUD), and 3) Arrive hypohydrated, progressively dehydrate during exercise and post exercise recovery (HYD). Following the exercise session participants were randomly allocated to either a prescribed or ad libitum rehydration group. The prescribed rehydration group received instruction to consume fluids matching 150% of their fluid losses (170) whereas the ad libitum rehydration group was permitted to consume fluids as those chose. All sessions were separated by at least 5 d to allow for participants to fully recovery before the next testing session. All sessions took place in a climate-controlled chamber (Model 2000; Minus-Eleven Inc., Malden, MA) with conditions set ambient temperature, 35.3±0.6°C and relative humidity, 31.3±2.0%.

**Participants**

Eleven healthy, recreationally active males (mean±SD; age, 22±3y; height, 178±6cm; \( \text{VO}_{2\text{max}}, 54.3±5.4\text{ml\cdotkg}^{-1\cdot\text{min}}^{-1} \); body fat, 11.6±3.9%) volunteered to participate in this study. All participants reported participating in exercise at least 4-5 days per week for a minimum of 30 minutes each day. Participants were excluded if they were observed to have one of the following: 1) Fever or illness at the time of testing, 2) History of cardiovascular, metabolic, or respiratory disease, 3) Current musculoskeletal injury that limits their level of physical activity, and 4) Consumption of >3 alcoholic beverages per day (>21 per week). Participants provided written
and informed consent to participate in the study, which was approved by the University’s Institutional Review Board, prior to beginning any aspect of the study.

**PROCEDURES**

**Familiarization Sessions**

Prior to the three exercise sessions, participants reported to the laboratory for a familiarization session that contained two stages. The first stage was a single visit to the laboratory to allow participants to become acquainted with the testing session procedures (movement assessment and postural control assessments, rectal thermometer to measure rectal temperature (T\textsubscript{REC}), heart rate monitor to measure heart rate (HR), and perceptual scales), assessment of cardiovascular fitness through a VO\textsubscript{2max} test, sweat rate and sweat electrolyte concentration assessment. The second stage required participants to arrive to the laboratory for three consecutive days to obtain an accurate baseline hydration status.

Upon arrival to the laboratory for the first stage of the familiarization session, participants provided a urine sample to assess hydration status and then performed a VO\textsubscript{2max} test on a motorized treadmill (NordicTrack, Icon Health & Fitness, Logan, UT) to ensure they meet the eligibility criteria (>45mL\textperiodcentered kg\textsuperscript{-1}\textperiodcentered min\textsuperscript{-1}) for inclusion into the study. Oxygen uptake (VO\textsubscript{2}) and related gas exchange was measured using open circuit spirometry using a PARVO Medics TrueOne 2400 Metabolic Measurement System (Parvo Medics, Inc., Salt Lake City, UT). To ensure euhydration prior to the familiarization session, participants were asked to consume 500 mL of water prior to going to bed and again upon waking the next day prior to their arrival to the laboratory.

Following the VO\textsubscript{2max} test, participants then provided a nude body mass (NBM), were familiarized to the postural and movement control assessments, and then completed 30 minutes
of exercise on a motorized treadmill (8 min, 40% VO$_{2\text{max}}$; 8 min, 60% VO$_{2\text{max}}$; 8 min, 40% VO$_{2\text{max}}$; 6 min, rest) in the climate-controlled chamber (ambient temperature, 35.3±0.6°C; relative humidity, 31.3±2.0%) that mimicked the exercise intensity of the testing sessions to obtain an accurate sweat rate. After exercise, participant’s sweat electrolyte concentration was assessed using previously published methods(10). A final nude body mass was taken to calculate total sweat losses during exercise to assess fluid needs during the testing sessions. Also, during this session, participants were instructed on the use of the RPE (Omni Scale), environmental symptoms questionnaire (ESQ), and fatigue scales. They were also instructed on the proper method of filling out a 24-hour diet record that will be utilized for the exercise sessions. Last, they were instructed and familiarized with the measures of neuromuscular control that were to be assessed prior to the exercise session (PRE), following the recovery portion of the exercise trial (RECOV) and the following day (FOLLOW).

The second stage of the familiarization session involved the participants to arrive to the laboratory for three consecutive mornings in a fasted state. They were instructed to collect all urine in a clean jug provided to them for a period of 24 hours prior to their arrival to the laboratory in addition to a pre-bedtime sample. Participants also recorded their 24-hour diet for each of the three days to gather an assessment of their normal nutritional intake. Upon arrival to the laboratory, participants returned the urine jug containing a 24-hour collection, the diet log containing a record of all foods and fluids consumed for the previous 24-hours and provided a nude body mass to the nearest 0.1 kg (Defender 5000, OHAUS, Parsippany, NY). Euvhhydration was defined as a urine specific gravity (USG) <1.020 (Model TS400; Reichert Inc., Depew, NY), urine osmolality ($U_{\text{OSM}}$) <500 mOsm•L$^{-1}$ (freezing point depression, model 3320; Advanced Instruments, Norwood, MA) and a change in nude body mass <2% from day to day.
Testing Sessions

For the testing sessions in which participants were to arrive in a euhydrated state, they were asked to consume an additional 500 mL of water before going to sleep and upon waking the next morning to ensure euhydration. For testing sessions with participants arriving hypohydrated, they were restricted from consuming fluids or water heavy foods for a period of 22 hours prior to the start of the trial. This was to ensure a level of hypohydration of ~1-2% of baseline body mass. To account for any potential confounding effects of time, participants were tested at the same time of day ± 1 hour.

Before each testing session, all participants collected their urine over a 24-hour period prior to testing for subsequent urinalysis. Participants also provided a pre-bedtime urine sample in a separate clean urine cup for urinalysis. Upon arrival to the laboratory, the following measures were collected: NBM, USG, U_{OSM}, T_{REC}, and HR. Participants performed pre-exercise (PRE) postural and movement control assessment tests and then entered the climate-controlled chamber for the exercise portion of the testing session.

Participants took a seated position inside the climate-controlled chamber for 15 minutes to equilibrate to the environmental conditions. Prior to the start of exercise, participants provided a blood sample, completed the perceptual scales (OMNI, ESQ, and Fatigue), and baseline measure of T_{REC} and HR were measured. Participants began exercise, performing six 30-minute cycles of exercise on a motorized treadmill ((8 min, 40% VO_{2max}; 8 min, 60% VO_{2max}; 8 min, 40% VO_{2max}; 6 min, rest). During exercise, measures of T_{REC}, HR, body mass with minimal clothing (BM), OMNI, and Fatigue were measured. The intention of this exercise protocol was to provide a duration of exercise permitting progressive dehydration in testing sessions in which no fluid was consumed. For sessions where participants minimized fluid losses, they consumed fluid
with a volume matching their calculated sweat rate fractioned out in boluses during every exercise cycle. After completion of the exercise protocol, a post exercise (POST) blood sample was taken and participants completed the ESQ questionnaire.

During the post-exercise recovery period (RECOV), participants rested for 60 minutes inside the climate-controlled chamber. Fluid replacement for EUR was a volume equal to up to 1% of their body mass losses based on the difference between pre and post BM. For EUD and HYD trial, participants were not provided fluid during the 60-minute recovery period. During RECOV, OMNI, Fatigue, T_{rec}, and HR were measured every 10 minutes. Participants provided a third blood sample at the completion of RECOV while remaining inside the climate-controlled chamber. After leaving the climate-controlled chamber, participants completed post-session (RECOV) postural and movement control protocols.

Upon leaving the laboratory, participants were given a clean urine jug for 24 h urine collection, a diet record and the activity tracker to monitor their sleep. They were also given an instruction sheet to follow prior to the follow-up (FOLLOW) testing session based on the randomly assigned rehydration group: the ad libitum group received instructions on what to bring for the follow up session, while the prescribed group received specific instruction on how much fluid they need to consume based on the difference between PRE and POST NBM.

For EUR, EUD, and HYD, participants returned to the laboratory 24-30 hours after completion of the exercise session for the FOLLOW testing. Participants collected all urine over the course of the 24-30 hours after completion of the exercise session as well as documented all fluid and food intake into a diet record. Upon arrival to the laboratory the following day, USG, U_{OSM}, NBM, Fatigue and KSD was measured. Following these measures participants provide a blood sample, completed the postural control assessments.
Movement and Postural Control Assessments

The movement postural control assessments were identical across all tested time points (PRE, RECOV, and FOLLOW). The assessments consisted of both the Balance Error Scoring System (BESS) and a jump landing test that was graded using the Landing Error Scoring System (LESS). The BESS is a validated test assessing postural control containing six positions: double leg firm (DL-F), double leg foam (DL-FO), single leg firm (SL-F), single leg foam (SL-FO), tandem stance firm (TD-F) and tandem stance foam (TD-FO). Each position was performed for 20 s and participants were instructed to keep their hands on their hips and their eyes closed for the duration of each stance tested. For SL-F and SL-FO, participants were instructed to stand on their dominant foot with their contralateral leg flexed at the hip and knee. TD-F and TD-FO required participants to stand with their non-dominant foot in front and in line with their dominant foot.

All BESS tests were graded in real time by two researchers who were proficient and had experience grading the BESS. For each stance, the total number of errors was calculated and the sums of the six stances were compiled to achieve an overall score. An error was tabulated for each of the following: hands lifted off the iliac crest, opening of the eyes, stepping down, stumbled, moved the hip in more than 30° flexion or abduction during the single-leg stance, lifting the forefoot or heel, or remaining out of the position for more than 5 s.

During the jump landing task, participants were instructed to jump down from a 30cm box with both legs to a distance equaling 25% of their standing height. Immediately upon landing they were instructed to jump straight up in the air for maximum vertical height. Participants performed three jump-landings with an additional jump performed if one of the three jumps was performed incorrectly. The jump-landing task was videotaped (Canon FS400, Canon
USA, Inc., Lake Success, NY) with cameras placed in front and to the side of the participant to capture movement in both the frontal and sagittal plane. The videos were graded at a later time by a single rater with experience grading the LESS-4 (ICC, 0.89) who was blinded from all test sessions using the LESS. Increasing scores on the LESS are associated with higher-risk lower extremity movement patterns, which have been correlated with increased incidence of lower extremity injuries (153).

**Perceptual Measures**

The environmental symptoms questionnaire (ESQ) is a validated scale assessing symptoms emanating from environmental stress(106, 166). We utilized a modified version of the ESQ that has been previously validated to assess symptomology related to heat stress(183). It consists of a 14-question assessment of symptoms related to environmental stress.

Fatigue was measured using an 11-point scale (0-10) with words anchored at the following numbers: 0-No fatigue at all, 1-Very small amount of fatigue, 2-Small amount of fatigue, 3-Moderately fatigued, 4-Somewhat fatigued, 5-Fatigued, 7-Very fatigued, 9-Extremely fatigued, and 10-Completely fatigued. Participants were asked “How fatigued do you feel right now?” and then prompted to indicate the level corresponding to their perceived level of fatigue at the time they were asked.

**Blood Collection Measures**

For each blood sampling time point (PRE, POST, RECOV and FOLLOW), participants provided 10 mL of blood taken from an antecubital vein using a butterfly needle equipped with an extension tube and hub (BD Vacutainer Safety-Lok Blood Collection Set, Becton Dickinson, Franklin Lakes, NJ). Blood samples were drawn into tubes pre-treated with lithium-heparin, and a serum tube (Vacutainer, Becton Dickinson, Franklin Lakes, NJ). The serum tube was placed in
an upright position and allowed to clot prior to placing in the centrifuge. They were then centrifuged for 15min at 3000 RPM at room temperature with the resulting plasma and serum aliquotted and stored at -80°C until analysis.

Plasma osmolality \( (\text{POsmo}) \) was measured in duplicate using freezing point depression (model 3320; Advanced Instruments, Norwood, MA). Percent changes in plasma volume (PV) were derived from measures of hematocrit (HCT) via the microhematocrit technique and hemoglobin (Hb) via a B-Hemoglobin photometer (HemoCue, Inc., Lake Forest, CA) using previously established calculations.(54)

**Statistical Analysis**

All statistical analyses were performed using SPSS Statistical Software (v.21. IBM Corporation, Armonk, NY). All values are presented as mean ± standard deviation unless otherwise noted. A mixed design (condition x trial x time) repeated-measures ANOVA was used to measure differences in dependent variables between rehydration conditions (Ad libitum and prescribed) and all trials (EUD, EUR, HYD) (Figure 3.1). Separate (trial x time) repeated-measures ANOVAs were used to evaluate hydration measures and sleep measures independent of the mode of rehydration. For statistically significant findings, Tukey’s post-hoc analysis was used to determine where differences lied between factors. Data were also compared using mean differences (MD), 95% confidence intervals (95%CI) and effect size (ES) using Cohen’s d were used to determine small (0.2-0.49), medium (0.5-0.79) and large (>0.8) effects. Logistic regression was used to identify variables predictive of increased risk of lower extremity injury as assessed by the LESS (LESS score ≥5). Significance was set a priori at p<0.05.

**RESULTS**
There were no differences in hydration status, measures of hydration status or measures of neuromuscular control between ad libitum and prescribed rehydration groups (p>0.05). Therefore, the data between ad libitum and prescribed groups have been pooled and only the trial x time analyses are shown below.

Tables 3.1 and 3.2 depict changes in body mass and other physiological variables during the exercise session. EUD and HYD resulted in greater losses of body mass and greater measures of $P_{\text{osmo}}$ at the POST and RECOV time point following exercise when compared to EUR (p<0.05). At POST, $T_{\text{rec}}$ was greater in HYD than EUD and EUR (p<0.05) with no differences between EUD and EUR (p>0.05). However, at RECOV, there were differences between HYD and EUR as well as EUD and EUR (p<0.05). At FOLLOW, there were no differences in body mass, percent body mass loss (%BML) from the 24h pre NBM measure, fluid consumed, or any urinary or hematologic measures of hydration status between EUD, EUR and HYD (p>0.05).

**LESS**

There were no differences in LESS scores at RECOV (4.55±1.9, 4.84±3.2, 3.1±1.9) or FOLLOW (3.45±2.2, 2.93±1.9, 4.81±2.7) between EUD, EUR, and HYD respectively (Figure 3.2) (p=0.082). Logistic regression found that those completing exercise with a large degree of hypohydration were 2.1 times more likely to have a LESS score ≥5 at FOLLOW (p<0.05), putting them into a category of increased risk. No other variables predicted LESS scores ≥5 at any time point.

**BESS**

There were no differences in total BESS score between groups or across time points (p=0.678) (Figure 3.3). Additionally, there were no significant differences in any individual component of the BESS between groups or across time (p>0.05).
Perceptual Scales

The score on the ESQ was significantly greater at POST and FOLLOW in HYD when compared to EUR (p<0.05) (Figure 3.4). There were no significant differences in ESQ between HYD and EUD at any time point (p>0.05). In addition, perceptual ratings of fatigue were significantly greater in HYD in comparison to EUR starting at minute 120 of exercise and continuing throughout the duration of the 1-hour recovery period following exercise (p<0.05) (Figure 3.5). There were no differences in ratings of fatigue between EUD and HYD (p>0.05). Furthermore, at FOLLOW, there were no differences in perceptual ratings of fatigue between any trial (p>0.05).

DISCUSSION

The purpose of this study was to examine the effects of exercise-induced hypohydration on neuromuscular control and aerobic capacity following 24 hours of recovery following either an ad libitum or prescribed rehydration plan. This is the first study to examine movement technique and postural control 24 hours following exercise-induced hypohydration. We found, that following an acute (1 hour) and prolonged (24 hours) bout of recovery from exercise in the heat, neuromuscular control remained unaffected, despite graded hypohydration of up to 5.5% of body mass. We did, however, observe that as the magnitude of hypohydration during exercise in the heat increased, there was a greater likelihood that movement technique would be classified as increased risk of lower extremity injury based on LESS score. Our results suggest that minimizing fluid losses during a prolonged bout of exercise in hot conditions will minimize the likelihood of increased risk of lower extremity injury following extending out to 24 hours following exercise in hot environmental conditions.
The LESS has been found to be a valid and reliable measure of assessing risk of lower extremity injury by examining 17 injury risk factors using a standardized jump landing task (154). The evaluation of gross movement errors provides a clinical evaluation of overall risk of lower extremity injury (i.e., medial knee displacement, hip or knee rotation, and movement within the sagittal plane), with an increase in one’s LESS score being indicative of poor movement technique. Evidence supports that a LESS score ≥5 increases the risk of injury to the anterior cruciate ligament (ACL) (153), which in addition to the financial costs associated with surgical reconstruction and rehabilitation, can cause long term health issues such as osteoarthritis (114).

DiStefano et al. (55), was the first to investigate the effect of individual and combined effects of hypohydration and hyperthermia on movement technique without the confounding variable of fatigue. Their results show that a combination of hyperthermia and hypohydration induced by exercise causes impairment of movement technique measured by the LESS, both immediately after exercise and following a 60-minute recovery period (55). The current study shows that 60-minutes following prolonged exercise in hot conditions, LESS scores were unaffected by hypohydration and hyperthermia, which conflict with the aforementioned findings.

Twenty-four hours following exercise in the heat, LESS scores were still similar between groups, suggesting that prolonged exercise in the does not inhibit neuromuscular control 24 hours following exercise. Padua et al. (153), found that those scoring ≥5 on the LESS had an increased risk profile of non-contact or indirect-contact ACL injury than those score <5 on the LESS. While there was no difference between groups or time points when measuring movement technique, we found that 24-hours following the exercise session, it was two times more likely to have LESS scores ≥5 when the magnitude of hypohydration was increased, despite returning to
baseline levels of euhydration within 24 hours. This suggests that the magnitude of hypohydration from the previous day’s exercise session could be responsible for the elevated risk profile as measured by the LESS when examined 24 hours post exercise, however, further research is needed to determine if this relationship has clinical significance when assessing one’s risk profile of an ACL rupture.

Prior research (35, 55, 73, 131, 147, 210) investigating the effects of hypohydration on balance has suggested that fatigue plays a contributing factor related to increase injury risk following exercise. However, other literature (155, 177, 187) has shown that the fatigue-related impairments of postural control are short-lived and can return to baseline within 15-20-minutes after cessation of exercise. Our results suggest that postural controlled measured by the BESS is not affected by hypohydration after exercise in the heat, which is in contrast to DiStefano et al. (55), where BESS scores remained elevated in the trial where they were hyperthermic and hypohydrated. This could attributed to the duration, intensity and elicited muscle damage that occurred during exercise (218), however it is unclear as to which factor contributed more to this observed difference in BESS scores.

Twenty-four hours following exercise, measures of balance returned to baseline levels with no statistically significant differences between trials. Our results coincide with those of Seay et al. (177), who found no effect of hypohydration on balance after a prolonged bout of recovery, despite differences in magnitude and mechanism of dehydration. The ability to return to baseline measures of balance 24 hours following exercise may be attributed to the ability for our participants to fully recover to baseline levels of euhydration. Future research examining prolonged hypohydration following exercise on balance may explain any potential relationship between hypohydration and balance and controlling for fatigue.
Our results indicated that participants were able to return to baseline levels of euhydration within 24 hours following exercise-induced hypohydration, independent of the mode of rehydration. This suggests that ad libitum fluid replacement over a 24-hour period following exercise-induced hypohydration was sufficient enough to allow our participants to return to a euhydrated state. Godek et al. (79), observed that football players exhibited progressive dehydration over the course of consecutive days of training. This was recognized as their inability to replace all fluid losses during and after daily training by ad libitum fluid replacement. While our results contrast the results from Godek et al., a potential explanation may be attributed to the fact that our participants habitually consumed >3.2L per day of fluid, which would create a high likelihood of being in a state of euhydration (156, 157). Future research is warranted to examine the ability of persons consuming <3L per day of fluid and their ability to return to a euhydrated state following exercise induced hypohydration of 3% and 5% of body mass losses.

This study was not without limitations. The subject sample size (n=11) may not have been sufficient to observe statistically significant differences between measures of neuromuscular control following both an acute and prolonged period of recovery. Additionally, the fact that our participants would be considered “high drinkers” based on their habitual daily fluid consumption (>3L), it is unclear as to how individuals consuming less fluid would respond to measures of neuromuscular control. Those consuming less fluid than observed in this study may not fully replace fluid losses within 24 hours when given an ad libitum rehydration plan, thus increasing the likelihood that would return the following day in a state of hypohydration. Lastly, there may be limited generalizability in that we tested only college-aged recreationally active males. Investigating females, adolescent athletes and older physically active populations...
may gather a better understanding of the effects of hypohydration and exercise in the heat on neuromuscular control.

Further research is needed to identify the mechanism(s) in which hypohydration and hyperthermia may play on neuromuscular control following exercise in the heat. A unique finding was that larger fluid losses during exercise in the heat increased the likelihood of an increased risk profile when assessing lower extremity movement patterns 24 hours following exercise, despite a return to a state of euhydration. It is recommended that during exercise in the heat, individuals should practice appropriate, individualized hydration strategies to prevent large fluid losses during exercise.

REFERENCES


Figure 3.1. Flow chart depicting random allocation to Ad Libitum and Prescribed rehydration groups following exercise.
<table>
<thead>
<tr>
<th>Trial</th>
<th>24h PRE</th>
<th>PRE</th>
<th>POST</th>
<th>RECOV</th>
<th>FU</th>
</tr>
</thead>
<tbody>
<tr>
<td>EUD</td>
<td>73.4±9.9</td>
<td>74.1±10.4</td>
<td>70.9±10.0</td>
<td>70.7±9.9</td>
<td>73.9±10.1</td>
</tr>
<tr>
<td>%Change</td>
<td>-0.837±1.2</td>
<td>3.5±1.1Φ</td>
<td>3.8±1.2Φ</td>
<td>-0.6±1.0</td>
<td></td>
</tr>
<tr>
<td>EUR</td>
<td>73.4±10.3</td>
<td>73.9±10.4</td>
<td>73.5±10.6</td>
<td>73.1±10.4</td>
<td>73.5±10.2</td>
</tr>
<tr>
<td>%Change</td>
<td>-0.654±1.1</td>
<td>-0.112±1.1</td>
<td>0.4±1.0</td>
<td>-0.2±0.9</td>
<td></td>
</tr>
<tr>
<td>HYD</td>
<td>73.6±9.8</td>
<td>72.3±10.1</td>
<td>69.8±10.0</td>
<td>69.6±10.9</td>
<td>73.3±10.1</td>
</tr>
<tr>
<td>%Change</td>
<td>1.8±0.6*</td>
<td>5.2±0.5*</td>
<td>5.6±0.6*</td>
<td>0.4±1.2</td>
<td></td>
</tr>
</tbody>
</table>

The absolute and relative change (% change) in body mass as a result of fluid loss across time points. Negative values associated with % change correspond to a body water gain compared to the 24-hour pre-exercise measure. Positive values associated with % change correspond to a body water deficit compared to the 24-hour pre-exercise measure. EUR=Euhydrated arrival, fluid intake matches sweat losses during exercise, and fluid replacement during recovery up to 1% of body mass loss; EUD=Euhydrated arrival, progressive dehydration during exercise and recovery; HYD=Hypohydrated arrival, progressive dehydration during exercise and recovery. * = Significant difference when compared to EUD and EUR trials (p<0.05). Φ = Significant difference when compared to only EUR (p<0.05).
Table 3.2. Physiological variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Condition</th>
<th>PRE</th>
<th>POST</th>
<th>RECOV</th>
<th>FU</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{REC}$</td>
<td>EUD</td>
<td>37.04±0.29</td>
<td>38.89±0.53</td>
<td>37.69±0.35(^\oplus)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EUR</td>
<td>37.16±0.32</td>
<td>38.51±0.43</td>
<td>37.02±0.24</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HYD</td>
<td>37.24±0.29</td>
<td>39.40±0.35(^*)</td>
<td>38.08±0.22(^*)</td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td>EUD</td>
<td>84±13</td>
<td>144±31</td>
<td>106±14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EUR</td>
<td>84±11</td>
<td>140±14</td>
<td>91±13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HYD</td>
<td>95±15</td>
<td>150±20</td>
<td>105±19(^\oplus)</td>
<td></td>
</tr>
<tr>
<td>24h $U_{VOL}$ (L)</td>
<td>EUD</td>
<td>3.1±1.6</td>
<td>2.0±1.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>EUR</td>
<td>3.0±1.5</td>
<td>2.2±1.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HYD</td>
<td>2.0±1.5</td>
<td>1.6±1.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24h $U_{OSMO}$ (mOsm•kg(^{-1}))</td>
<td>EUD</td>
<td>404±176</td>
<td>634±270</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>EUR</td>
<td>395±219</td>
<td>480±171</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HYD</td>
<td>544±226</td>
<td>684±247</td>
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<tr>
<td>24h USG</td>
<td>EUD</td>
<td>1.011±0.004</td>
<td>1.018±0.008</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>EUR</td>
<td>1.011±0.006</td>
<td>1.014±0.005</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HYD</td>
<td>1.015±0.006</td>
<td>1.020±0.006</td>
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<td></td>
</tr>
<tr>
<td>24h $U_{COL}$</td>
<td>EUD</td>
<td>3±1</td>
<td>4±1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>EUR</td>
<td>3±1</td>
<td>4±1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HYD</td>
<td>4±1</td>
<td>4±1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$P_{OSMO}$ (mOsm•kg(^{-1}))</td>
<td>EUD</td>
<td>294±6</td>
<td>305±8(^\oplus)</td>
<td>305±6(^\oplus)</td>
<td>297±4</td>
</tr>
<tr>
<td></td>
<td>EUR</td>
<td>294±5</td>
<td>287±5</td>
<td>289±6</td>
<td>294±6</td>
</tr>
<tr>
<td></td>
<td>HYD</td>
<td>303±9(^*)</td>
<td>314±8(^*)</td>
<td>310±7(^*)</td>
<td>294±6</td>
</tr>
<tr>
<td>PV Change (%)</td>
<td>EUD</td>
<td>-14.3±4.1</td>
<td>-10.8±4.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>EUR</td>
<td>-6.2±5.0</td>
<td>-4.7±3.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HYD</td>
<td>-12.0±5.3</td>
<td>-9.8±4.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluid Consumed (L)</td>
<td>EUD</td>
<td>3.2±2.1</td>
<td>3.2±1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>EUR</td>
<td>4.5±2.4</td>
<td>3.2±2.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HYD</td>
<td>1.6±1.3(^*)</td>
<td>3.3±1.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Physiological variables between trials across time. Negative values associated with plasma volume change correspond to a loss of plasma volume compared to the pre-exercise measure. EUR=Euhydrated arrival, fluid intake matches sweat losses during exercise, and fluid replacement during recovery up to 1% of body mass loss; EUD=Euhydrated arrival, progressive dehydration during exercise and recovery; HYD=Hypohydrated arrival, progressive dehydration during exercise and recovery. \(^*\)=Significant difference when compared to EUD and EUR trials (p<0.05). \(^\oplus\)=Significant difference when compared to only EUR (p<0.05).
Figure 3.2. LESS scores between time points and conditions. EUD=Euhydrated arrival, progressive dehydration during exercise and recovery; EUR=Euhydrated arrival, minimize fluid losses by matching sweat rate during exercise and recovery; HYD=Hypohydrated arrival, progressive dehydration during exercise and recovery.
Figure 3.3. BESS scores between time points and conditions. EUD=Euhydrated arrival, progressive dehydration during exercise and recovery; EUR=Euhydrated arrival, minimize fluid losses by matching sweat rate during exercise and recovery; HYD=Hypohydrated arrival, progressive dehydration during exercise and recovery.
Figure 3.4. Total score on the Environmental Symptoms Questionnaire over at the Pre-exercise, Post-exercise, and Recovery time point. *=Significant difference between HYD and EUR trials (p<0.05). ESQ=Environmental Symptoms Questionnaire. EUD=Euhydrated arrival, progressive dehydration during exercise and recovery; EUR=Euhydrated arrival, minimize fluid losses by matching sweat rate during exercise and recovery; HYD=Hypohydrated arrival, progressive dehydration during exercise and recovery.
Figure 3.5. Perceptual feelings of fatigue over the course of exercise, recovery, and the following day. * = Significant difference between HYD and EUR trials (p<0.05). IPE = Immediately post exercise. Follow = Follow up trial the day following the exercise session. EUD = Euhydrated arrival, progressive dehydration during exercise and recovery; EUR = Euhydrated arrival, minimize fluid losses by matching sweat rate during exercise and recovery; HYD = Hypohydrated arrival, progressive dehydration during exercise and recovery.
Appendix A: Karolinska Sleep Diary

Bedtime (hr.): ____________

Time of awakening (hr.): ____________

Time falling asleep? ____________

How did you sleep?

Very poorly - 1  2  3  4  5-very well

Feeling refreshed after wakening?

Not at all - 1  2  3  4  5-Completely

Calm Sleep?

Very restless - 1  2  3  4  5-very calm

Slept throughout the allotted time?

Woke up much to early - 1  2  3  4  5-yes

Ease of waking up?

Very difficult - 1  2  3  4  5-very easy

Ease of falling asleep?

Very difficult - 1  2  3  4  5-very easy

Amount of dreaming?

None - 1  2  3  4  5-much

Number of awakenings? ____________
Appendix B: Pittsburgh Sleep Quality Index

Sleep Quality Assessment (PSQI)

What is PSQI, and what is it measuring?
The Pittsburgh Sleep Quality Index (PSQI) is an effective instrument used to measure the quality and patterns of sleep in adults. It differentiates “poor” from “good” sleep quality by measuring seven areas (components): subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medications, and daytime dysfunction over the last month.

INSTRUCTIONS:
The following questions relate to your usual sleep habits during the past month only. Your answers should indicate the most accurate reply for the majority of days and nights in the past month. Please answer all questions.

During the past month,

1. When have you usually gone to bed?
2. How long (in minutes) has it taken you to fall asleep each night?
3. What time have you usually gotten up in the morning?
4. A. How many hours of actual sleep did you get at night?
   B. How many hours were you in bed?
5. During the past month, how often have you had trouble sleeping because you

| A. Cannot get to sleep within 30 minutes | Not during the past month (0) | Less than once a week (1) | Once or twice a week (2) | Three or more times a week (3) |
| B. Wake up in the middle of the night or early morning | |
| C. Have to get up to use the bathroom | |
| D. Cannot breathe comfortably | |
| E. Cough or snore loudly | |
| F. Feel too cold | |
| G. Feel too hot | |
| H. Have bad dreams | |
| I. Have pain | |
| J. Other reason(s), please describe, including how often you have had trouble sleeping because of this reason(s): | |

6. During the past month, how often have you taken medicine (prescribed or over the counter) to help you sleep?

7. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?

8. During the past month, how much of a problem has it been for you to keep up enthusiasm to get things done?

9. During the past month, how would you rate your sleep quality overall?

| Very good (0) | Fairly good (1) | Fairly bad (2) | Very bad (3) |

Scoring

| Component 1 | #9 Score | C1 |
| Component 2 | #3 Score (<15 min (0), 16-30 min (1), 31-60 min (2), >60 min (3)) + #4b Score (if sum is equal =0, 1=1, 2=2; 3=3, 5=5) | C2 |
| Component 3 | #4 Score (>7, 0; 6-7 (1), 5-6 (2), 4 (3)) | C3 |
| Component 4 | (total # of hours asleep) / (total # of hours in bed) x 100 | C4 |
| Component 5 | # sum of scores 6b to 8j (0=0, 1-9=1, 10-18=2, 19-27=3) | C5 |
| Component 6 | #6 Score | C6 |
| Component 7 | #7 Score + #8 score (0=0, 1=2; 3=4; 5=5) | C7 |

Add the seven component scores together Global PSQI ________

A total score of “5” or greater is indicative of poor sleep quality.

If you scored “5” or more it is suggested that you discuss your sleep habits with a healthcare provider.
Appendix C: Environmental Symptoms Questionnaire

### ENVIRONMENTAL SYMPTOMS QUESTIONNAIRE

<table>
<thead>
<tr>
<th>Subject #:</th>
<th>Date:</th>
</tr>
</thead>
</table>

#### How Do You Feel Questionnaire

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

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

5. SOURCE: Modified from Kobrick and Sampson (1979) and Sampson and Kobrick (1980).
Appendix D: Fatigue Scale

Fatigue Scale

**INDICATE YOUR LEVEL OF OVERALL FATIGUE RIGHT NOW**

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No Fatigue At All</td>
</tr>
<tr>
<td>1</td>
<td>Very Small Amount of Fatigue</td>
</tr>
<tr>
<td>2</td>
<td>Small Amount of Fatigue</td>
</tr>
<tr>
<td>3</td>
<td>Moderately Fatigued</td>
</tr>
<tr>
<td>4</td>
<td>Somewhat Fatigued</td>
</tr>
<tr>
<td>5</td>
<td>Fatigued</td>
</tr>
<tr>
<td>6</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Very Fatigued</td>
</tr>
<tr>
<td>8</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Extremely Fatigued</td>
</tr>
<tr>
<td>10</td>
<td>Completely Fatigued</td>
</tr>
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</table>