The Impact of Adequate Water Intake on Exercise Performance and Mood in Women and Men

by

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ABSTRACT

The purpose of this study was to evaluate the impact of improved hydration on endurance performance and mood in physically active adults. Participants (n = 72; age, 21.0 ± 3.0; 22.2% female) completed two two-mile run trials separated by exactly a week. Before each trial, participants provided a urine sample from the day before the run and a sample from the morning of the run. These samples were analyzed for urine osmolality (UOsm), urine specific gravity (USG), and urine color (U_{col}). UOsm and USG levels determined if the participants were placed in either the euhydrated or underhydrated group after the first trial. Those assigned to the euhydrated group were instructed to maintain their current fluid intake levels and those in the underhydrated group were instructed to increase fluid intake levels before the second trial. However, results were grouped by if they improved or maintained their hydration or not. The subjects also completed a Profile of Mood States (POMS) questionnaire before and after each trial to determine mood. Based on conditioning requirements for group assignment, 38% of subjects were classified as underhydrated. There were significant differences between the two trials for both subjects that improved and worsened their hydration in UOsm, USG, U_{col}, and thirst ($P < 0.05$). The group with improved hydration ran -15 ± 67 sec faster in the second trial, while the group that worsened hydration ran 4 ± 26 sec slower in the second trial. When these differences were compared between the two groups with a t-test, there was a trend for statistical differences with a one-way t-test analysis ($P = 0.06$). When results were split by sex no statistically significant differences were observed (male: -10.8 ± 63.6 sec; female: -29.4 ± 94.8 sec; $P > 0.05$). Improved hydration did not result in statistically significant difference in TMD or any of the individual mood sub-scales for either group for both males and females ($P > 0.05$). In conclusion, increased fluid intake to optimize hydration status may affect endurance exercise in young, healthy adults in a two-mile run, but no effect was seen on mood.

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CHAPTER 1

INTRODUCTION

Water is arguably the most essential yet the most understudied macronutrient in the human body, accounting for anywhere from 50 to 80 percent of body mass (James, 2019; Suh, 2021; He, 2020; Suh, 2019). Any significant decrease in this amount of water can lead to physical, mental, or emotional dysfunction, threatening survival (Masento, 2014; Hooper, 2014; Kavouras, 2012; Zhang, 2019). Maintaining proper hydration status is important for any living species, yet humans continue to consistently not adequately consume this critical nutrient. This deficiency can lead to an increase in chronic health issues and heat-related illnesses. Unfortunately, it can be complicated to determine one's specific water requirements for optimal hydration, as biological sex, age, body composition, overall health status, electrolyte intake, physical activity level, and environmental factors all play a role (Stookey, 2020). Therefore, making water recommendations inaccurate and seemingly impossible to establish for an entire population.

While more studies are being performed to look at endurance performance and mood outcomes of dehydration, the literature is deficient in studies that look at both of these outcomes together. Along with replicating past studies that look at just one of these as a dependent variable, the current study will look at whether increased water intake can increase both endurance performance and mood simultaneously in physically active individuals.

Past studies have also come to conclusions through the opposite effect of dehydrating their subjects and seeing decreased performance and mood results in both men and women (Young, 2019; Benton, 2011; Benton, 2015; Ramos-Jiménez, 2014; Montain, 1998; Casa, 2010; James, 2017). The current study, however, will be increasing fluid intake to optimize hydration status over a period of one week and hypothesizing improvement in performance and mood. Figure 1 shows the different aspects that can lead to increased risks of acute dehydration, which can also be used to safely yet quickly dehydrate subjects in hydration studies. The components in Figure 1 can be used to describe both voluntary and involuntary dehydration. The current study took a similar approach to Kavouras et al., where they educated children on the importance of hydration at a camp while increasing drink availability in certain areas of the camp, ultimately, enhancing performance in the children that increased fluid intake following these interventions (2012). Goulet et al. went above a euhydration state and chose a pre-exercise hyperhydration method, concluding that this can delay dehydration and improves endurance in cyclists (2008). The current study is also looking specifically at how a two-mile timed trial run can be affected, similar to intervention strategies used by Casa et al., where the subjects ran four 12-km timed trial runs (2010). James et al. also evaluated endurance performance, however, their study used an ergometer rather than timed trial runs to measure endurance performance (2017).

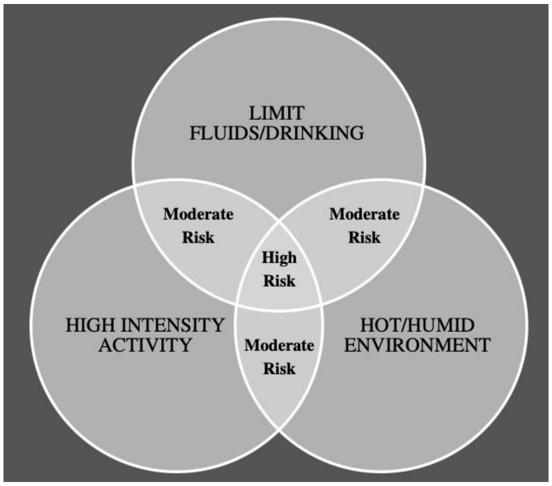


Figure 1. Components That Lead to Voluntary and Involuntary Acute Dehydration (adapted from Nuccio; 2017)

Purpose of Study and Hypothesis

Thus, the aim of the present study was to investigate the impact of improved hydration on aerobic exercise performance and mood in physically active men and women. The primary hypothesis for the present study was that aerobic endurance exercise performance and mood will improve with one week of increased water intake in physically active adults, with the null hypothesis being that performance and mood will not improve with increased water intake in the chosen population.

Definition of Terms

To understand research related to hydration and mood, one must have an exceptional understanding of specific concepts and words. Tests used in this research include urine specific gravity (USG), urine osmolality (UOsm), freezing point depression osmometry, and the Profile of Mood States (POMS) questionnaire. USG is the concentration of particles and density within urine in comparison to water (Flasar, 2008) using refractometry. UOsm is the concentration of particles dissolved within the urine (Najem, 2021) using freezing point depression osmometry. Freezing point depression osmometry involves using an osmometer to measure at what temperature a liquid freezes (Zhang, 2019). Lastly, the POMS questionnaire measures mood by looking at different sub-scales of mood through a series of words and statements where one must rate how they feel on a zero to four scale.

By using these urine assessments, clinicians can conclude whether a subject is hyperhydrated, hypohydrated, dehydrated, or euhydrated. The terms “dehydration” and “hypohydration” can be commonly used interchangeably throughout literature, when in fact, they represent different things. Dehydration is the process of the body losing or a loss of water due to discharging more water than taking in (Hew-Butler, 2018; Nuccio, 2017), while hypohydration is the sustainment of this decreased total body water (TBW) or water deficit (James, 2019). Therefore, chronic dehydration leads to a state of hypohydration. On the other side of the hydration spectrum, euhydration is a normal or optimal state of balanced hydration within the body (Hew-Butler, 2018). Any TBW level above this euhydrated state is considered hyperhydration. This state comes with a sustained increase in body water in excess (James, 2019).

Limitations and Delimitations

The findings of the current study may be limited by the use of a convenience sample for participants, subject compliance, researchers not being blinded to the group assignments, not having an established gold standard for measuring hydration status and mood, multiple clinicians running urine analyses, hydration levels varying from day-to-day, and significant environmental differences between the trials. Possible compliance issues could include subjects not properly replicating their diet for the second trial as instructed, taking urine samples not within the specified times, drinking caffeine or alcohol 24-hours before run trials, or not increasing water intake if assigned to the dehydrated group. The gold standard for hydration status measurement is highly debated within the scientific community, therefore, adding to the present study's limitations. Mood also does not have an established gold standard but the POMS questionnaire is widely used in mood research.

Through established inclusion and exclusion criteria, results cannot be generalized to other populations such as inactive individuals, people younger than 18 or older than 35, individuals with excluding conditions such as irritable bowel syndrome (IBS) or diabetes, or students at other universities other than Arizona State University (ASU). Aerobic endurance exercise was also used as physical activity within the present study, therefore, the results may also not be generalizable to other types of physical activity and exercise.

CHAPTER 2

REVIEW OF LITERATURE

Importance of Water

As the most abundant macronutrient in the human body, it is alarming that such a critical component of cells, tissues, and organs is overlooked in research and scarcely studied within the scientific community (Suh, 2021). Total body water (TBW) is the percent of water content within the body that contributes to weight, coming from both intracellular and extracellular water within the tissues. TBW can make up anywhere from 50 to 80 percent of body weight (BW) (James, 2019; He, 2020; Masento, 2014). Many factors contribute to TBW such as gender, age, health, weight, and water intake. Typically, adult female's TBW can range anywhere from 50 to 60 percent of their weight, while adult males TBW is slightly higher around 60 to 70 percent of their weight, leading to the conclusion that to prevent dehydration and sustain optimal hydration, men need more fluid than women (Ramos-Jiménez, 2014). Looking at how age affects TBW, water contributes to around 70 to 75 percent of newborns' total weight, 60 percent of children’s weight, and 50 to 55 percent of older adults' weight (Suh, 2019; Hooper, 2014). Anywhere from five to ten percent of TBW is turned over every day to help the body maintain stable fluid homeostasis (Suh, 2019), and any decrease in TBW can harm this necessary body function. Without water, in conjunction with electrolytes, many physiological processes within the body cannot occur to assist with survival, development, and homeostasis. These processes include cellular function, metabolism, osmotic pressure regulation, electrolyte balance, body temperature regulation, circulation, lymphatic system function, waste removal from cells and the body, digestion, lubrication, urinary tract function, and brain function (Masento, 2014; Hooper, 2014; Zhang, 2019). With 75 percent of one's brain mass coming from water, this high fluid-containing organ can be severely affected by decreased intake of fluids (He, 2020; Zhang, 2019). The brain, along with the rest of the human body, is so dependent on water, that death from lack of this macronutrient can occur in just a few days to within a week, which is faster than the absence of any other nutrient our body requires for survival (Hooper, 2014).

There are many biomarkers to determine hydration status, as shown in Table 1. Both blood and urine can be used in the assessment, but no gold standard for hydration status has been established (Benton, 2011). It is important to investigate and discuss the importance, key factors, analysis, and effect of water on the human body so that experts can better understand the impact this macronutrient has on endurance performance, mood, and overall health so that enhanced dehydration prevention and rehydration strategies can be developed.

Table 1. Markers of Hydration Status (adapted from Hew-Butler, 2018; Emerson, 2017)

Variable	**Normal Ranges**
Serum [Na^+] (mmol/L)	135 - 145
Urine [Na^+] (mmol/L)	40-220
Serum [K^+] (mmol/L)	3.5 - 5
Urine [K^+] (mmol/L)	25-120
Serum osmolality (mOsm/kg)	275 - 295
Urine osmolality (mOsm/kg)	300 - 900
Urine specific gravity	1.005 - 1.030

Physiological Breakdown of Dehydration

The human body is made to maintain homeostasis in all bodily systems. To maintain fluid balance when water intake levels are low, the body must conserve water through homeostatic measures involving hormones and the renal system to decrease water loss (Masento, 2014). This process involves increasing renal water absorption, stimulating thirst, and modifying the production of urine and its osmolality (James, 2019; Suh, 2021; Masento, 2014; Hooper, 2014). Thirst is an important component of hydration, as it facilitates a behavioral response to find and intake fluids to cease the feeling (James, 2019; Suh, 2021; Masento, 2014). The body does this in response to osmolality changes by releasing arginine vasopressin (AVP) (James, 2019; Suh, 2021; Masento, 2014; Hooper, 2014). This hormone, also known as antidiuretic hormone (ADH), is the main fluid regulatory hormone in the body and is highly associated with UOsm (Suh, 2019). Suh et al. also state that long-term high plasma AVP levels are correlated with anxiety and depressive disorders (2021). One of the main causes of high sodium levels in the body is decreased water intake and/or increased water loss (Hooper, 2014). This decrease in fluid intake raises sodium levels in the extracellular fluid (ECF) outside of the cell, and the body must equalize the osmolality between both the ECF and intracellular fluid (ICF) (Hooper, 2014). To do this, the ICF must exchange water for a portion of the excess sodium in the ECF, causing ICF osmolality levels to rise and the cell to shrink due to less fluid within it (Hooper, 2014). Osmoreceptors within the cell detect this, which leads to the release of AVP to activate the kidney medulla to retain water and produce more highly concentrated urine (Hooper, 2014).

Many factors with low water intake can lead to increased cardiovascular (CV) system strain. With a lack of fluid intake, decreased circulating blood volume or "hypovolemia" can occur (James, 2019). Other factors such as diet, environmental temperature, evaporation levels, activity level, and certain diseases can affect total fluid volume within the body. A decrease in volume also decreases cerebral blood flow, brain volume, stroke volume, and increases heart rate (Nuccio, 2017). These CV dysfunctions are exacerbated in hotter conditions (James, 2019). Decreased peripheral blood flow can be detrimental in hot environments or during exercise, as our body uses blood flowing to the skin to sweat and dissipate heat to cool off. With diminished evaporation, our body's main source of releasing heat, the CV system begins to overwork and eventually fail.

In addition to voluntary water loss through lack of intake, water is lost involuntarily through urine, feces, sweat, respiration, and skin (James, 2019). But can be gained back through food, drink, and metabolic or endogenous water formation within the body (James, 2019; He, 2020; Suh, 2019). Different levels of fluid loss have progressively different effects on the body. A fluid loss of less than one percent of body weight (BW) can have negative effects on the brain or one's mood and cause U_{col} to darken (He, 2020). With a fluid loss between one and two percent of BW, plasma osmolarity and thirst levels increase (He, 2020). And a loss of between two and four percent of BW can lead to low blood pressure, orthostatic intolerance, and decreased work efficacy (He, 2020). Fluid loss above eight percent of BW can cause mental and central nervous system (CNS) dysfunction, and a loss above 20 percent of BW can lead to death (He, 2020).

When the CNS and endocrine systems are affected by dehydration, the hypothalamic-pituitary-adrenal (HPA) axis is activated (Suh, 2021). The HPA axis is a system that responds to stress such as the body experiencing a threat or uncomfortable environment and when activated, stimulates the hypothalamus and amygdala (Suh, 2021). With dehydration, situations such as feelings of thirst or being in a hotter environment can be seen as a threat to survival, and through the HPA axis in our brain, we seek fluids and a more comfortable, cooler environment (Suh, 2021).

Impact of Dehydration on Health

A significant proportion of the United States population does not meet water intake recommendations, along with third-world countries in which they do not have access to clean, proper water sources (Suh, 2021). Sustained lack of this important nutrient can increase the risk of heat illnesses such as heat cramps, heat syncope, heat exhaustion, and heatstroke (Masento, 2014; Zhang, 2019; Hew-Butler, 2018). Heat syncope is caused by the venous pooling of blood, which reduces the amount of blood flow to the brain (González-Alonso, 1997). Lack of fluids can also lead to renal or urinary complications such as urinary tract infections (UTIs), kidney stones, and kidney failure (Masento, 2014; Zhang, 2019; Hew-Butler, 2018). As mentioned earlier, CV diseases, such as hypertension or stroke, can stem from consistent dehydration (Masento, 2014; Zhang, 2019; Hew-Butler, 2018). It only takes as little as a loss of two percent of BW from dehydration for the body to experience decreased thermoregulation (Hew-Butler, 2018).

Age is an important factor clinicians must take into consideration when understanding the effects of dehydration on one’s body. Infants, children, adults, and older adults all differentiate in body fluid percentage, dehydration signs and symptoms, aerobic capacity, sweat rates, body surface area, mass, and fluid regulation (Masento, 2014; Hooper, 2014; Kavouras, 2012). For example, newborn babies are typically around 70 percent body fluid, while children are around 60 percent, and older adults are even less at 50 percent body fluid (Hooper, 2014). With a much higher body water content, children are more likely to experience dehydration, with 60 percent of children around the world not meeting water intake guideline recommendations (Suh, 2019). Only 20 to 30 percent of older adults tend to be dehydrated (Suh, 2019). According to Kavouras et al., children tend to become dehydrated due to a lower max aerobic capacity and increased sweat rates due to a greater body surface area (BSA) to mass ratio (2012). This causes faster absorption of heat when the temperature of the air is higher than the temperature of their skin. Older adults, however, have an increased risk for dehydration due to other physiological changes such as decreased muscle mass, reduced kidney function, cognitive and physical disabilities, and decreased feelings of thirst due to polypharmacy or hormonal changes (Hooper, 2014). While the causes of increased dehydration in the younger and older populations may be different, the signs and symptoms are fairly similar. Signs and symptoms of dehydration in these populations include decreased skin elasticity, sunken eyes, reduced or abnormal skin color, decreased or dark urine, dry mouth or skin, and rapid heartbeat or "tachycardia" (Hooper, 2014). Hooper et al. also state that children may experience decreased capillary refill time, abnormal respiratory patterns, ill appearance, and abnormal radial pulse, while older adults may experience confusion, headaches, lethargy, dizziness, and orthostatic hypotension (2014).

Impact of Dehydration on Performance

Studying the effects of dehydration on performance has some complications and confounding variables. Specific methods for inducing dehydration in subjects, such as intense exercise and exposure to heat, can be uncomfortable or unfamiliar for them (James, 2019; Suh, 2021). This is a possible confounding variable in studies and could be the true cause of decreased performance results.

Another commonly found confounding variable within studies is subjects' awareness of their hydration status. If a patient knows they are well-hydrated, they may perform better. The same goes for subjects knowing they are dehydrated, either naturally or induced, and performing worse. If a subject is not blinded to their hydration status, they may assume they should be thirsty or become distracted. James et al. discuss this in their study, stating that thirst affects performance rather than hypohydration (2019). This same study aimed to eliminate the confounding variable of subjects knowing their hydration status by blinding their water intake by using intravenous (IV) or gastric tube delivery methods for fluids (2019). These blinded hydration methods also tested the theory of activated oropharyngeal receptors relieving thirst when fluids were ingested orally.

During physical activity, sweat loss rates are highly correlated with the intensity of the exercise (Nuccio, 2017). But on average, humans can lose one to two liters of sweat per hour (James, 2019), leading to fast hypohydration if fluids are not replaced properly. Getting to this hypohydration state could cause decreased muscle blood flow, impaired muscle metabolism, decreased cerebral blood flow, increased core body temperature, increased heart rate, increased CV strain, increased glycogenolysis, increased lactate levels, and limited peak oxygen uptake (James, 2019; Nuccio, 2017). Figure 2 shows the relationship between these physiological outcomes from hypohydration and how they affect performance. And when exposed to a hot environment, skin temperature increases even more (James, 2019). When the core temperature is elevated and skin blood flow rates are increased, this displaces blood away from the central blood volume which increases the strain on the CV system. Peripheral dilation allows heat to dissipate through the skin, but during physical activity, this process also competes with muscles for blood which causes an increased strain on the CV system (James, 2019). Losing the ability to evaporate is detrimental to the human body, as this is its primary way of heat loss (Nuccio, 2017). It has been concluded that dehydration impairs thermoregulatory functions and exercise performance independently of thermal, dietary, and metabolic stressors.

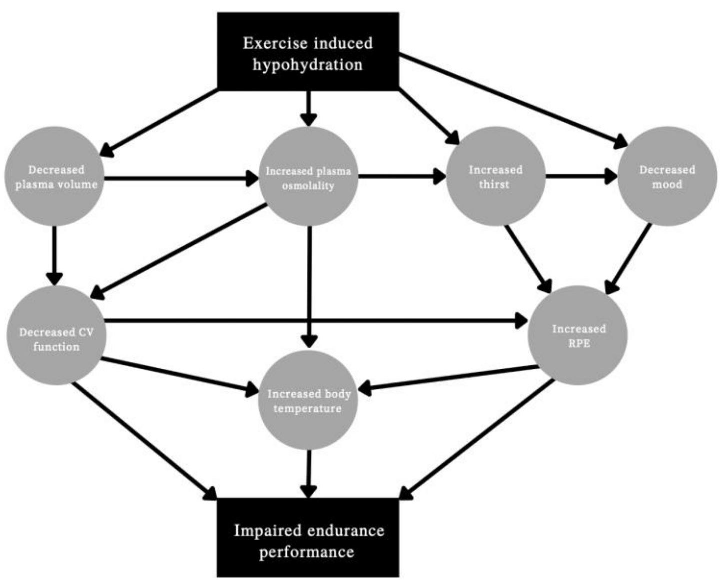


Figure 2. Physiological Effects of Hypohydration on Endurance Performance (adapted from James, 2019)

Impact of Dehydration on Mood

Like performance, studying and effectively measuring how dehydration affects overall mood can be difficult in the sense that limiting the confounding variables can be complicated. Subjects being uncomfortable when being introduced to unfamiliar methods of purposeful dehydration, such as exercising and heat exposure, can cause emotions such as nervousness and anger, which ultimately leads to discomfort and possible distraction of cognitive and physical tasks (James, 2019; Suh, 2021). Distraction during cognitive tasks and impaired performance may also come from the physical aspects of dehydration such as thirst, headaches, and fatigue (Nuccio, 2017). Dehydration also increases perceived exertion and tension (Masento, 2014; Zhang, 2019; Nuccio, 2017). With the exception of these outlying variables, there is no agreed-upon consensus on a psychological gold standard assessment tool for measuring fluctuations in mood, however, the POMS questionnaire is one of the most widely used during mood-related research (Liska, 2019). Zhang et al. (2019) and He et al. (2020) used POMS as a way of measuring in their studies to see if water deprivation had a negative effect on mood.

Overview

As mentioned earlier, the lack of literature on water does not correlate with the importance it has as a macronutrient within the human body. And while individual studies examine the effect of hydration levels on overall health, physical performance, cognition, and mood, we must look at these results simultaneously to observe a possible predominant relationship. We must also use the opposite approach and increase fluid intake to optimize hydration in participants to observe possible improvement. As it may unfortunately never be possible to fully eliminate the confounding variables involved in this type of research, consistency of continued experiments and published literature may be persuasive enough for the scientific community as a whole to realize the importance of this macronutrient concerning these functions. Other demographics must also be considered when researching this topic such as age and activity level. Older and younger populations thermoregulate differently than young adults. Therefore, the risks and hydration upkeep must increase to prevent heat illness and chronic disorders stemming from dehydration. Athletes and other physically active populations must take into consideration the amount of fluids they lose during activity so that they may stay optimally hydrated for optimal performance physically and mentally. But it must be kept in mind that there is a fine line between underhydration and overhydration. Too much water can be just as detrimental as too little water, so staying within the optimal levels between these high and low ranges is best for general health, performance, and mood.

CHAPTER 3

METHODOLOGY

Participants

Physically active, healthy females and males aged 18 to 35 that currently participate in ROTC and club sports at ASU were eligible to participate in the current study. Prior to being enrolled in the study, subjects completed a health history questionnaire [Appendix A] and a 26-question water frequency questionnaire. They were excluded based on recommendations from the medical director if they self-reported the use of certain medications (diuretics, antidepressants, injectable insulin) or were diagnosed with certain conditions (IBS or diabetes) which may have an impact on body fluid balance or thirst perception. Female subjects were excluded if they reported they were currently pregnant or lactating. Subjects were also excluded from analyses for not completing the second run trial, forgetting a urine sample, and turning in incomplete questionnaires. After receiving Institutional Review Board (IRB) approval [Appendix B], recruitment flyers were posted, and emails were sent out immediately containing a QR code [Appendix C]. Participants were considered enrolled in the study once they passed all screenings, satisfied both the inclusion and exclusion criteria and signed the informed consent statement via DocuSign.

Approximately 200 subjects were recruited and of those, 99 were screened and signed the consent form. 82 subjects participated in the study and of those, seven dropped out after completing the first trial with three not providing all of the required urine samples. Complete data collection was obtained on 72 participants in total.

All subjects were compensated with a pre-paid Tango gift card after completing the study via email. The gift card amount varied between how much they participated in the study and how fast their average run times were.

Study Design

Subjects participated in an endurance test on two separate occasions, separated by exactly one week in this randomized crossover study. If a subject participated in the first run trial and then was unable to attend the second, they were asked to start the process over at a later date so that their run trials were exactly a week apart. This situation only happened with one subject. During both trials, subjects completed a two-mile timed-trial run. The day before the first trial, subjects took a urine sample between the times of 4 PM and 8 PM and reported their perception of thirst at the time of urine collection with a 175 mm visual analog scale (VAS) [Appendix D]. The left anchor of the VAS (0 mm) indicated the absence of thirst with no desire to drink anything labeled "not thirsty at all", while the very right anchor (125 mm) represented the most thirst sensation they had ever had, labeled "extremely thirsty". The line also extended past the right anchor up to 175 mm if subjects felt as though their thirst level went beyond that point. Subjects were instructed to make one vertical mark with a writing utensil of their choice on the VAS scale to show their current level of thirst. Subjects repeated these steps for urine collection and thirst perception on the morning of the run. These same urine samples and thirst perception VAS procedures were used for trial two, with each subject bringing four urine samples (two PM and two AM) and four completed VAS (two PM and two AM) throughout the entirety of the study. Subjects also provided a 24-hour food log [Appendix E] from the day before the first trial, after being instructed to refrain from exercising and caffeine or alcohol intake. Subjects were asked to replicate their food log in 24 hours before the second trial. The subject's height was taken before the first trial run and they were weighed both before and after each trial run. Lastly, subjects completed the validated 35 question Adult Short POMS questionnaire (POMS 2®-A Short, Multi-Health Systems Inc.) [Appendix F] before and after each trial run. Responses were combined to determine total mood disturbances (TMD) as well as the seven mood sub-scales which include anger-hostility (AH), confusion-bewilderment (CB), depression-dejection (DD), fatigue-inertia (FI), tension-anxiety (TA), vigor-activity (VA), and friendliness. TMD scores range from -20 to 100 and are calculated through the following equation: (AH + CB + DD + FI + TA) - VA. Anger and hostility relate to the feeling of aggression. Confusion and bewilderment explain a state of being lost or confused. Depression and dejection are feelings of sadness or melancholy. Feelings of fatigue and inertia can be described as a tired, weak, or heavy feeling with maybe some disorientation. Tension and anxiety relate to the feeling of persistent fear or worrying. The feeling of vigor and activity describes a state of energy and enthusiasm. And friendliness is the feeling of being friendly and welcoming to others.

After the first trial, subjects were assigned to either the euhydrated or underhydrated group, based on the USG of their self-provided PM urine sample (euhydrated as <1.020 and underhydrated as ≥ 1.020). Subjects classified as euhydrated were instructed to maintain their current water intake levels and subjects classified as underhydrated were instructed to increase their water intake to meet the specific criteria of urinating at least seven times a day and having straw-like colored urine. Each subject also received a Brita® Premium 26oz Filtering Water Bottle at the conclusion of the first trial. Data and results were then analyzed by both hydration level improvement and sex.

The primary outcomes of the study were the completion time of the two-mile run and TMD scores from the POMS questionnaire. The secondary outcome measures were UOsm, USG, and U_{col}. And lastly, tertiary outcomes were thirst perception, heart rate immediately after the runs, height, and pre-and post-run body weight.

Biochemical Analysis

UOsm, USG, and U_{col} were used to calculate hydration status biomarkers during both trials. UOsm was measured using freezing point depression with an $A_2O^{®}$ Advanced Automated Osmometer (Advanced Instruments, Norwood, MA). USG was measured using refractometry with an ATAGO® Digital Pocket Refractometer (Atago, 3810 (PAL-1), Bellevue, WA). And U_{col} was measured using an eight-level urine color chart, with "1" representing the lightest and "8" representing the darkest color of urine. USG was measured immediately with the PM urine samples from the first trial run so we could inform the subjects what their group assignment was after their run and before they left. UOsm and Ucol for all samples and USG for the three other urine samples were measured at room temperature a couple of hours after the run trials in the lab. With the exception of samples from the second run trial on November 1st, 2021. USG and were assessed on these samples, however, the osmometer machine malfunctioned. These urine samples were stored in the laboratory refrigerator for two days until a technician was able to come to fix the machine. Urine samples remain stable for up to a week in a refrigerator and the samples were being frozen in the osmometer anyway, therefore, to our knowledge, we do not have reason to believe this affected our results.

The osmometer machine used was located in the hydration science lab in the Wexford Innovation Center building at the downtown ASU campus. The lab is run by Stavros Kavouras, Ph.D., FACSM, FECSS with the assistance of his Ph.D. student, Abigail Colburn, MS. The present study began obtaining data in April 2021, when Abigail originally coordinated and analyzed all urine samples using the osmometer, refractometer, and urine color chart. In September 2021, Dr. Kavouras' graduate student, Amanda Doyle, LAT, ATC, took over the role of coordinating the study and analyzing the urine specimens received. Abigail trained Amanda on how to use each urine assessment, specifically going through cleaning procedures with the osmometer and refractometer, and proper visual inspection of U_{col} such as using good lighting with the overhead lab lights and sunlight coming in through the windows with the urine color chart held right next to the container holding urine.

Sample Size Calculation

To detect significant differences in the endurance run, a total of 101 participants was required to reject the null hypothesis with mean differences between the first and second measurements between both groups of euhydrated and dehydrated. This number is based off sample sizes from previous studies examining similar variables. In anticipation of potential subject attrition and an approximate drop-out rate of ten percent, it was planned to recruit 120 subjects. It was estimated that 100 subjects would give the present study enough power to examine the study aims based on calculations from previous studies and the preliminary outcome of the endurance run.

Statistical Analysis

Data was entered using a personal computer device with JMP® Pro 16 (SAS Institute Inc), a statistical software, and accessed for normality through visual inspection and measurement of histogram skewness, where outliers were removed. A second authorized personnel re-entered the same data into the software, where a computerized cross-control accessed data conformity. Data is presented as mean ± standard deviation (SD). Once normally distributed, a two-way analysis of a variance model was used to check the difference between the two groups and pre- and post- intervention results. Normality was tested using the Shapiro-Wilk test. Log-transformation was used to test differences in non-normally distributed variables. Demographic characteristic differences and changes between the two were tested with a t-test. The threshold for statistical significance was a type I error of 0.05 or less.

CHAPTER 4

RESULTS

Participants

The subjects' baseline characteristics are shown in Table 2. Results have also been separated by sex, as past studies have shown significant result differences in hydration, exercise performance, and mood between males and females.

Table 2. Subject Characteristics (mean ± SD)

	Male	**Female**	**Total**
N	56	16	72
Age (y)	20.9 ± 3.0	21.6 ± 2.9	21.0 ± 3.0
Height (m)	1.79 ± 0.07	1.66 ± 0.08	1.75 ± 0.09
Weight (kg)	76.6 ± 11.5	61.2 ± 6.8	73.2 ± 12.4
BMI (kg/m^2)	24.0 ± 3.0	22.7 ± 2.7	23.7 ± 3.0

N, number of subjects; BMI, body mass index; y, years; m, meters; kg, kilograms

Hydration

The subjects' baseline hydration data by sex is shown in Table 3. Hydration status was assessed using the provided PM sample from the day prior, as this has been shown to be the most equivalent of a 24-hour urine sample. Based on conditioning requirements for group assignment (<1.020 USG indicating euhydrated, ≥1.020 USG indicating underhydrated), 38% of subjects were classified as underhydrated. Of those classified as underhydrated, 41% were male and 25% were female. There were only significant differences between males and females for U_{col} (P = 0.040). When urine data was analyzed by trial and improvement status, there were significant differences between all urine biomarkers (Table 4). Environmental data averages for both trials can be found in Table 5 where the highest environmental readings from each day during the time the subjects were running were used. There were significant differences between trials in ambient temperature (P = 0.023), globe temperature (P = <0.001), and relative humidity (P = <0.001).

Table 3. Baseline Hydration Data by Sex (mean ± SD)

	Male *(n = 56)*	**Female** *(n = 16)*	***P*-value**
UOsm (mmol/kg)	720 ± 320	575 ± 355	0.060
USG	1.019 ± 0.009	1.015 ± 0.010	0.057
U_{col} **(1-8)**	3.46 ± 1.21	2.94 ± 1.61	0.040*
Thirst (mm)	48.42 ± 24.58	50.56 ± 30.10	0.385
Underhydrated (%)	41%	25%	

UOsm, urine osmolality; mmol, millimoles; kg, kilogram; USG, urine specific gravity; U_{col} , urine color; mm, millimeters; *, significant difference between sexes

Table 4. Hydration Results by Improvement Status and Trial (mean ± SD)

	Improved			**Worse**		
	1st Trial	**2nd Trial**	***P*-value**	**1st Trial**	**2nd Trial**	***P*-value**
UOsm (mmol/kg)	816 ± 288	529 ± 286	<0.001*	486 ± 295	692 ± 304	<0.001*
USG	1.022 ± 0.007	1.014 ± 0.007	<0.001*	1.012 ±0.008	1.018 ± 0.008	<0.001*
U_{col} **(1-8)**	3.84 ± 1.10	2.84 ± 1.18	<0.001*	2.57 ± 1.26	3.29 ± 1.36	0.002*

Thirst (mm)	41.82 ± 25.79	33.86 ± 22.35	0.001*	49.04 ± 26.07	40.21 ± 22.83	0.038*

UOsm, urine osmolality; mmol, millimoles; kg, kilogram; USG, urine specific gravity; U_{col} , urine color; mm, millimeters; *, significant difference between trials

Table 5. Average Environmental Characteristics from Each Trial (mean ± SD)

	1st Trial	**2nd trial**	***P*-values**
TA (°C)	21.92 ± 2.98	21.19 ± 2.95	0.023*
TG (°C)	23.14 ± 3.21	21.51 ± 3.73	<0.001*
WBGT (°C)	16.39 ± 2.57	15.97 ± 2.54	0.064
RH (%)	39.23 ± 6.54	45.39 ±11.05	<0.001*

TA, ambient temperature; TG, globe temperature; WBGT, wet bulb globe temperature; RH, relative humidity; *, significant differences between trials

Endurance Performance

For overall two-mile run time performance, in the second run trial, those that increased their hydration status (n = 44) ran the two-mile run in 13.43 ± 1.90 minutes as opposed to their first run trial time average of 13.67 ± 2.13 minutes (*P* = 0.168) (Table 6). In the first run trial, those that worsened their hydration status (n = 28) ran the two miles in 14.07 ± 1.05 minutes while in the second run trial they ran it in 14.13 ± 2.12 minutes (*P* = 0.483). When data was analyzed by sex and trials, on average, male subjects ran their first run trial in 13.28 ± 1.89 minutes and 13.19 ± 1.90 minutes in the second run trial (*P* = 0.469) (Table 7). On average, female subjects ran their first run trial in 15.76 ± 1.59 minutes and 15.48 ± 1.85 minutes in the second run trial (*P* = 0.377). Run time differences for those that improved their hydration can be found in Table 8 and for those that worsened their hydration status can be found in Table 9. It was found that the time to complete the run in males was 10.8 ± 63.6 seconds faster (*P* = 0.313) in the group that improved their hydration (n = 35) compared to the group of males whose hydration became worse (n = 21) and finished 4.8 ± 26.4 seconds slower (*P* = 0.429). This was similar in the female subjects, where the time to complete the runs was 29.4 ± 94.8 seconds faster (*P* = 0.376) in females that improved than hydration (n = 9) than in the ones who decreased their hydration (n = 7) and finished 0.06 ± 27.6 seconds slower (*P* = 0.987). There were no significant differences found in any run time performance between hydration status improvement, trial, or sex (*P* > 0.05). Figure 3 shows mean run times for both hydration improvement groups in each trial.

Table 6. Run Times by Trial and Hydration Improvement (mean ± SD)

	1st Trial	**2nd Trial**	***P*-value**
Improved *(n = 44)*	13.67 ± 2.13 min.	13.43 ± 1.90 min.	0.168
Worsened *(n = 28)*	14.07 ± 1.05 min.	14.13 ± 2.12 min.	0.483

Table 7. Run Times by Trial and Sex (mean ± SD)

	1st Trial	**2nd Trial**	***P*-value**
Males *(n = 56)*	13.28 ± 1.89 min.	13.19 ± 1.90 min.	0.469
Females *(n = 16)*	15.76 ± 1.59 min.	15.48 ± 1.85 min.	0.377

Table 8. Run Time Difference for Subjects that Improved Hydration (mean ± SD)

	Time Δ	***P*-value**
Males *(n = 35)*	-10.8 ± 63.6 sec.	0.313
Females *(n = 9)*	-29.4 ± 94.8 sec.	0.376

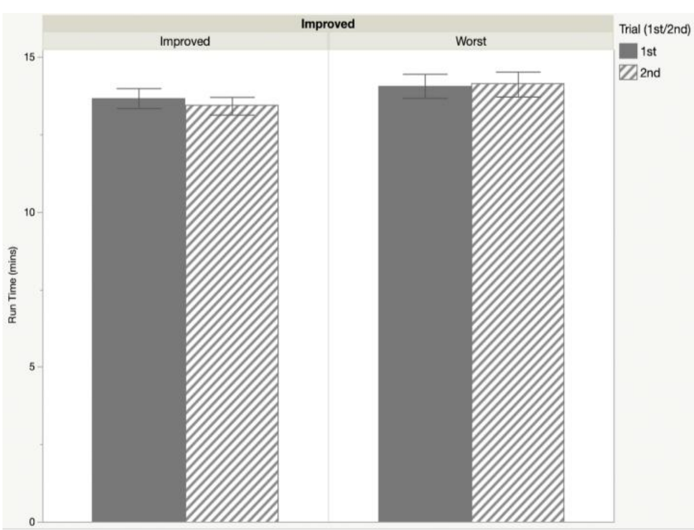
Δ, mean difference; minus sign (-), decreased time (improved performance)

Table 9. Run Time Difference for Subjects that Worsened Hydration (mean ± SD)

	Time Δ	***P*-value**
Males *(n = 21)*	+4.8 ± 24.6 sec.	0.313
Females *(n = 7)*	+0.06 ± 27.6 sec.	0.987

Δ, mean difference; plus sign (+), increased time by (worsened performance)

Figure 3. Mean Run Times for Both Groups in Both Trials



Mood

Pre- and post- run POMS mean difference scores separated by hydration status improvement and trials are shown in Table 10. These same variables separated by sex and trials are shown in Table 11. There were no significant differences found in any sub-scale or overall TMD for hydration status improvement, trial, or sex ($P > 0.05$).

Table 10. Pre- and Post- Run TMD and Sub-Scale Score Mean Differences by Improvement and Trial (mean ± SD)

	Improved			**Worse**		
	1st Trial	**2nd Trial**	***P*-value**	**1st Trial**	**2nd Trial**	***P*-value**
AH Δ	0.23 ± 2.78	0.16 ± 1.48	0.571	-0.59 ± 1.45	-0.19 ± 1.14	0.356
CB Δ	0.65 ± 1.74	0.41 ± 1.66	0.445	0.37 ±1.57	-0.03 ± 1.93	0.350
DD Δ	0.20 ± 1.79	-0.09 ± 1.20	0.295	-0.04 ± 1.34	-0.15 ± 1.46	0.660
F Δ	-2.21 ± 3.32	-1.43 ± 2.47	0.126	-1.11 ± 3.58	-0.39 ± 2.33	0.217
FI Δ	4.27 ± 4.81	3.59 ± 4.61	0.253	2.86 ± 4.58	2.50 ± 5.61	0.681
TA Δ	0.02 ± 2.57	-0.02 ± 1.89	0.908	-0.54 ± 1.66	-0.75 ± 2.37	0.658
VA Δ	1.27 ± 3.38	0.34 ± 3.56	0.091	1.14 ± 3.90	0.93 ± 3.57	0.711
TMD Δ	3.83 ± 10.77	4.17 ± 9.88	0.749	0.64 ± 9.15	0.81 ± 8.14	0.675

AH, anger-hostility; CB, confusion-bewilderment; DD, depression-dejection; FI, fatigue-inertia; F, friendliness; TA, tension-anxiety; VA, vigor-activity; TMD, total mood disturbance; Δ, mean difference

Table 11. Pre- and Post- Run TMD and Sub-Scale Score Mean Differences by Sex and Trial (mean ± SD)

	Males			**Females**		
	1st Trial	**2nd Trial**	***P*-value**	**1st Trial**	**2nd Trial**	***P*-value**
AH Δ	-0.26 ± 2.45	-0.56 ± 1.39	0.916	0.50 ± 2.07	0.33 ± 1.23	0.607
CB Δ	0.46 ± 1.76	0.21 ± 1.80	0.424	0.81 ±1.38	0.31 ± 1.74	0.228
DD Δ	0.07 ± 1.73	-0.05 ± 1.38	0.615	0.25 ± 1.24	-0.40 ± 0.91	0.117
F Δ	-1.39 ± 3.40	-0.86 ± 2.35	0.189	-3.13 ± 3.36	-1.63 ± 2.80	0.118
FI Δ	3.98 ± 4.91	3.20 ± 5.38	0.181	2.81 ± 4.10	3.06 ± 3.57	0.770
TA Δ	-0.43 ± 2.32	-0.03 ± 2.25	0.639	0.63 ± 1.89	-0.44 ± 1.50	0.094
VA Δ	1.32 ± 3.69	0.77 ± 3.57	0.189	0.88 ± 3.18	-0.13 ± 3.52	0.359
TMD Δ	2.18 ± 10.84	2.57 ± 9.88	0.707	4.13 ± 8.16	4.07 ± 7.02	0.614

AH, anger-hostility; CB, confusion-bewilderment; DD, depression-dejection; FI, fatigue-inertia; F, friendliness; TA, tension-anxiety; VA, vigor-activity; TMD, total mood disturbance; Δ, mean difference

CHAPTER 5

DISCUSSION

In the present study, we examined the effects of increased and decreased hydration on endurance performance and mood. Hydration status during exercise can be affected voluntarily and involuntarily by limited fluid intake, high intensity activity, and a hot or humid environment (Nuccio, 2017). Changes in hydration status were confirmed in this study through assessing multiple urine biomarkers including UOsm, USG, U_{col}, and thirst, however, only USG was used for group assignment. All four of these secondary outcomes measures showed significant hydration differences between both trials in subjects that improved and worsened their hydration status.

After both run trials, those that improved their hydration status ran the two miles faster than those whose hydration status worsened. Concluding the second run trial, 62.5% of the male subjects (n = 35) and 56.3% of female subjects (n = 9) had increased their hydration status and decreased their average run times. The positive impact of increased hydration status on run time results are consistent with another study using optimization of hydration levels as the independent variable to improve endurance performance in timed run trials of a specific distance (Kavouras, 2012). However, this other study did not assess the effects on mood.

In agreement with the present study, TMD scores through POMS questionnaires have been shown to vary with hydration status (Suh, 2021; He, 2020; Zhang, 2019). These same studies reported data for each sub-scale component of TMD as well, showing significant effects in some but not all sub-scales. However, the specific sub-scales that were affected varied for each study. One counterbalanced, crossover trial study by looking specifically at cellular dehydration showed significant differences in the CB, DD, and FI in 49 adult subjects of both sexes (Suh, 2021). Another study looking at dehydration and rehydration effects using 12 male college students showed significant differences in both FI and VA (Zhang, 2019). Lastly, a randomized control trial study the effects of amount and frequency of fluid intake only showed a significant difference with the VA sub-scale with 92 adult subjects (He, 2020). However, the present study observed no statistically significant increase in TMD scores or its' sub-scale components from improved hydration status.

Previous studies reported that dehydration induced by exercise could decrease performance and mood (James, 2019; Zhang, 2019; He, 2020). Though subjects significantly improved hydration according to the hydration biomarkers assessed, the influence of hydration status improvement on endurance performance in the form of a two-mile timed trial run and mood using the POMS questionnaire still remains unclear. A one-tail t-test analysis of run time differences calculates a *p*-value of 0.060. While this is not statistically significant, there is a trend towards significance and perhaps a larger sample size with enough statistical power could show significance. In terms of TMD and its' sub-scales, hydration status and sex differences have been reported in previous studies (Suh, 2021; He, 2020; Zhang, 2019). All of the validated measures for mood in the present study showed no statistical significance between improvement groups or sex.

CHAPTER 6

CONCLUSION

The present study indicated that increased fluid intake to optimize hydration status may affect endurance exercise in young, healthy adults in a two-mile run through a one-way analysis of run time differences, but no effect was seen on mood. The underlying mechanism to explain these run time differences with no effect on mood remain to be determined but may be related to increased rate of perceived exertion by the ability to run faster and exhaust the body more. Future studies should continue to conduct similar designs with larger sample sizes, with different populations and forms of exercise, and be more effective in improving hydration status. Continued research looking at different populations and perspectives could help show causal conclusions and significance.

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APPENDIX A

IRB APPROVAL

ASU Knowledge Enterprise Development

APPROVAL: EXPEDITED REVIEW

Stavros Kavouras
Nutrition
(602) 496-2547
Stavros.Kavouras@asu.edu

Dear Stavros Kavouras:

On 3/21/2021 the ASU IRB reviewed the following protocol:

Type of Review:	Initial Study
Title:	The impact of adequate Water intake on Exercise performance and MOood in women and men
Investigator:	Stavros Kavouras
IRB ID:	STUDY00013646
Category of review:	
Funding:	Name: Arizona State University (ASU), Grant Office ID: GF2226, Funding Source ID: n/a
Grant Title:	GF2226;
Grant ID:	GF2226;
Documents Reviewed:	• Consent form cleared, Category: Consent Form; • flyer, Category: Recruitment Materials; • food diary, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • Grant Award letter, Category: Grant application; • Letter of award, Category: Sponsor Attachment; • Medical History + PAR-Q, Category: Screening forms; • POMS, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • ROTC study support, Category: Other; • Study Protocol cleared, Category: IRB Protocol; • Survey Review approval letter, Category: Other; • Thirst, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • Water intake questionnaire, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions);

The IRB approved the protocol from 3/21/2021 to 3/20/2022 inclusive. Three weeks before 3/20/2022 you are to submit a completed Continuing Review application and required attachments to request continuing approval or closure.

If continuing review approval is not granted before the expiration date of 3/20/2022 approval of this protocol expires on that date. When consent is appropriate, you must use final, watermarked versions available under the "Documents" tab in ERA-IRB.

In conducting this protocol you are required to follow the requirements listed in the INVESTIGATOR MANUAL (HRP-103).

Sincerely,

IRB Administrator

cc:

Jason Siegler
Hunter Kleinschmidt
Emma Haas
Van Dexter Calo
Taylor Shiflet
Abigail Colburn
Floris Wardenaar

APPENDIX B

MEDICAL HISTORY QUESTIONNAIRE

Medical History

aadoyle14@gmail.com (not shared)
Switch account

Name

Your answer

Date

MM DD YYYY
/ /

Do you have a medical condition that necessitates you drink more or less water than you would otherwise choose?

- Yes
- No

Do you currently restrict or supplement your sodium intake above and beyond normal healthy eating?

- Yes
- No

Are you currently taking any medications that alter your body water regulation (i.e., diuretics)?

- Yes
- No

Do you have irritible bowel syndrome or any other disease that results in frequent diarrhea?

- Yes
- No

Do you currently use injectable insulin therapy?

- Yes
- No

Are you currently taking aniti-depressant medication (i.e., Prozac)?

- Yes
- No

Have you ever had surgery on your digestive tract (excluding appendectomy)?

- Yes
- No

Has your weight fluctuated more than 5lbs in the past month or have you made significant dietary changes?

- Yes
- No

Are you currently breastfeeding?

- Yes
- No

Have you ever been hospitalized for either dehydration or hyponatremia (i.e., water intoxication)?

- Yes
- No

Please check the box next to any of the following illnesses with which you have ever been diagnosed or for which you have been treated, and describe in the next question.

- High blood pressure
- Asthma
- Bladder problems
- Coronary artery disease
- Elevated cholesterol
- Epilepsy (seizure)
- Anemia
- Lung problems
- Diabetes
- Kidney problems
- Heart problems
- Chronic headaches

If you selected any illnesses in the previous question, please describe each one below. If you have been diagnosed with or treated for diabetes, how many years have you been diagnosed/treated?

Your answer

Have you had any other significant illnesses not listed previously?

- Yes
- No

Do you currently have any illness?

- Yes
- No

Do you know of any other reason why you should not participate in this study?

- Yes
- No

Please list all medications or drugs (including recreational) you are currently taking (i.e., drug/supplement/vitamin name, dose, and frequency [e.g., daily, 2x/day, etc.]). Make sure to include over-the-counter medications and birth control pills.

Your answer

Please list all allergies you have (i.e., substance name and reaction).

Your answer

How many bowel movements do you typically have?

- >1/day
- 1/day
- 5/week
- 3-4/week
- <3/week

What do you consider your physical activity level to be?

- Sedentary (no exercise)
- Inactive-occasional light activity (walking)
- Active-regular light activity and/or occasional vigorous activity
- Heavy work-regular vigorous activity

Has your doctor ever said that you have a heart condition?

- Yes
- No

Has your doctor ever said that you have high blood pressure?

- Yes
- No

Do you feel pain in your chest at rest, during your daily activities of living, OR when you do physical activity?

- Yes
- No

Do you lose balance because of dizziness OR have you lost consciousness in the last 12 months? Please answer NO if your dizziness was associated with over-breathing (including during vigorous exercise).

- Yes
- No

Have you ever been diagnosed with another chronic medical condition (other than heart disease or high blood pressure)?

- Yes
- No

If you have ever been diagnosed with another chronic medical condition (other than heart disease or high blood pressure), please list condition(s) and medications here.

Your answer

Do you currently have (or have had within the past 12 months) a bone, joint, or soft tissue (muscle, ligament, or tendon) problem that could be made worse by become more physically active? Please answer NO if you had a problem in the past, but it does not limit your current ability to be physically active.

- Yes
- No

If you currently have (or have had within the past 12 months) a bone, joint, or soft tissue (muscle, ligament, or tendon) problem that could be made worse by become more physically active, please list

Your answer

Submit　　Clear form

APPENDIX C

RECRUITMENT FLYER AND EMAIL

"The impact of water intake on exercise performance and mood (WEXMO)"

The study involves running of a 2-mile run at two days one week apart (days still need to be scheduled).
You will be asked to provide urine samples and fill out some questionnaires.
The week in between the runs you will receive a water bottle with instructions to increase your water intake.

Upon completion of the study **you will receive $75** in the form of gift cards.
Additionally, you will have a chance to also receive $10-$40 based on your running performance.

If you are interested scan the QR code or visit http://bit.ly/wexmo21
We will contact you with consent form and medical history forms.

APPENDIX D

THIRST VISUAL ANALOG SCALE

AFTERNOON (4:00 pm – 8:00 pm • Day before Run)

Subject #:____ Date : _______ Trial: 1st ☐ - 2nd ☐

How Thirsty do you feel now?

not at all

I do not want to drink anything

extremely thirsty

The thirstiest I had ever been till now

First Morning (Day of Run)

Subject #: ______ Date : _________

How Thirsty do you feel now?

not at all

I do not want to drink anything

extremely thirsty

The thirstiest I had ever been till now

APPENDIX E

24-HOUR FOOD LOG

Food Log

1 **All foods** consumed should be recorded.

2 **Be very specific**. Make sure you include:
- the **type** of food
- the **amount** of each food
- the **preparation method** (i.e., fried, baked)
- the **brand name** of the food (if applicable)
- the **time** it was eaten
- the restaurant you ate it at (i.e., Subway, Applebees, Red Robin)

3 Record food/beverage consumption **after each meal/snack** instead of waiting until the end of the day.

4 **Save labels** from packages of food you eat and return them with your food record forms (this will greatly assist and enhance our analysis of your true nutrient intake).

5 Use nutrient descriptors (e.g., low-fat, low-carb, fat-free, light, reduced calorie, etc.).

6 **Include miscellaneous items** such as condiments (ketchup, salad dressing, mayonnaise, jams, creams, sugar), and chewing gum.

7 Record all **vitamins, minerals, herbals, supplements** (powders, shakes, bars, etc.).

8 Be careful about **"amount"** we would like these values as specific as possible. For packaged food providing "1 serving" is adequate, for home made food please use standard measuring cups and spoons, weights and/or volumes. Examples: cup (c), Tablespoon (Tbs), teaspoon (tsp), fluid ounces (fl oz),weight ounces (oz), inches (in), milliliter (mL), gram (g), or centimeter (cm).

EXAMPLE* **Subject #:** **Date: 1/1/2001**

Time	Detailed food description: brand name, restaurant, method of preparation, flavor, condiments etc.	Amount
8:30 AM	Egglands best eggs, pan-fried	3 eggs
8:30 AM	Wright brand bacon, pan-fried	2 pieces
8:30 AM	Wonder bread, toasted with butter	2 slices
12:30 PM	Cheeseburger from Chilis, with ketchup and lettuce	1 cheeseburger
12:30 PM	Baked potato from Chilis, with butter and salt	1 potato
7:00 PM	Steak, grilled with salt and olive oil	½ lb. steak
7:00 PM	Asparagus	6 pieces

Repeat this diet the day before each testing dat

Subject #: **Date:** **Trial:**

Time	Detailed food description: brand name, restaurant, method of preparation, flavor, condiments etc.	Amount

APPENDIX F

PROFILE OF MOOD STATES QUESTIONNAIRE

POMS2® – Adult Short

By Juvia P. Heuchert, Ph.D. & Douglas M. McNair, Ph.D.

ID:

Administration Date MM DD YYYY

Administration Time HH MM ○ AM ○ PM

Shade circles like this: ●
Not like this: ⊙ ○ ⊗ ✓

To the Respondent:
Below is a list of words that describe feelings that people have. Please read each word carefully, then shade in the circle that best describes:

how you feel RIGHT NOW.

Shade circles like this: ●
Not like this: ⊙ ○ ⊗ ✓

		Not at all	A little	Moderately	Quite a bit	Extremely
1.	Friendly	⓪	①	②	③	④
2.	Tense	⓪	①	②	③	④
3.	Angry	⓪	①	②	③	④
4.	Worn out	⓪	①	②	③	④
5.	Lively	⓪	①	②	③	④
6.	Confused	⓪	①	②	③	④
7.	Considerate	⓪	①	②	③	④
8.	Sad	⓪	①	②	③	④
9.	Active	⓪	①	②	③	④
10.	Grouchy	⓪	①	②	③	④
11.	Energetic	⓪	①	②	③	④
12.	Panicky	⓪	①	②	③	④
13.	Hopeless	⓪	①	②	③	④
14.	Uneasy	⓪	①	②	③	④
15.	Unable to concentrate	⓪	①	②	③	④
16.	Fatigued	⓪	①	②	③	④
17.	Helpful	⓪	①	②	③	④

		Not at all	A little	Moderately	Quite a bit	Extremely
18.	Nervous	⓪	①	②	③	④
19.	Miserable	⓪	①	②	③	④
20.	Muddled	⓪	①	②	③	④
21.	Bitter	⓪	①	②	③	④
22.	Exhausted	⓪	①	②	③	④
23.	Anxious	⓪	①	②	③	④
24.	Good-natured	⓪	①	②	③	④
25.	Helpless	⓪	①	②	③	④
26.	Weary	⓪	①	②	③	④
27.	Bewildered	⓪	①	②	③	④
28.	Furious	⓪	①	②	③	④
29.	Trusting	⓪	①	②	③	④
30.	Bad-tempered	⓪	①	②	③	④
31.	Worthless	⓪	①	②	③	④
32.	Vigorous	⓪	①	②	③	④
33.	Uncertain about things	⓪	①	②	③	④
34.	Drained	⓪	①	②	③	④
35.	Enthusiastic	⓪	①	②	③	④

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Please ensure you have answered every item.
Thank you for completing this questionnaire.

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