The Relationship and Seasonal Changes of Hydration Measures in Collegiate Wrestlers

Thesis

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By

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Abstract

**Purpose:** The purpose of this study is to evaluate collegiate wrestlers and their state of hydration before and after a Division I collegiate wrestling season. **Methods:** Eight collegiate wrestlers (mean ± SD; 19.88 ± 1.05 yrs) gave informed consent to participate in the investigation. Measurements were obtained during the middle of the season and after the season. Height and body mass, a urine sample, a venous blood sample and a VAS thirst scale were obtained at each visit. Prior research had indicated that all hydration measures may not give the same answer and was a focal point of this study. Therefore, hydration status was evaluated using two different testing criteria, urine specific gravity (USG) [two methods] via refractometry and plasma osmolality using a biochemical assay. **Results:** Body mass was not significantly ($p \leq 0.05$) different between the two testing time points. Measurements for USG, regardless of methods were not significantly different between visits but plasma osmolality was significantly different with test visit 1 at the beginning of the season, producing significantly higher values than after the season. For USG a slight level of hypohydration existed but plasma osmolality indicated a bit higher level. No changes were seen between perceived level of thirst between the two testing time points. The two methods of measuring USG were highly correlated $r > 0.95$. However, USG and plasma osmolality were not correlated. **Conclusion:** The primary findings of this study were that significant differences exist from during the season compared to after season for plasma osmolality which is not reflected by USG despite no body mass differences. Finally, USG and the gold standard measure of hydration, plasma osmolality do not track hydration in a similar manner and
are not correlated. Thus, consistent with prior research these two measures should not be
considered to be identical in their evaluation of hydration status.

Key Words: DEHYDRATION, URINE SPECIFIC GRAVITY, BLOOD PLASMA
OSMOLALITY, COLLEGIATE WRESTLERS.
Dedication

This document is dedicated to my loving family and friends for supporting me through this crazy adventure.
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Chapter 1 Introduction

The sport of wrestling makes up 23% of the approximately 2,500 National Collegiate Athletics Association (NCAA) Division I student athletes. While wrestling is a sport that requires substantial strength, endurance, and power, it also necessitates consistent fluctuation in weight as a part of participation. Changes in body composition and hydration status can significantly affect the performance of these athletes. Increasing concern for collegiate wrestlers has been recognized with regards to their weight reduction practices and fluid intake restrictions. The NCAA has taken steps to mitigate these challenges and has implemented a minimal weight program (i.e. minimum weight loss for the season at 5% body fat) and official, day of, pre-competition weigh-ins 1-2 hours in advance help ensure the safe participation of the athletes. The NCAA has also identified a urine specific gravity (USG) less than or equal to 1.020 g/cm³ as an indicator of proper hydration. Anything greater than 1.020 g/cm³ is considered “failed” and the wrestler must be retested no sooner than 24 hours after the initial assessment. The inherent challenge in utilizing USG values for assessment lies in their capacity to shift after relatively modest consumption of fluids (591-1,000 mL) and an overnight fast. More recent studies have shown that the consumption of 1,000 mL of water reduced USG to acceptable values (1.026 vs. 1.013 g/cm³) thus allowing athletes to unknowingly participate at potentially harmful levels of dehydration.

The Ohio State University Division I wrestling team, represents an elite group of wrestlers at the top of the collegiate ranks and a group of which hydration status is an
important element for optimal performance. Urine specific gravity and blood plasma osmolality are common tests used to measure an individual’s hydration status. To gain an understanding of the differences of hydration in the sport of wrestling, comparisons of hydration status during and after the season are needed to help evaluate the different measurements of hydration status. Measurement of plasma osmolality is considered the “gold standard” test for hydration status, ranging from 280-285 mOsm·kg$^{-1}$ for healthy individuals but for wrestlers in particular it has been seen that this can regulate at a higher level than normal$^{68}$.

Wrestlers often do not feel the effects of their hypohydration but when compared to baseline, measures of grip strength, isokinetic fast velocity knee extension torque, isokinetic slow velocity elbow extension torque and plasma dopamine concentrations tests are significantly lower after just one match$^{42}$. Secondary to hypohydration, participation in multiple matches over a short period of time can lead to deficits of fatigue, vertical jump, reaction time, and other physiological markers. Thirst may produce subjective feelings of fatigue or loss of vigor, but attempts to measure somatosensory gating and performance are thus far inconclusive$^{54}$.

Urine specific gravity (USG) has been used predominately in large groups as a time efficient, safe, non-invasive, inexpensive, and perceived accurate indicator of hydration. Urine specific gravity has been observed to be just as predicative as plasma osmolality in detecting low levels of exercise-induced dehydration. However, recent
studies have called into question the equivalency of the two measures, because their interpretation of hydration or the level of dehydration has varied in athletes. In a study done by Oppliger et al. it was observed that of the 27 subjects they tested, 31.3% of the euhydrated subjects (TN) were correctly classified for the 1.020 cut-off for USG and 80.0% of the dehydrated (TP) were correctly classified. This study alone shows a significant variation in the correlation of USG and plasma osmolality.

*Purpose:*

The primary purpose of this investigation was to examine the hydration state of collegiate wrestlers during their wrestling season and as well as after the season using two different measurement methods to determine if hydration levels indicated the same clinical interpretation. A secondary purpose was to determine if the two methods were correlated in their measurements. It was hypothesized that a state of chronic dehydration would exist during the season and would continue after the season. It is also hypothesized that the two measures of hydration would not be significantly correlated.

*Definition of Terms:*

**Urine Specific Gravity** – A urine test that compares the density of urine to the density of water.

**Blood Plasma Osmolality** – Plasma osmolality is a measure of different solutes in plasma. Osmolality is defined by osmoles per kilogram of water.

**Dehydration** – An abnormal depletion of body fluids.
Euhydration – Normal state of body water content; absence of absolute or relative hydration or dehydration.

Hypohydration – The provision of less than the normal amount of water in the body to meet its metabolic demands; dehydration of the human or animal body.

Hyperhydration – Excess water content in the body.

Collegiate Wrestlers – NCAA Division I varsity wrestling athletes.
Chapter 2: Related Literature

Physiology of Wrestling

General Characteristics

The general physiological profile of a successful wrestler consists of high anaerobic power (mean $\geq 6.1$ W/kg for arms; mean $\geq 11.5$ W/kg for legs), high anaerobic capacity (mean $\geq 4.8$ W/kg for arms; mean $\geq 7.4$ W/kg for legs), high muscular endurance; average to above average aerobic power (range 52 to 63 ml/kg/min), average pulmonary function (range 1.90 to 2.02 L/kg/min for $V_{Emax}$), normal flexibility; a high degree of leanness (range 3.7 to 13.0% fat) minus heavyweights, and a predominately mesomorphic somatotype. While optimal body composition is a concern of many wrestlers, athletes in the United States are more focused on weight reduction than the Europeans and Asians wrestlers. There is evidence to support a relationship between leanness and success in this athlete; where the lightest wrestlers appear to be the leanness. Body composition has generally been assessed using the underwater weighing methods or with field testing that includes skinfold thickness, skeletal width measurements, and most recently bioelectrical impedance analysis.

Anaerobic Characteristics

According to Stine et al. and Cisar et al., scholastic/collegiate wrestlers with a greater isokinetic strength profile have been shown to be more successful. Power (the amount of work accomplished per unit of time) can be seen as an advantage in wrestling because while opponents are matched for weight, not necessarily height or body mass,
their relative power may be different. Power in wrestlers is associated with quick, explosive maneuvers that lead to control of the opponent. The sources of these quick movements originate from the phosphagens (ATP-PC) and glycogen (anaerobic glycolysis). Power is most commonly tested with the Margaria Stair Climb (test duration approx. 1 sec) and the Wingate Anaerobic Test (test duration 30-40 seconds). Anaerobic power may help distinguish elite wrestlers from non-elite by as much as 13% for similar weight, age and wrestling experience. While flexibility of a wrestler may not differentiate them from other power-based athletes such as weightlifters and gymnasts, it can make a difference between successful and unsuccessful wrestlers. Stine et al\textsuperscript{84} have shown that sit and reach measurements for a cohort of successful wrestlers was greater when compared to moderately and less successful wrestlers\textsuperscript{84}. Interestingly, while reaction time, or the speed at which a person moves in response to a stimulus, may seem critical in most sports, it is not considered to be a critical attribute for success in wrestling\textsuperscript{43, 67, 84}.

\textit{Aerobic Characteristics}

Aerobic training is known to improve cardiovascular fitness and possibly muscular endurance, thereby improving performance in sport. In the wrestling athlete, aerobic training plays a key role in the fluctuation of body composition, or ‘making weight’. Since aerobic training requires a high volume of energy expenditure, it promotes the reduction of body fat and subsequently bodyweight. This mode of exercise creates a metabolic heat load that induces sweating for the purpose of rapid weight loss\textsuperscript{28}. 
Historically, long-distance running (road work) has been the predominant way to help in rapid weight loss but at the cost of whole body power capabilities pushing most wrestlers in the recent years to use cycling as exercise instead which does not seem to impact power as much.

A hallmark feature of aerobic function in the wrestling athlete is the size of the left ventricular wall and septum\[^{55}\]. This cardiovascular feature has been shown to be greater in wrestlers than non-athletes and endurance athletes. The left ventricle must increase the contractile force to generate enough pressure to overcome the increased peripheral resistance, which leads to an adapted increase in mass of the ventricle wall. This hypertrophy is due to increased afterload during the isometric contractions performed frequently in wrestling\[^{55}\]. Peak oxygen consumption is not significantly different between successful and less successful wrestlers at any level of competition\[^{32,57,84}\].

In general, cardiovascular fitness is assessed by VO\(_2\) max, which measures peak oxygen uptake while a progressive workload is placed on the athlete. The test is terminated when a state of exhaustion is reached by the participant. Wrestlers have a peak VO\(_2\) between 50-60 ml/kg/min when using a treadmill running protocol\[^{55}\]. When compared to untrained and trained athletes, elite wrestlers have a peak oxygen uptake that is considered average to slightly above average, but are still below average compared to endurance athletes. However, there does appear to be a difference in peak oxygen uptake
among the different styles of wrestling; VO\(_2\) max is specifically higher in freestyle wrestlers\(^{57,76}\) than in Greco-Roman competitors\(^{20,88}\).

While most of the energy comes from anaerobic processes, it has been reported that there is a 21.5% reduction in glycogen concentrations after a 6-minute freestyle match (i.e. aerobic mechanism). From this it can be concluded that there is a fairly uniform recruitment pattern of slow twitch, oxidative fast twitch and glycolytic fast twitch muscle fibers during wrestling. Blood lactate concentrations also rose almost 10 fold post competition, while blood pH decreased from 7.31 to 7.06\(^{35}\).

There are many acute changes in blood chemistry during and directly following wrestling competition. Grassi et al.\(^{23}\) showed an elevation in creatine kinase, or creatine phosphokinase (CPK). This increase is often associated with muscle damage that is obtained during contact or eccentric contractions\(^ {74,92}\). There is also an increase in nonessential fatty acids and alanine aminotransferase (glutamic pyruvic transaminase) levels was observed; while no changes in aspartate aminotransferase (glutamic oxaloacetic transaminase), triglycerides, HDL-cholesterol and total cholesterol were seen\(^{23}\). Farris studied the effects of wrestling on total blood count, noting significant increases in red blood cells, neutrophils, and lymphocytes with a moderate increase in polymorphonuclear cells. The increase in red and white blood cells counts can be related to the transmission of plasma out of the vascular space as a result of increased hydrostatic pressure. Under the intense, static contractions, which increase systolic blood pressure
and osmotic pressure, production of metabolites by the muscle increases, leading to value changes in the aforementioned blood values\textsuperscript{18}.

**Body Mass (Weight) Loss**

*Methods*

Weight class recommendations and weigh-in measures have been enacted to ensure the physical safety of athletes by reducing vast weight differences between opponents and lowering associated injury risks. Many wrestlers choose to compete in a class lower than their natural weight with hopes of gaining advantages in strength, power and leverage over an opponent that does not reduce weight for the same class. These strategies for losing weight can come at a price to the wrestler’s physiology and performance.

The most common techniques for losing weight include the reduction of food intake and reduction or abstention of water intake in conjunction with excess exercise to burn calories and induce sweating (metabolic dehydration), sitting in a sauna (thermal dehydration)\textsuperscript{82, 103}, and spitting\textsuperscript{99}. Less commonly wrestlers will use laxatives or purging to empty the gastrointestinal tract\textsuperscript{99, 103}; diuresis-induced water loss by consuming a high protein diet, taking contrast showers\textsuperscript{79} or ingesting pharmacological agents\textsuperscript{82, 103}, or at the international level, bloodletting/doping\textsuperscript{3}. 

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**Effects**

The effect of weight loss on performance in wrestlers has been studied with respect to isometric strength, isokinetic strength, isotonic strength, anaerobic power, anaerobic capacity, muscular endurance, peak oxygen uptake (peak VO$_2$) and physical work capacity estimated from heart rate. Ironically, while not all studies agree, many studies have shown that there is no statistical difference between anaerobic power, strength or peak VO$_2$ uptake before and after rapid weight loss, but there are several theories as to why performance is still compromised. First, while muscle is not dependent upon blood-borne nutrients such as glucose and oxygen for short-duration exercise bouts, there should not be a limitation based on the decreased blood flow to the muscle during dehydration$^{27}$. Second is the thought that the excitability of the muscle is fairly resistant to perturbations in the mineral and water content in the dehydrated state which implies that the nervous system can still recruit the muscles for contraction$^9$. Lastly, the rapid weight loss will not decrease muscle concentrations of ATP and phosphocreatine for brief, intense efforts$^{34}$. In contrast to this short, high-power performance, sustained or repeated performance lasting more than 30 seconds at near-maximal efforts, appears to deteriorate with rapid weight loss$^{30,40,98}$. During the dehydrated state, the reduced blood flow to muscles can slow nutrient exchange, waste removal and heat dissipation from the muscle between contractions which can impede its ability to recover. Muscle glycogen is significantly decreased with rapid weight reduction$^{34}$. Also noticed is the buffering capacity of the muscle and blood may be compromised after such rapid weight loss$^{31}$. This may lead to decreased recovery after
intense activity as well as decreased energy reserves, which will subsequently affect physical performance. However, research is inconclusive regarding the connection between the success of a wrestler and their weight loss. More successful wrestlers have been shown to lose more weight than their less successful cohort, although results are not statistically significant\textsuperscript{84}.

\textit{Diet}

There are two different methods recommended to classify food intake. The Recommended Daily Allowance (RDA) designed by National Institutes of Health (NIH), is based on how intake acts as the primary energy source for the brain. The Acceptable Macronutrient Distribution Range (AMDR) is based on a person’s intake as a source of kilocalories (kcals) to maintain bodyweight. RDA for carbohydrates is 130 g/day, whereas the AMDR is 45-65 g/day for the 19-50-year-old healthy males. This is important to note in reference to wrestlers because they are most often trying to lose body weight and carbohydrates tend to be the first thing that they cut. Total fat AMDR is 20-35 g/day, with the recommendation for no saturated and trans fatty acids. Protein and amino acids should be consumed per AMDR of 10-35 g/day. It is recommended that healthy adults (ages 19-50) should consume 1.5g sodium and 2.3 chloride each day or 3.8g salt to replace lost salt, with an average daily sodium intake is 3.5-6g/day. Adults should also consume 4.7g potassium each day. However, most Americans don’t meet these recommendations. See Appendix A for more information on RDA for micronutrients.
Limited research has been performed in assessing the dietary habits of collegiate wrestlers. In a study which observed 42 wrestlers at two universities, diets were composed of approximately 15% protein, 33 to 37% fat and 43 to 47% carbohydrates depending on the time of the season (pre, mid, post)\(^\text{83}\). In a similar study observing wrestlers at a single university, diet consumption ranges of 29 to 39% fat, 40 to 53% carbohydrate and excess of 1g of protein per kg bodyweight per day\(^\text{79}\), a stark contrast to the aforementioned values. However, both studies display drastic changes in daily kcal intake compared to the national average. Steen and McKinney\(^\text{83}\) observed that for one 53.6kg wrestler, daily energy intake ranged from 334 kcal on the day before a match to 4214 kcal the next day after making weight. Regardless of the wrestlers attempt to reduce bodyweight, in-season testing observed deficiencies of vitamin B\(_6\), vitamin A, vitamin C, zinc, magnesium, thiamine and iron, as well as calcium, riboflavin and niacin.

*Endocrine Response to Weight Loss*

Weight loss may affect the endocrine function of wrestlers. Low concentrations of serum testosterone and prolactin, exclusively in collegiate wrestlers during the competitive season, have been reported\(^\text{85}\). More recent studies have shown that during weight loss, collegiate wrestlers experience a decrease in insulin-like growth factor I (IGFI) and an increase in growth hormone\(^\text{50}\). Wrestlers that undergo continuous ‘weight cycling’ may experience permanent suppression of resting metabolic rates. This is not necessarily true for wrestlers that gain and lose weight more appropriately; it has been seen that once the weight was regained, the resting metabolism returned to the ‘normal’
preseason rate\textsuperscript{51}. During exercise after rapid weight loss, the body will experience an increase in fat utilization and a decrease in carbohydrate oxidation. This is supported by evidence of 8 to 11\% increase in fat oxidation in college wrestlers\textsuperscript{50}. Evidence of elevation in plasma glycerol levels before exercise and attenuated lactate response after standard exercise has also been reported in college wrestlers\textsuperscript{30,50}. There is a reduction in the utilization of carbohydrates that, based on adaptations of wrestlers, could be due to the reduction of muscle glycogen\textsuperscript{34} or weight-loss-induced elevation of plasma free fatty acids, which subsequently suppresses glycolysis\textsuperscript{66}. While many adaptations are made, there is no resultant metabolic changes at rest for hemoglobin concentrations, hematocrit, lactic acid concentration and pH after rapid weight loss\textsuperscript{30} even though blood base excess was significantly lower than at rest before weight loss occurs. This decrease in base excess is thought to be caused by excess ketone body production, which is caused by restricted food intake\textsuperscript{4} and is proven present in wrestlers; 17 to 18\% of scholastic wrestlers exhibit ketonuria after rapid weight loss\textsuperscript{105}.

**Homeostasis**

*Hormones and Osmoregulation*

The human body has a high concentration of water, approximately 60\% of a man’s body weight and about 50\% of a women’s weight. Two-thirds of the body’s water content can be found in the intracellular fluid. The other one-third is found in extracellular fluid, which is composed of mostly plasma (containing 92\% water) and interstitial fluid. There are four major electrolytes found in the human body: sodium (major extracellular
electrolyte), potassium, chloride and bicarbonate. These electrolytes are classified as either positively (cation) or negatively (anion) charged and work together to maintain water homeostasis of the body, proper pH, optimal heart function, and participate in various other physiological reactions.

The prime determinant of free water excretion is the regulation of urinary flow by circulating levels of arginine vasopressin (AVP), also known as ‘antidiuretic hormone’ (ADH). AVP is a nine-amino acid peptide that is synthesized in [magnocellular] neural cells located in the supraoptic (SON) and paraventricular (PVN) nuclei in the hypothalamus. Once synthesized, AVP is then transported to the posterior pituitary gland where it is stored with neurosecretory granules until specific stimuli causes its secretion into the bloodstream. Osmoreceptors located in the brain but outside the blood-brain barrier detect changes in the plasma osmotic pressure and transmit electrochemical signals to activate the synthesis and release of AVP from the SON and posterior pituitary. Baroreceptors located in the heart detect changes in the blood volume and send afferent signals to both the SON and PVN to increase the synthesis and release of AVP. Antidiuresis (the reduction or suppression of the excretion of urine) occurs through activation of the circulating vasopressin V2 receptors in the nephron and vasoconstriction V1a receptors in arterioles, which is enhanced through the insertion of a water channel called aquaporin-2 into the apical membranes of collecting tubule principal cells.
The primary renal response to AVP is an increase in water permeability of the kidney collecting tubules. Having increased water retention due to AVP can lead to concentrated urine, increased plasma volume and reduced plasma osmolality. AVP secretion is stimulated by low plasma volume and increased plasma osmolality\textsuperscript{11}. Increased plasma osmolality is one of the main physiological driver of AVP secretion from the posterior pituitary gland\textsuperscript{69}.

Hyperhydration can occur from excessive ingestion of hypotonic fluid, electrolyte deficiency, and/or renal failure\textsuperscript{26}. During exercise, core temperature is increased because of increased metabolic heat production, which leads to the initiation of evaporative cooling in an attempt to reduce the rise in temperature. Exercising in the heat leads to marked reduction in plasma volume and an increase in plasma osmolality that is caused in part by dehydration resulting from sweat loss\textsuperscript{58}.

Euhydration is defined as a body mass $\pm 0.2\%$ of normal in temperate environments and $\pm 0.5\%$ of normal in hot environments or during exercise\textsuperscript{24}. When there is an increase in temperature during exercise (such as wrestlers training in extra layers) the chances of dehydration increases which can result in heat syncope due to venous pooling and reduced blood flow to the brain\textsuperscript{5}, or heat exhaustion due to hypotension and central nervous system fatigue\textsuperscript{39}. These large changes can lead to an increase in core body temperature and initial symptoms of central nervous system dysfunction that can progress to systemic inflammation and even death\textsuperscript{45}.
Often many athletes start exercise in a dehydrated state. While this can impair performance, it is often difficult for athletes to determine their true state of hydration to begin with. If fluid intake is less than fluid loss, dehydration can occur, most commonly at the end of an exercise bout. Rapid ingestion of a large volume of hypotonic fluid may increase blood volume and consequently reduce plasma osmolality and AVP release\(^6^9\), resulting in increased urine production making an individual “appear” hydrated. Surprisingly, water alone is not effective in maintaining fluid balance during recovery because it instead leads to a large reduction in plasma sodium concentrations and osmolality\(^5^8\). Ideally, we should avoid large reductions in plasma osmolality by adding certain solutes like sodium (main cation of ECF), potassium (main cation of ICF), carbohydrates; and protein.

Urine output has been shown to decrease as sodium drink concentrations increase\(^4^7, 5^3, 7^7\). As such, it is essential that sodium concentration is greater than or equal to that of the sweat lost in order to maintain positive fluid balance. The effectiveness of sodium in a rehydration drink is due to the mechanisms of plasma sodium concentrations, osmolality and AVP release. The presence of potassium in sports drinks have been shown to improve rehydration when the drinks also contain chloride (major anion of ECF)\(^4^8, 6^5\). The addition of carbohydrates, especially glucose, can enhance post-exercise rehydration\(^1^6, 1^7, 6^4\). Carbohydrate consumption is reported to decrease urine output and the resultant maintenance of euhydration post-exercise due to a reduction in gastric
emptying and therefore overall fluid absorption but does not necessarily result in fluid balance compared to hypotonic drinks\(^8\). Collectively, the addition of carbohydrates to rehydration drinks enhances fluid retention if the concentration of carbohydrate and volume of fluid uptake are sufficiently high\(^{14}\).

Milk is the common choice of protein in many post-exercise drinks. This added element has been shown to enhance post-exercise rehydration compared to carbohydrate-electrolyte drinks and/or water\(^{13,36,75,78,96}\). Milk contains sodium, potassium, carbohydrates, and protein, which can all independently influence results. The protein in milk helps achieve fluid balance and influences muscle protein synthesis post-exercise\(^{101}\). Protein-containing drinks may enhance post-exercise rehydration as they slow the overall rate of fluid uptake, increasing plasma albumin content and thus oncotic pressure\(^{14}\).
Figure 1. Schematic diagram outlining the major factors involved in restoring and maintaining fluid balance during the post-exercise period. ECF, extracellular \textsuperscript{14}.

Alcohol may affect homeostasis more than we think. Because of its influence on the release of AVP\textsuperscript{56} as well renal absorption\textsuperscript{46,87}, alcohol has strong diuretic effects. For this reason, it is not advised to ingest alcohol prior to exercising nor solely drink alcohol-containing drinks post-exercise. While urine volume still increases after consumption of alcohol, there is evidence of renal water conservation that could lead to further long-term complications. Although alcohol is unlikely to inhibit the rehydration process, it is not recommended for consumption between bouts of exercise on the same day\textsuperscript{14}.

It can also be argued that ingesting food post-exercise along with any drink will adequately rehydrate your body as well. Evans et al.\textsuperscript{15} conducted a study that shows no
difference of fluid retention between water and a sports drink when a standard meal was co-ingested during the rehydration period.

**Thirst**

Thirst acts as the body’s defense mechanism to increase water consumption in response to perceived deficits of body fluids. This sensation is controlled in the anterior hypothalamus as a response to physiological changes in osmolality of plasma and cerebrospinal fluid, while also receiving sensory input from esophageal and gastrointestinal receptors to create a sensation which drives the drinking behavior. Thirst can be stimulated by either hypertonic-hypovolemia (water loss > salt loss with sodium values >150 mEq/L, serum osmolality >290 mOsm/L, and urine osmolality >400 mOsm) or isotonic-hypovolemia (equal sodium and water loss with normal serum sodium levels 135-145 mEq/L, serum osmolality >290 mOsm/L, and urine osmolality >500 mOsm). Hypotonic dehydration is another form of dehydration more commonly seen because the salt loss is greater than the water loss, resulting in laboratory values of serum sodium <120 mEq/L, serum osmolality <290 mOsm/L, and urine osmolality >500 mOsm/L. Hypovolemia (a decrease in the volume of circulating blood, caused by traumatic injury or severe dehydration) is important in thirst stimulation due to depletion of the intravascular fluid causes decreased plasma volume and often occurs with thermoregulatory sweating during exercise. In many cases, hypovolemia leads to cellular dehydration by increasing the osmolality, which creates an environment resulting in fluid shift between compartments. Hypovolemia is a stimulus for AVP secretion in humans.
AVP plays a critical role in fluid regulation and thirst, and is secreted after stimulation of baroreceptors and osmoreceptors in the hypothalamus as well as peripheral baroreceptors and the presence of angiotensin II. However, it has been speculated that AVP response to decreases in blood volume are absent, as small changes in plasma osmolality below the thirst-stimulation threshold are not detectable in the human body. This can be suggestive of homeostasis accomplished more so by regulated free water excretion than by regulated water intake.\(^{94}\)

Analogous studies in humans using quantitative estimates of subjective symptoms of thirst have confirmed that increases in plasma osmolality of similar magnitudes are necessary to produce the thirst sensation.\(^{70,90}\) While stimulated thirst does not represent a major regulatory mechanism, unregulated fluid ingestion supplies adequate water in excess of true ‘need’, which is then excreted via osmoregulated pituitary AVP secretion. However, plasma osmolality will fall to levels that stimulate thirst in order to stimulate water intake and osmolality when fluid intake goes unregulated. Day-to-day conditions of body homeostasis are maintained by unregulated fluid intake in association with AVP-regulated changes in urine flow, which occur before the threshold for regulated thirst. If not, this is regulated by thirst-induced fluid intake that occurs as a primary defense mechanism against dehydration.\(^{95}\) Osmotically stimulated thirst is generally set at 5-10 mOsmol/kg higher than that of AVP secretion (290-295 mOsmol/kg H\(_2\)O) or when decreases in body water reach approximately 1.7-3.5\(^{\circ}\)\(^{49,71,95}\)
Hydration Status

**Urine Specific Gravity**

Urine specific gravity (USG) is considered a reliable method for clinicians to check the kidneys’ ability to function; more specifically, it examines how your kidneys are diluting the urine to determine hydration status\(^ {25}\). Urine specific gravity measures the concentration of particles in urine and the density of the urine compared to the density of water. The results of USG can be falsely decreased from factors such as lithium consumption, drugs such as isotretinoin, diuretics, and antibiotics (including penicillin), moderate amounts of protein in the urine, unsanitary testing cups or equipment, and cold urine specimens\(^ {6}\).

Decreased USG can be a result of excessive fluid intake, diabetes insipidus, glomerulonephritis, pyelonephritis, aldosteronism, and/or edema from brain injury. Increases in USG can be caused by excessive water loss due to illness, diabetes mellitus, and inappropriate secretion of antidiuretic hormones and/or congestive heart failure. Dehydration can reduce cardiac output and decrease overall endurance capacity, and can have adverse effects on anaerobic capacity and performance\(^ {37,93}\). Despite the negative effects, hypohydration and fluid restriction are still some of the most common methods for rapid weight loss prior to competition\(^ {62,93,97,98,104}\). While it is possible to alter short term hydration status, measuring via USG, this does not hold true for athletes that lose greater than 2% of their original body mass during a practice\(^ {10}\). The average range for
USG is 1.005 to 1.030 g/cm$^3$ where euhydration measured are considered 1.013-1.020 g/cm$^3$ and dehydration is defined as USG >1.020 g/cm$^3$.

Hypohydration in wrestlers has been observed throughout all levels of competition. Wrestlers that participated in the collegiate world championship had a higher competition mean body weight compared with training body weight during the four days prior to competition weigh-ins. Urine specific gravity values were found between 1.031 and 1.036 g/cm$^3$ in these athletes$^{44}$. Levels of USG have been found to fluctuate in these athletes throughout the competitive season. Significant elevations in USG were observed mid-season one day prior to competition when compared to three time points from pre-season to post-season$^{68}$.

**Blood Plasma Osmolality**

Plasma Osmolality is a method of measuring the electrolyte balance within the body. Osmolality is the measure of osmoles of solutes per kilogram in plasma. Plasma and urine osmolality can be measured by using an Osmometer that measures the freezing point depression of a sample, but can also be calculated by the following formula (Equation 1.1):

Plasma osmolality = 2 × Sodium + Glucose + Urea (all concentrations in $\frac{mmol}{L}$).
Serum osmolality is partly controlled by a substance called antidiuretic hormone (ADH) or previously described AVP, which is used to conserve body water by reducing the loss of water in urine. Once stimulated by increased plasma osmolality, AVP binds to receptor cells in the collecting ducts of the kidney and promotes reabsorption of only water back into circulation, creating concentrated urine. On the other hand, a decrease in serum osmolality will reduce the secretion of ADH, leading to an increase in water excreted as urine (diluted urine). The plasma osmolality increases with hypohydration and decreases with hyperhydration which will determine the rate at which antidiuretic hormones are secreted.

Previous studies have shown plasma osmolality levels of elite high school wrestlers to be significantly higher than the normal range of about 280-285 mOsm·kg⁻¹. Wrestling is a constant challenge for the body’s regulatory center. A compensatory response is seen to endure low fluid intake during training which can lead to post competition plasma osmolality values of about 320 mOsm·kg⁻¹. This drastic change in plasma osmolality values, if sustained over time, supports the idea of a new “normal” in the osmoregulatory centers of the hypothalamus in wrestlers. Thus, the importance of understanding the physiology of the sport of wrestling really can start with the careful examination of hydration measurements.
Chapter 3: Methods

Experimental Approach to the Problem

In this study varsity wrestlers at the Ohio State University who compete at the National College Athletic Association (NCAA) Division I level volunteered to participate in the study. Testing was completed on two separate days, one in the middle of the season and one a week after the season, approximately three months apart. On each of the two test days the athletes reported to the Human Performance Laboratory Physical Activity and Education building on the Ohio State University Main Campus.

Participants

All participants are adult males over the age of 18 that participated in varsity wrestling at the Ohio State University. Each participant completed two test visits, one in the middle of the season and one the week after the NCAA Championship. Each wrestler signed a consent form (Appendix B) that was approved by the Ohio State University’s Institutional Review Board for use of human subjects in research after the risks and benefits of the study were explained to them. This was obtained at least one week prior to initial testing to allow for adequate time to complete the pre-test body weight log. Participant characteristics are shown in Table 1.
Table 1. Characteristics of collegiate wrestlers (n=8).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>19.88 ± 1.05</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>174.35 ± 5.55</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>83.52 ± 18.99</td>
</tr>
<tr>
<td>BMI</td>
<td>27.18 ± 4.37</td>
</tr>
</tbody>
</table>

Testing Overview

Upon check-in, their weight and hydration logs were collected, along with their current height and weight. The wrestlers then produced a urine sample for immediate specific gravity analysis using a hand-held refractometer. Once their urine was collected and analyzed, they completed the VAS Thirst Scale (see Appendix C). Next, a blood sample was obtained and processed per protocol. The participants were able to perform their daily activity without restrictions following completion of the testing battery. Post season testing was conducted in the identical manner.

Test Days

On test day the wrestlers came in the morning. Upon arrival, they were asked to step on a clinical scale/stadiometer (Seca 763 scale/stadiometer) (Seca, Inc., Chino, CA) to confirm current height and body weight. Weight and hydration logs were provided prior to testing in order to assess their weight fluctuation for the week prior and fluid
intake 24-hours prior to testing and collected the morning of each test visit. The athletes
then provided a urine sample which was compared using a pen refractometer and a
manual refractometer. Once the urine sample was obtained we had each athlete complete
the VAS thirst scale and await their blood draw. Blood samples were obtained by a
trained phlebotomist. Participants were seated comfortably while approximately 10mL
of blood was taken from the antecubital vein via 23-gauge butterfly needle into a sodium
heparin vacutainer. Blood was centrifuged using a Sorvall Legend XT centrifuge
(Thermo Fisher Scientific Inc., Waltham, MA, USA) at 2000 rpm for 10 minutes and
plasma was harvested and aliquoted into 1.5mL osmometer specific tubes. Plasma was
then immediately assayed for plasma osmolality.

**Assay Measurements / Instrumentation**

**Urine Specific Gravity**

A High Precision TS400 Clinical Refractometer (Reichert Inc., Buffalo, NY) was
used as the digital refractometer for all experiments using a human USG scale. We
compared the values of the manual Reichert refractometer with those from the Atago
Pen-Urine S.G Refractometer (Atago U.S.A., Inc., Bellevue, WA) for accuracy and
validity of both instruments. All studies were conducted with the instrument and
materials at ambient (room temperature) to prevent any delays for specimen temperature
equilibration. All samples were provided as midstream samples at approximately (within
1 hour) the same time for each test. Samples were tested within 2 hours of collection or otherwise refrigerated for no more than 8 hours prior to testing.

**Blood Plasma Osmolality**

Each participant’s blood was drawn once at each visit and was approximately 10mL (~2.0 teaspoons) for each blood draw. A trained phlebotomist performed all of these blood draws and no adverse reactions were seen. Plasma osmolality was measured for each participant using the Micro-Osmette Osmometer (Precision Systems Inc., Natick, MA). Plasma osmolality samples were collected in a 10mL Sodium Heparin tube per recommendation from Precision Systems Inc. and tested following their protocol for the μOsmette™ Model 5004 Automatic Osmometer. Each sample was tested in triplicate and averaged to get the final value unless one value was an outlier.

**Height and Body Mass (Weight)**

At each test visit, participants stood upright on a standard weight scale (Seca 763 scale/stadiometer) (Seca, Inc., Chino, CA). The measuring arm was lowered onto the top of their heads measuring their height in centimeters. The participants then stood on a bodyweight scale in their underwear with no shoes, and their bodyweight was recorded in kilograms. Each participant was asked to track their weight for the 7 days prior to each test visit using the bodyweight scale provided in their practice facility. Their weight was recorded on the log they were provided (Appendix D).
**Fluid Consumption / Hydration Log**

Participants were asked to fill out a hydration log (Appendix D) for the 24 hours prior to each test visit indicating when, how much, and what kind of fluids they consumed.

**Thirst Scale**

Participants completed a visual analog thirst scale (see Appendix C) at each visit prior to the blood draw but following urine sample production to measure their perceived level of thirst. There was a categorical scale associated with the VAS that was also completed at the same time.

**Statistical Analyses**

The data was presented as mean ± SD. Data was analyzed using a dependent t-test to determine differences between mid- and post-season time points for the various analyzes under consideration. Pearson-product moment correlation coefficients were determined to examine the relationship between selected variables. An SPSS software version 25 was used for the analyses. Significance in this investigation was P ≤ 0.05.
Chapter 4: Results

It was the purpose of this investigation to assess the hydration status of college wrestlers by measuring the following variables: (1) urine specific gravity and (2) blood plasma osmolality. The wrestlers were tested in the middle (TV1) of and after (TV2) their collegiate wrestling season.

Urine Specific Gravity

Firstly, USG was tested via manual and pen refractometers for comparison. The same sample was tested on both measurements tools at each visit and compared using a paired sample t-test. When looking solely at USG, the comparison of manual vs pen refractometer showed significant differences at both testing time points (p=0.000). It also showed a strong correlation between the two as well with values of 0.985 (p=0.000) and 0.998 (p=0.000) respectively. A comparison for the two methods of testing is shown in Figures 2a and 2b.

Secondly, in both conditions (mid and post season), no significant differences in USG were found to exist among the samples taken (Figure 3). The mean for test visit 1 (TV1) was 1.023 ± 0.008 and 1.019 ± 0.011 for test visit 2 (TV2). The difference between TV1 and TV2 presents with a P-value of 0.188.
Figure 2. Comparison of Urine Specific Gravity Methods: Manual vs. Pen Refractometer – Test Visit 1.

Figure 3. Comparison of Urine Specific Gravity Methods: Manual vs. Pen Refractometer – Test Visit 2.

Figure 4. Comparison of USG between TV1 and TV2.
Blood Plasma Osmolality

Results of plasma osmolality based off a paired sample t-test showed a significant difference between TV1 and TV2 in regards to plasma osmolality resulting in a P-value of 0.001 (P ≥0.05). Values for each visit ranged from 288.67 to 301.67 mOsm·kg⁻¹ and 269 to 289.33 mOsm·kg⁻¹ respectively. Comparing post-season (TV2) to mid-season (TV1) it was seen that plasma osmolality is actually lower post-season with a mean of 279.96 ± 5.81 and 294.46 ± 4.79 respectively.

Figure 5. Comparison of Plasma Osmolality between TV1 and TV2.
Figure 6. Comparison of USG and Plasma Osmolality mid-season (TV1).

Figure 7. Comparison of USG and Plasma Osmolality post-season (TV2).

Body Weight

Testing weight was taken two to three days after competition but averaged for the week prior to testing to evaluate changes in weight prior to competition. When compared to their competitive weight class, the wrestlers were an average of 3.31 ± 1.66 kg heavier
at TV1 and 3.76 ± 3.99 kg heavier at TV2. When compared between the visits there was no significant difference seen between their weights (p=0.766).

**VAS Thirst Scale**

The thirst scale was analyzed by measurements both categorically as well as on a 100mm scale. A paired sample t-test was run for both methods of analysis; no significant differences were seen between test visits (P=0.192 and P=0.685). However, a correlation was seen between the numeric and categorical values for thirst given at TV1 with a Pearson Correlation value of 0.946 (p=0.000). When analyzed categorically, the scale was broken down into 4 sections to determine the wrestlers perceived level of thirst at the time of the test. Two (12.5%) wrestlers complained of low thirst (VAS ≤ 3), 13 (81.25%) wrestlers complained of moderate thirst (VAS = 4-6), and 1 (6.25%) wrestler complained of severe thirst (VAS ≥7). The mean categorical thirst score was 5.31 ± 1.16 (range: 3 to 7), which is considered in range for the moderate level of thirst distress. While there was no specific hypothesis regarding the VAS thirst scale, it is important to note the results of it. Ten (62.5%) wrestlers considered themselves to be at least somewhat thirsty while 4 (25%) did not. When evaluated further it was discovered that the individuals who perceived themselves most thirsty were indeed the most dehydrated at each test visit.
Chapter 5: Discussion

The primary findings of this study were that collegiate wrestlers in our sample were more dehydrated in the middle of the season when compared to after the season. The magnitude of this dehydration was greater when viewed from the measurement of plasma osmolality than USG. Furthermore, the two measurement techniques were not correlated, leading one to question the sole use of USG for the assessment of hydration status.

The controversy of the measurement technique was recently brought to the forefront in a study by Sommerfield et al.\textsuperscript{81} that showed the lack of similarity between measures of USG and plasma osmolality. The reasons for this difference may be based on the theory that wrestlers have created a new osmol-regulatory set point, which is not reflected in the USG measurements\textsuperscript{104}. This could indicate that wrestlers have a larger reservoir or higher level of overall body water as a conduit to rapid weight loss. Changes in urinary output can be seen throughout the day as observed by Zambraski et al.\textsuperscript{105} when wrestlers were tested at weigh-in, before the match and post competition, revealing their ability to rehydrate post weigh-in before their match and go back into a dehydrated state post competition. Plasma osmolality does not tend to fluctuate as quickly as seen by Kraemer et al.\textsuperscript{42} when testing at rest across a tournament but has been observed at values reaching as high as 320 mOsm·kg\textsuperscript{-1} for some post-match. Furthermore, the plasma osmolality values were significantly higher during this time in the middle of the season.
The USG measures and the plasma osmolality measures were not correlated reflecting a dramatic difference in the two measurement techniques.

The USG values did show minimal much change from mid- to post-season, with a value of $1.02300 \pm 0.008452$ indicating only a minor change in the hydration status. This was in contrast to a mean plasma osmolality value of $294.458 \pm 4.791$, which indicates a dramatically higher level of dehydration in these athletes at rest. In a study by Ratamess et al.\textsuperscript{68} looking at season changes in USG they showed significant elevations only the day prior to a meet. Based on their data, it brings in to question whether USG can accurately reflect hydration status during a training week when plasma osmolality tends to trend higher. It is known that dramatic body mass loss of 5-6% can result in performance deficits during a tournament format for both strength and power\textsuperscript{42}. Interestingly, the two methods of measuring USG were highly correlated to each other but both were not significantly related to plasma osmolality. This lends some validation to the pen method, but it still has the same problems as the manual method and is not correlated to the gold standard of hydration, plasma osmolality.

As noted in the paper by Sommerfield et al.\textsuperscript{81} different athletes, including wrestlers can find unity of the two measures while many others do not. In other words, the matching of USG and plasma osmolality might reflect the same level of dehydration while in others or other times it may not. The disparity in this study was greater in the
beginning of the season than it was after when 4 out of 8 returned to normal ranges for both USG and plasma osmolality.

Surprisingly, the wrestlers’ thirst ratings did not differ significantly between the two visits. When evaluated further it was discovered that the individuals who perceived themselves most thirsty were indeed the most dehydrated at each test visit. As we know “thirst” is a key factor in telling the body that they are hypohydrated and it can persist into dehydrated conditions as well\(^9\). This is in response to rise in core body temperature, food intake (prandial) and signals from the circadian clock. The perceived level of thirst, however, occurs in higher-order centers in the brain, such as the anterior cingulate cortex (ACC) and insular cortex (IC), which receive information from midline thalamic relay nuclei\(^2\).

In some cases such as cycling, hypohydration impairs performance despite thirst not being reflective on the effects\(^1\). However, different in wrestling than other sports, reduction in water intake is the primary method of body mass loss\(^8\). Thus, thirst may still be stimulated to signal for hydration. The changes in body mass were not different between the testing points and may reflect the findings in perceived level of thirst. Changes in thirst ratings are subjective and individual but when compared after a fluid bolus, showed positively correlated changes in plasma osmolality. With 24-hour deprivation, there is an upward shift in plasma osmolality, plasma sodium, and hematocrit. With oral rehydration, plasma osmolality decreased in as little as 5 minutes.
after drinking, and decreased to below pre-deprivation values within 15 minutes. The wrestlers that needed to make weight for their initial competitions or were closer to their weight class at the time of testing may potentially reflect more recent weight loss which helps to explain thirst ratings.

In summary, this study showed a significant change in plasma osmolality between test visits but failed to discover a significant difference in USG values. We observed a strong correlation between types of USG methods (manual vs. pen) at both testing time points. Overall, we concluded that the majority of wrestlers seemed to return to a more ‘normal’ state of hydration post-season when compared to mid-season.
25. Health BM. Urine specific gravity test: How well your kidneys are diluting your urine; 2017.


91. Thompson E, Dalkin A. Serum Osmolality: WebMD Medical Reference from Healthwise; 2015.


### Appendix A: Recommended Daily Allowance (Micronutrient) Summary Males 19-50yr

<table>
<thead>
<tr>
<th>RDA Vitamins (Per Day)</th>
<th>RDA Mineral (Per Day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A – retinol</td>
<td>900 µg</td>
</tr>
<tr>
<td>Calcium</td>
<td>1000 mg</td>
</tr>
<tr>
<td>Vitamin C – Ascorbic Acid</td>
<td>90 mg</td>
</tr>
<tr>
<td>Chromium</td>
<td>35 µg</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>5 µg</td>
</tr>
<tr>
<td>Copper</td>
<td>900 µg</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>15 mg</td>
</tr>
<tr>
<td>Fluoride</td>
<td>4 mg</td>
</tr>
<tr>
<td>Vitamin K</td>
<td>120 µg</td>
</tr>
<tr>
<td>Iodine</td>
<td>150 µg</td>
</tr>
<tr>
<td>Vitamin B₁ - thiamin</td>
<td>1.2 mg</td>
</tr>
<tr>
<td>Magnesium</td>
<td>400/420 mg</td>
</tr>
<tr>
<td>Vitamin B₂ – riboflavin</td>
<td>1.3 mg</td>
</tr>
<tr>
<td>Manganese</td>
<td>2.3 mg</td>
</tr>
<tr>
<td>Vitamin B₃ – niacin</td>
<td>16 mg</td>
</tr>
<tr>
<td>Molybdenum</td>
<td>45 µg</td>
</tr>
<tr>
<td>Vitamin B₅ – pantothenic acid</td>
<td>5 mg</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>700 mg</td>
</tr>
<tr>
<td>Vitamin B₆ – pyridoxine</td>
<td>1.3 mg</td>
</tr>
<tr>
<td>Selenium</td>
<td>55 µg</td>
</tr>
<tr>
<td>Vitamin B₁₂</td>
<td>2.4 µg</td>
</tr>
<tr>
<td>Zinc</td>
<td>11 mg</td>
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<tr>
<td>Biotin</td>
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<tr>
<td>Potassium</td>
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<tr>
<td>Choline</td>
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<tr>
<td>Sodium</td>
<td>1.5 g</td>
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<tr>
<td>Folate – folic acid</td>
<td>400 µg</td>
</tr>
<tr>
<td>Chloride</td>
<td>2.3 g</td>
</tr>
</tbody>
</table>
Appendix B: Consent
The Ohio State University Consent to Participate in Research

Study Title: Tracking of Hydration Status Using Blood Plasma Osmolality and Urine Specific Gravity

Principal Investigator: William J. Kraemer, Ph.D.

This is a consent form for research participation. It contains important information about this study and what to expect if you decide to participate. Please consider the information carefully. Feel free to discuss the study with your friends and family and to ask questions before making your decision whether or not to participate.

Your participation is voluntary. You may refuse to participate in this study. If you decide to take part in the study, you may leave the study at any time. No matter what decision you make, there will be no penalty to you and you will not lose any of your usual benefits. Your decision will not affect your future relationship with The Ohio State University. If you are a student or employee at Ohio State, your decision will not affect your grades or employment status.

You may or may not benefit as a result of participating in this study. Also, as explained below, your participation may result in unintended or harmful effects for you that may be minor or may be serious depending on the nature of the research.

You will be provided with any new information that develops during the study that may affect your decision whether or not to continue to participate. If you decide to participate, you will be asked to sign this form and will receive a copy of the form. You are being asked to consider participating in this study for the reasons explained below.

1. Why is this study being done?
You are invited to participate in a research study that will track your hydration status before, during and after the wrestling season.

2. How many people will take part in this study?
Up to 50 people may participate each year.

3. What will happen if I take part in this study?
You will complete three test visits, one during pre-season, one during the season and one post-season. At each test visit, you will provide a blood and urine sample.

Location of testing
The testing protocols and procedures will take place at The Ohio State University, Columbus Campus.

Urine Collection
You will be asked to provide a urine sample, up to 3 ounces, in a urine cup at each visit.

Blood Draws
Blood will be drawn one time at each visit. Approximately 10ml (~1.7 teaspoons) of blood will be taken at each blood draw. A trained phlebotomist will perform all blood draws.

Surveys/Questionnaires
Fluid consumption will be recorded 24-hours prior to testing and collected the morning of the test. Weight will also be recorded every day for the 7 days prior to testing as well to evaluate either loss or gain of body weight in comparison to their “goal” weigh-in weight.

4. How long will I be in the study?
5. **Can I stop being in the study?**
You may leave the study at any time. If you decide to stop participating in the study, there will be no penalty to you, and you will not lose any benefits to which you are otherwise entitled. Your decision will not affect your relationship with The Ohio State University, the wrestling team coaches and staff, and your status on the wrestling team. There may also be reasons why your study participation can be stopped without your consent including if you fail to adhere to the study’s requirements.

6. **What risks, side effects or discomforts can I expect from being in the study?**
This study has no more risk than minimal risk. Blood draws do have certain risks including localized soreness, bruising, and physical discomfort or anxiety during needle insertion. In extremely rare cases, infection may occur at the insertion site. Every effort will be made to maximize your safety and only trained phlebotomist will perform the blood draws.

7. **What benefits can I expect from being in the study?**
There are no direct benefits to you. However, you may learn more about your individual hydration status throughout the wrestling season.

8. **What other choices do I have if I do not take part in the study?**
There are no other choices to take part in the study.

9. **Will my study-related information be kept confidential?**
Your study-related information will be kept confidential in accordance with IRB rules and regulations. Your test results will be shared with the wrestling coaches and staff. In addition, there may be circumstances where this information must be released. For example, personal information regarding your participation in this study may be disclosed if required by state law. Your records may also be reviewed by the following groups (as applicable to the research): Office for Human Research Protections or other federal, state, or international regulatory agencies; U.S. Food and Drug Administration; The Ohio State University Institutional Review Board or Office of Responsible Research Practices; The sponsor supporting the study, their agents or study monitors; and Your insurance company (if charges are billed to insurance).

**Publications and/or Presentations**
We may publish and/or present our findings. All identifying information will be removed from any data beforehand so that participants cannot be linked to the information published and/or presented. Information will be presented in summary format and participants will not be identified in any publications or presentations.

**Data Analysis**
The research personnel when possible will analyze data. However, some data may need to be sent to appropriate persons for analysis (e.g., Blood sent to Quest Diagnostics for analysis). This means that your data may leave our lab / possession for analysis. Any data out of our lab / possession will have all identifying information removed and will be assigned a unique code. Only approved and authorized research personnel will have access to the information that links the code to participants.

10. **What are the costs of taking part in this study?**
You may need to pay for parking if you do not have an OSU parking pass.

11. **Will I be paid for taking part in this study?**
There is no payment for participation in the study.

12. **What happens if I am injured because I took part in this study?**
If you suffer an injury from participating in this study, you should notify the researcher or study doctor immediately, who will determine if you should obtain medical treatment at The Ohio State University Wexner Medical Center. The
cost for this treatment will be billed to you or your medical or hospital insurance. The Ohio State University has no funds set aside for the payment of health care expenses for this study.

13. What are my rights if I take part in this study?
If you choose to participate in the study, you may discontinue participation at any time without penalty or loss of benefits. By signing this form, you do not give up any personal legal rights you may have as a participant in this study. You will be provided with any new information that develops during the course of the research that may affect your decision whether or not to continue participation in the study. You may refuse to participate in this study without penalty or loss of benefits to which you are otherwise entitled.
An Institutional Review Board responsible for human subjects / participants research at The Ohio State University reviewed this research project and found it to be acceptable, according to applicable state and federal regulations and University policies designed to protect the rights and welfare of participants in research.

14. Who can answer my questions about the study?
For questions, concerns, or complaints about the study you may contact:
William J. Kraemer, Ph.D. | A54 PAES Building | 305 Annie & John Glenn Ave | Columbus, OH 43210 | (614) 688-2354 | kraemer.44@osu.edu

For questions about your rights as a participant in this study or to discuss other study-related concerns or complaints with someone who is not part of the research team, you may contact:
William J. Kraemer, Ph.D. | A54 PAES Building | 305 Annie & John Glenn Ave | Columbus, OH 43210 | (614) 688-2354 | kraemer.44@osu.edu

Signing the consent form
I have read (or someone has read to me) this form and I am aware that I am being asked to participate in a research study. I have had the opportunity to ask questions and have had them answered to my satisfaction. I voluntarily agree to participate in this study.
I am not giving up any legal rights by signing this form. I will be given a copy of this combined consent and HIPAA research authorization form.

Printed name of subject

Signature of subject

Date and time

Printed name of person authorized to consent for subject (when applicable)

Signature of person authorized to consent for subject (when applicable)

Date and time

Investigator/Research Staff
I have explained the research to the participant or his/her representative before requesting the signature(s) above. There are no blanks in this document. A copy of this form has been given to the participant or his/her representative.

Printed name of person obtaining consent

Signature of person obtaining consent
Witness(es) - May be left blank if not required by the IRB

Printed name of witness

Signature of witness

Date and time

__________________________________________  AM/PM

__________________________________________  AM/PM

__________________________________________  AM/PM

Printed name of witness

Signature of witness

Date and time
Appendix C: VAS Thirst Scale
Thirst Scales

Visual Analog Scale (VAS):

How thirsty do you feel right now?

not thirsty                                very thirsty

Numerically identify your perceived thirst by using the 9-point thirst scale ranging from 1 ("not thirsty at all") to 9 ("very, very thirsty").

Categorical Scale (CS):

How thirsty do you feel right now?

1 Not thirsty at all  2 Not very thirsty  3 Not thirsty  4 Somewhat not thirsty  5 Neutral  6 Somewhat thirsty  7 Thirsty  8 Very thirsty  9 Very, very thirsty

From: Thirst and hydration status in everyday life
Nutr Rev | © 2012 International Life Sciences Institute
Appendix D: Weight and Hydration Logs

1. Hydration Log

<table>
<thead>
<tr>
<th>Date:</th>
<th>Time:</th>
<th>Type of Fluid (water/Gatorade/etc)</th>
<th>Amount of Fluid (oz)</th>
</tr>
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<tr>
<td></td>
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2. Weight Log

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