VALIDITY OF ULTRASOUND VELOCITY TO DETECT CHANGES IN THE HYDRATION STATUS OF MALE AND FEMALE ATHLETES DURING ACUTE DEHYDRATION AND REHYDRATION

A Thesis
by
MASON C. CALHOUN

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Abstract

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HYDRATION STATUS OF MALE AND FEMALE ATHLETES DURING ACUTE
DEHYDRATION AND REHYDRATION

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Recent work by Utter, Mcanulty, Sarvazyan, Query, & Landram, (2010) determined

that UV could be used to measure changes in the hydration status of male collegiate wrestlers

undergoing acute dehydration. However there was a large amount of individual variability

amongst participants. Additionally, Utter et al. (2010), only examined male athletes and not

female athletes leaving a significant population gap in the potential use of UV for hydration

status in a collegiate athletic setting. This paper further explores the validity of ultrasound

velocity (UV) as a less invasive form of hydration assessment using improved technology

than that used by Utter et al. (2010) in both male and female athletes.

Key Words: ultrasound velocity, dehydration, athletes, plasma osmolality, urine

specific gravity, total body water

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Abstract

Recent work by Utter, Mcanulty, Sarvazyan, Query, & Landram, (2010) determined that UV could be used to measure changes in the hydration status of male collegiate wrestlers undergoing acute dehydration. However there was a large amount of individual variability amongst participants. Additionally, Utter et al. (2010), only examined male athletes and not female athletes leaving a significant population gap in the potential use of UV for hydration status in a collegiate athletic setting. This paper further explores the validity of ultrasound velocity (UV) as a less invasive form of hydration assessment using improved technology than that used by Utter et al. (2010) in both male and female athletes.

   Key Words: ultrasound velocity, dehydration, athletes, plasma osmolality, urine specific gravity, total body water

Validity of Ultrasound Velocity to Detect Changes in the Hydration Status of Male and
Female Athletes during Acute Dehydration and Rehydration

Recently, scientists proposed that ultrasound velocity (UV) through human muscle

tissue could detect dehydration in the field setting. The method they are currently studying

uses two calipers with transducers to transmit and receive ultrasound signals through muscle

tissue. Previous research has demonstrated that as water is lost from muscle tissue UV

increases. Changes in the speed of sound through animal muscle tissue at different water

contents displayed a 3 m/s increase in ultrasound velocity with every 1% reduction in water

loss from the tissue (Sarvazyan, Tatarinov, & Sarvazyan, 2005). Additional in vivo work

with edema patients undergoing dialysis showed a less inversely proportional relationship

with a 5% decrease is body mass resulting in a 5 m/s increase in UV. A recent study by Utter

et al. (2010), demonstrated that ultrasound velocity through human muscle tissue can

determine changes in acute dehydration and rehydration status in male collegiate wrestlers.

Changes in hydration status, as a change in percent body weight, were positively correlated

with P_{osm} (r = 0.27, $p < 0.05$). UV measurements in both in vivo studies were only taken at

the soleus muscle. The present study will use a two-site approach with all subjects having

UV measures conducted at the soleus and biceps muscle. Additionally, both of the in vivo

studies had large individual subject variability resulting in lower than expected correlation

values. In light of previous research, it is unclear whether UV can accurately detect changes

in human hydration status due to dehydration and rehydration. Additionally, no

standardized, noninvasive, reproducible tool to accurately assess hydration status exists

(Armstrong, 2005). Finally, there is no data that examines the change in UV with exercise

induced dehydration and rehydration using a two site measurement approach. Thus, additional investigation into the ability of UV to detect changes in human hydration status is clearly needed.

Human hydration status is a measure of total body water (TBW). TBW is defined as the combined volumes of water within the extracellular compartment which is comprised of blood plasma and interstitial fluid (ECF) and intracellular fluid (ICF) compartments. Intracellular volume and extracellular volume account for approximately 38% and 25% of total body mass, respectively. This means that TBW accounts for roughly 63% of total body mass (Armstrong, 2007). However, this value is subject to change due to the influence of body composition. In adults, roughly 70% of lean tissue mass and 10% of fat mass is comprised of water, with females having lower amounts of TBW than males due to higher body fat content (Shoeller, 1989; Sawka, Cheuvront, & Carter, 2005). It is established that TBW is a dynamic fluid matrix that is constantly in flux with respect to position changes, daily activity, dietary intake, exercise, and natural perturbations (Armstrong, 2007). Moreover, approximately 5% to 10% of TBW is turned over daily through obligatory water loss avenues (Sawka et al., 2005). Despite this turnover, humans maintain a stable state of TBW through thirst and hunger mechanisms despite differences in activity levels and age as long as water and food is readily available (Sawka et al., 2005). The regulation of TBW is achieved by modulating urine output, generating metabolic water, and consuming fluids to match loses incurred from exercise sweat loss and obligatory sources (Cheuvront, Kenefick, Charkoudian, & Sawka, 2013). However, there are large individual differences in the amount of TBW that constitutes an adequate state of hydration. It is known that exposure to heat stress and physical activity increases the need for fluid intake to maintain TBW levels

(Sawka et al., 2005). Of recent interest is the measurement of TBW volumes at single time points in order to identify an individual's current hydration status. This issue is of particular interest amongst athletic populations in which inadequate amounts of TBW can lead to performance decrements and increase health risks.

Euhydration and hypo-hydration are two terms that are used to describe TBW as a hydration status. Euhydration is a term used to describe TBW fluctuations that have little variation from a mean basal TBW value. Hypo-hydration is defined as a negative net balance of water intake to TBW loss resulting in a TBW value outside that of normal fluctuations from the basal mean (Armstrong, 2005). Dehydration is a term used to describe the act of losing TBW. One type of dehydration is hypertonic hypovolemia that occurs when fluid lost as sweat during activity or exercise leads to an inadequate state of TBW (Cheuvront, Ely, Kenefick, & Sawka, 2010). Because TBW is a dynamic fluid state, shifts between the ECF and ICF that occur during dehydration and rehydration dictate an individual's state of hydration. There is research to suggest the fluid shifts during exercise induced dehydration are different between men and women (Eijsvogels, Scholten, Duijnhoven, Thijssen, & Hopman, 2013; Gibson, Stuart-Hill, Pethick, & Gaul, 2012; Weitkunat, Knechtle, Knechtle, Rüst, & Rosemann, 2012), but these findings have yet to be confirmed by additional research. A recent review paper by Cheuvront et al. (2013) determined that during acute exercise induced dehydration, the ratio of blood plasma to TBW loss was 1:10. The authors believed the low ratio was a result of ICF shifting into the ECF due to an osmotic gradient occurring at a roughly 2% reduction in plasma volume. Some researchers believe that this ICF to ECF shift leads to selective water loss from the interstitial fluid to maintain cardiac stability and preserve plasma volume (Patterson, Stocks, & Taylor,

2014; Cheuvront et al., 2013). However, additional research found that during exercise there

are reductions in intra-vascular fluid, interstitial fluid, and ICF (Hamouti, Coso, & Mora-

Rodriguez, 2013). Additionally, it has been suggested that during exercise there is an

increase in extracellular fluid tonicity. This increase in tonicity is matched with increases in

osmolality of the ICF causing a net shift of water into the interstitial fluid and muscle cells

from the intravascular space (Armstrong, Maughan, Senay, & Shirreffs, 2013). While debate

exists, it appears that researchers' believe exercise causes water shifts of intravascular fluid

compartment and ICF to the interstitial fluid compartment where it is lost as sweat. This

causes exercise induced dehydration which can then be detected in blood and urine samples.

The issue of exercise induced dehydration and its ability to be measured is of particular

interest to athletic trainers and coaches who have athletes that deal with acute hypertonic

dehydration occurring with moderate-duration, vigorous exercise.

Exercise induced dehydration results in negative effects on exercise performance

(Judelson et al., 2007; Kraft et al., 2012; Aldridge, Baker, & Davies, 2005; Goulet, 2012,

2013 ; Sawka et al., 2007; Smith, Newell, & Baker, 2012). According to a position statement

released by the National Athletic Trainers' Association (NATA), a 2% to 3% decrease in

body weight due to dehydration prior to the start of exercise is associated with impaired

oxygen consumption, physical work capacity, reflex function, temperature regulation,

muscular strength, and muscular endurance (Turocy et al., 2011). Additionally, dehydration

of 2% body mass increases ratings' of perceived exertion (RPE) and decreases cognitive

motor capability (Sawka et al., 2007; Smith et al., 2012). Two recent reviews by Judelson et

al. (2007) and Kraft et al. (2012) on dehydration and anaerobic exercise performance

concluded that dehydration decreases anaerobic exercise performance but is largely mode

dependent. Intermittent high intensity anaerobic activity was affected at a 2% body mass loss and maximal isometric strength at 4%. Single work bouts of anaerobic exercise greater than 30 s in duration along with other types of anaerobic activity seem to be effected at a body mass reduction of 2.5%. The authors also concluded that hot water submersion dehydration versus exercise induced dehydration, may affect the performance decrements observed and could confound the dehydration thresholds reported. While there appear to be confounding factors and limitations, the current research still indicates that dehydration has detrimental effects on several factors influencing athletic performance. Additionally, any measurement at a single time point may not truly represent hydration status and there is no method to directly measure intracellular fluid volumes (Armstrong, 2007). Still, many indirect measurement techniques are used to identify hydration status.

Current indirect measures consist of laboratory and field assessments. Research suggests that laboratory methods using serial dilution techniques, neutron activation analysis, and blood plasma osmolality (P_{osm}) are the most accurate and precise methods available (Armstrong, 2007; Kavouras, 2002; Popowski et al., 2001; Sawka et al., 2007; Schoeller, 1989; Tam & Noakes, 2013). Serial dilution techniques using stable isotopes take approximately three to four hours to equilibrate evenly into all body water compartments and were not available for use in this study (Armstrong, 2005). The use of P_{osm} to detect changes in dehydration is based on the concept that water lost from blood plasma during dehydration causes an increased solute concentration and plasma osmolality. There is research by Armstrong et al. (2007) and Armstrong, Maughan, Senay, & Shirreffs (2013) questions P_{osm}'s ability to detect changes in hydration status on the basis of arginine vasopressin and angiotensin II induced renal water retention, differences in TBW losses incurred (hypo-

hydration verses dehydration), and differences in individual plasma protein concentrations. However, recent work by Cheuvront et al. (2013) provides evidence that P_{osm} was superior to urinary indices even with large intra-individual and inter-individual variation within the individuals tested, and consideration to the influence of arginine vasopressin and angiotensin II. The authors also found that P_{osm} was the only reliable measure for predicting hydration status from a single measure (Cheuvront et al., 2010). However, P_{osm} requires blood sample collection and has low portability, high invasiveness, and high time constraints which make it of little use in the field (Armstrong, 2007; Stachenfeld, 2014). Thus, body mass and urinary measures are more widely used in the field setting.

Research by Armstrong (2005) has questioned body mass as an accurate measure of TBW loss due to dehydration. The arguments by Armstrong (2005) were made on the basis of normal weight fluctuations, bowel movements, and loss of mass due to substrate utilization. Armstrong (2005) also noted that body mass measurements require true baseline values and three consecutive measurements to accurately determine daily body mass at any given time point. However, a recent paper by Baker, Lang, & Kenney (2009) testing the efficacy of body mass as a marker of TBW loss determined that it was more highly correlated than urine and plasma markers when the concerns raised by Armstrong (2005) are controlled for. This is important to note because change in body mass is the primary factor that many other indirect TBW measures such as blood and urinary markers are correlated with. The two most common urine based markers are Urine Specific Gravity (U_{sg}) and Urine Osmolality (U_{osm}). Urine osmolality is used to assess dehydration based upon increasing urine solute concentration as water is lost from the body (Shirreffs, 2000). Urine specific gravity is based on the mass per volume of urine and is determined using a refractometer that

allows the clinician to determine specific gravity as compared to that of water (Armstrong, 2005). At present U_{sg} provides the most practical option for field based dehydration assessment. A study by Oppliger, Magnes, Popowski, & Gisolfi (2005) found that U_{sg} and U_{osm} correctly identified hydration status in only 65% and 63% of subjects, respectfully, when compared to plasma osmolality. Conversely, research by Hamouti, Coso, & Mora-Rodriguez (2013) found U_{sg} to be as sensitive as P_{som} during an acute bout of dehydrating exercise. A study by Cheuvront, Ely, Kenefick, & Sawka (2010) determined that U_{osm} and U_{sg} where useful in detecting dehydration in multiple, but not in single time point measurement settings. As evidenced by the previous studies, current field assessments appear logistically difficult to administer and lack the accuracy and precision needed to adequately evaluate hydration status. However, they do provide a well-researched basis of comparison for new forms of dehydration assessment techniques as is the case in this study. The purpose of the present study was twofold. First, is to confirm UV as a valid form of hydration assessment for both male and female collegiate athletes in the field setting. The second purpose is to determine if individual subject variability can be reduced using the soleus and biceps muscles as measurement sites. I hypothesize that UV will be able to detect states of euhydration and dehydration in both subject populations with decreased individual variability due to the two site measurement approach. Validation of UV to detect acute changes in hydration status due to exercise will have direct applications to coaches and athletic trainers in the field because it will allow them to rapidly determine an athlete's hydration status throughout practice and competition.

Statement of the Problem

        Current field based methods to assess human hydration status in athletic populations lack the precision and accuracy of laboratory based methods while still requiring the use of urine samples. Recent development of a hydration monitor to evaluate human hydration status without the need for any blood or urine samples may provide an alternative to urine based field assessments. However, recent research on early prototypes of the device has revealed significant individual variability in ultrasound velocity measurements in human muscle tissue. Finally, there is no data that examines the change in UV with exercise induced dehydration and rehydration or in female athletes using a two site measurement approach. Thus, additional investigation into the ability of UV to detect changes in human hydration status is clearly needed.

Hypothesis

        The null hypothesis is that ultrasound velocity will not be able to detect changes in hydration status or reduce individual variability following acute dehydration and rehydration in male and female collegiate athletes with a two site measurement approach. The alternative hypothesis is that UV will be able to detect states of euhydration and dehydration in both subject populations with decreased individual variability due to the two site measurement approach.

Significance

        Validation of UV to detect acute changes in hydration status due to exercise will have direct applications to coaches and athletic trainers in the field because it will allow them to rapidly assess athlete's hydration status throughout practice and competition. Additionally, this device could be used to ensure adequate hydration in elderly individuals in assisted

living communities, patients participating in cardiac rehabilitation programs, and by soldiers

on extended campaigns in the field.

Definition of Terms

Total Body Water: The total amount of water stored in the intravascular space, interstitial

fluid, and intracellular compartments at any given time

Euhydration: A state of adequate total body water content within the normal limits of total

body water fluctuations around a basal set point value

Hypo-Hydration: A state of inadequate total body water content

Dehydration: The act of losing total body water through exercise, heat induced, or

obligatory water loss pathways.

Ultrasound Velocity: The speed of sound calculated as the time it takes a burst of ultrasound

to travel through a medium that is between a transducer and transmitter

Osmolality: The number of osmols of solute per liter of solvent and is common measure of

concentrations within fluid mediums

Plasma Osmolality: number of osmols of solute per liter of blood plasma

Urine Osmolality: number of osmols of solute per liter of Urine

Urine Specific Gravity: Density of urine as compared to the specific density of water

Literature Review

Human hydration status as assessed by total body water (TBW) presents a difficult problem due to number of water containing compartments in humans and the complicated interactions between those compartments. This problem is further complicated due to the substantial individual variability between sexes, races, and due to activity status. Because of the complicated nature of human TBW compartmentalization and individual variability, current indirect field based methods have been unable to accurately assess hydration status. Conversely, lab based methods, while accurate, are time consuming and highly invasive. Additionally, there is currently no method to directly measure intracellular fluid volume.

Currently, urine specific gravity (U_{sg}) offers the best field based assessment in terms of portability, accuracy, precision, and low invasiveness. However, ultrasound velocity (UV) has been proposed as a new form of hydration assessment. If proven, UV is advantageous to current field measures because it does not require tissue samples. Recent research revealed that UV can identify changes in hydration status. However, there is a large amount of intra- and inter-individual variability in the measurements that have been obtained. Moreover, UV differences between sexes have yet to be investigated. In light of previous research, it is unclear whether UV can accurately detect changes in human TBW during acute dehydration and rehydration. An in depth review of possible sources of error and variability are explored in the present paper in order to establish the potential validity of UV as method for human hydration status assessment.

Total Body Water

  Race differences in total body water. Research examining TBW is expansive.

Therefore, the information presented here only reflects what is related to ultrasounds ability

to assess TBW content as it relates to the specific subjects in this study. A descriptive study

by Chumlea et al. (2001) compiled four data sets from various locations in the United States

and determined that significant age and sex differences in TBW content exist. TBW was

measured using deuterium or tritium dilution and body composition with dual x-ray

absorptiometry (DXA) machine. They examined 604 White men, 128 Black men, 772 White

women, and 191 Black women all 18 to 90 years of age. Mean values for TBW, total body

fat (TBF) and fat free mass (FFM) were compared between sex and races. On average black

individuals had larger TBW values than white individuals at all ages. High values for TBW

were also associated with higher values of TBF and FFM in all subjects Chumlea et al.,

(2001). Earlier research by (Aloia, Vaswani, Flaster, & Ma, 1998) corroborated these

findings by examining 72 Black women and 128 White women to determine changes in

TBW values and compartmentalization with age. Using similar methods as Cameron et al.

(2001), the authors concluded that race plays a role in TBW due to the ratio of TBF to FFM

difference observed. The authors also recommended that body water estimation be adjusted

for age, weight, and race (Aloia et al., 1998). Additional work by Raman et al. (2004) found

significant differences in TBW between black men and white men suggestive of greater

water retention in black individuals as compared to white individuals.

  The athletes examined in the present study are of diverse racial origins, and as such,

they may exhibit differences in body composition and levels of FFM. Differences in FFM

and subsequently TBW may affect measures of the device in the current study because it is

designed to assess the water content within the muscle tissue. Additionally, it appears that there may be differences in TBW between sexes.

      Daily and weekly variability in total body water.   Additional information from the study by Raman et al. (2004) revealed that daily water turn over, as measured by a ratio of urine output to water intake as food and fluid, was greater in white individuals then black individuals. A study by Bartoli, Davies, Pate, Ward, & Watson (1993) measured TBW in 10 males 21 to 32 years four times using deuterium dilution. Measurements were taken with at least seven days separating each session to understand weekly variability in TBW turn over. The authors found that individual TBW values varied as much as eight liters for each individual over the four time points. Combining these findings with those of Raman et al. (2004) it appears that daily and weekly TBW is highly variable amongst individuals and that there are differences in TBW turnover rates between races. It also seems that any baseline TBW level, as assessed by a single measurement, may not represent an individual's true TBW value in a completely euhydrated state. In the case of the present study, the immediate values used to indirectly assess TBW are used to screen for hypo-hydration. However, due to the relatively large variability in TBW values, one athlete's average TBW content maybe different than that of another athlete even when in a euhydrated state. Because cut-off values for UV regarding what constitutes a hydrated state have not been clearly established, this may lead to the misidentification of an athlete being hypo-hydrated or euhydrated at baseline when in fact they are not. This suggests that in order to have a true baseline value, each athlete should be assessed over multiple days to have a more accurate indication of what is, and is not, a euhydrated state for each individual. This was not done in the present study because the current investigation is only concerned with changes from baseline incurred from

exercise induced dehydration and subsequent rehydration. Additionally, multiple measures of

TBW were used to reduce the chance of committing Type I and II errors.

Total Body Water Shifts

      Changes in position. A study by Gibson, Beam, Alencar, Zuhl, & Mermier (2014)

examined 64 subjects, 32 of whom were men. TBW, ECF, and ICF values were determined

using multi-frequency bioelectrical impedance spectroscopy (BIA). BIA measurements were

taken in the supine and standing positions at 5 min intervals for 30 min. TBW, ECF, and ICF

were calculated at each five minute interval using information gathered from the BIA

measurements. The authors found that in the supine position ECF decreased with time while

ICF increased. When standing, ECF increased with time and decreases in ICF were not

significant. At each time point supine values for ICF and ECF differed from standing values.

Men had a larger decrease in the percent of ICF across time points for the standing condition

when compared to women Gibson et al. (2014). The findings of this study show that position

effects where water is stored in the body and complete equilibration of TBW may take up to

30 min following a shift in position. In the present study subjects move from a seated

position to a standing position prior to being assessed by the ultrasound monitor and having

blood drawn. Subjects will be standing for various time intervals prior to being assessed by

the ultrasound monitors. If the ultrasound device is placed correctly on the limb ICF and

interstitial fluid should be dominant water compartments measured. Therefore, if ICF values

do not change during the standing condition, increases in interstitial fluid values upon

standing would be a result of water influx from the vascular space. This may cause an

overestimation of water content during rehydration and dehydration potentially leading to

Type I and Type II errors. Additionally, if ECF generally increases when shifting from

supine to standing conditions it may be possible that slight increases in blood plasma osmolality could be a result of the potentially still equilibrating fluid compartments. These potential errors may be most prevalent in the soleus muscle due to its location and the effects of gravity on shifting blood volume to the lower extremities. However, it should be noted that these conclusions are only based the findings of a single study due to the sparseness of this topic amongst the literature.

Total body water shifts with exercise. A recent study examined nine well trained male cyclists aged 13 to 33 years. The subjects refrained from exercise 24 hr prior to the day of testing. Subjects ate a standard breakfast rich in carbohydrates along with water to ensure euhydration. Upon arrival subjects urine specific gravity was determined using a refractometer. If it was determined that the subjects were hypo-hydrated their testing was rescheduled. If euhydrated, subjects were then weighed and asked to give blood, urine, and muscle biopsies for baseline data. Each subject then exercised for 150 min. Blood samples, sweat samples, gas exchange values, and weights were recorded at four time points during exercise. Blood samples, weight, urine, and muscle biopsies were immediately collected, 1 hr, and 4 hr post exercise. The researchers found that after exercise subjects lost an average of 4.7% of body weight or 2.75 L of water by sweating at a rate of 1.06 liters per hour. TBW explained 86 % of water lost even with metabolic water production accounted for. During exercise plasma volume (PV) decreased progressively while total muscle water (TMW), as determined by analyzing pre exercise and post-exercise muscle biopsies, was unchanged.

Conversely, at 1 hr and 4 hr post-exercise PV increased while TMW decreased. Muscle electrolyte concentrations were unchanged immediately post-exercise, while serum electrolyte concentrations increased during exercise. At four hours post exercise muscle

electrolyte concentration increased while serum electrolyte concentrations decreased. During

exercise ECF decreased while ICF increased from pre exercise levels. At one hour post

exercise ECF had increased to close to baseline levels while ICF decreased. There were no

significant differences between the one and four hour time points (Mora-Rodríguez,

Fernández-Elías, Hamouti, & Ortega, 2014). The findings of this study suggest that the ICF

does not contribute water to sweat during exercise and that ICF increases in the working

muscle due to the production of metabolic water. It also demonstrates that at one hour post

exercise ICF is retracted to resupply intravascular water when no fluid to maintain hydration

is ingested.

The findings of Mora-Rodríguez et al. (2014) are similar to early work in the field by

Costill, Cote, & Fink (1976) who found that no changes in muscle water content were

detected after 120 min of cycling resulting in an average 3.4% decrease in body weight. In

this study, Costill et al. (1976) took muscle biopsies from the thigh at 30 min post-exercise

and subjects did not consume any fluids during or after exercise. The combined findings of

these studies evidence that intra-muscular water content is stable after exercise induced

dehydration without fluid replacement at similar body weight losses as the present study.

Additionally, decreases in muscle water exhibited at the one hour time point are in line with

current thinking that post exercise pressure decreases and increased protein content within

the intravascular space causes ICF to shift into the vascular space to normalize blood volume.

Additional work by (Sanders, Noakes, & Dennis, 2001), examined six cyclists who

performed three separate 4 hr rides to elicit exercise induced dehydration. The authors found

that with lower sodium intakes during exercise greater water loss occurred from the ECF

with little change to the intracellular space. Conversely, the researchers found that increased

sodium intake caused an attenuation of water losses from the ECF and at the highest intakes an expansion of the ICF was noted (Sanders et al., 2001). In the present study, no sodium was administered during exercise. Therefore, it is presumable that most of the water losses incurred were from the extracellular space. However, unlike the study by Mora-Rodríguez et al. (2014), there was not dietary recall or a standardized meal consumed by the subjects in the present study. This leaves the possibility that some athletes could have consumed high amounts of sodium prior to practice and thus during exercise may have had varied water losses and shifts from different compartments. A standardized meal controlling for sodium intake was not used in this study because in a field based setting it is unlikely that athletes will consume the same pre exercise meal consistently. Thus, changes in water losses associated with potentially high sodium intakes prior to exercise are a potential limitation to the present methods.

The previous research is in line with current thinking that exercise induced dehydration, when no fluid or electrolytes are consumed during exercise, causes water losses to occur from the intravascular and interstitial spaces. Moreover, because the changes in intramuscular water were minimal or non-existent during exercise induced dehydration, the ability of UV as it is being used in this study will rely predominantly on detecting losses of water from the interstitial not intracellular space. Additionally, the possibility of intramuscular water increasing during exercise may provide another source of error in detecting dehydration immediately post exercise.

Sex Differences in Exercise Induced Dehydration

  Differences in body mass loss. A study by Weitkunat et al. (2012) examining 20 male and 11 female open-water swimmers competing in an ultra-endurance swimming event

found that males had larger body mass reductions than females. However changes in plasma

sodium concentrations were similar between sexes with both sexes exhibiting decreases

indicative of exercise induced hyponatremia. No significant correlations were found between

fluid intake and plasma sodium concentration, plasma volume, or hematocrit. Subject body

mass, body fat (skinfolds), arm and leg circumferences, hematocrit, plasma sodium

concentrations, plasma volume, and urine specific gravity were determined before, during,

and after the event. Caloric, sodium, and fluid intake were also tracked using food logs from

the support crew and dietary reference tables Weitkunat et al. (2012). These results were

similar to those of another study by Eijsvogels, Scholten, Duijnhoven, Thijssen, & Hopman,

(2013).

   The study consisted of 98 volunteers who walked 30-50 km at a self-selected pace.

Body weight and blood sodium levels were recorded prior to and following the exercise bout.

Fluid intake was also recorded throughout the exercise bout. The authors found that men

exhibited a larger decrease in body mass than women. Post exercise blood sodium

concentrations in men were also higher than in women. Finally, fluid intakes were lower for

men than women during the exercise bout (Eijsvogels et al., 2013).

   In both studies male subjects lost greater body mass than females despite similar

exercise environments. However, Weitkunat et al. (2012) found no differences between

plasma sodium levels indicating that even when water is available, women and men

dehydrated at comparable levels. This is in contrast with the findings of Eijsvogels et al.

(2013) who found the opposite. However, because males drank less frequently than females,

it is likely that water ingestion differences are what accounted for the higher plasma sodium

concentrations in the study by (Eijsvogels et al., 2013). In the present study both males and

females were asked to lose the same percentage of body weight though exercise induced

dehydration. Because water intake is prohibited during exercise in this study, we believe that

a comparable change in body mass between both sexes is feasible.

Differences in sweat rates. A study by Ichinose-Kuwahara et al. (2010) examined

the sweat rates of 37 subjects during different exercise intensities. The authors examined

eight trained and nine untrained males to ten trained and untrained females during cycling

exercise. Sweat rates were calculated using the ventilated capsule method. Sweating rates

were higher in trained verses untrained subjects. Males exhibited a greater increase in

sweating rates at higher exercise intensities than females. Increased sweat rates with

increased exercise intensity were a product of increased sweat gland output in trained

females, while in untrained females additional sweat glands were activated (Ichinose-

Kuwahara et al., 2010). In the present study, I examined trained females, and based on the

findings of Ichinose-Kuwahara et al., (2010), trained females may need to exercise for a

longer duration or at higher intensities than their male counterparts in order to achieve the

desired degree of exercise induced weight loss for the present study.

Pre-practice hydration status. Another study by Volpe, Poule, & Bland (2009)

examined 138 male and 125 female athletes measured pre-practice hydration status using

urine specific gravity. The authors recorded the current menstrual cycle phase and

determined three levels of hydration as euhydrated, hypo-hydrated, or significantly hypo-

hydrated. They found that a greater percentage of men (47%) were hypo-hydrated than

women (28%). The authors also found that 13% of students were significantly hypo-

hydrated, 53% were hypo-hydrated, and 34% were euhydrated. Additionally, there were no

differences observed between phases of the menstrual cycle (Volpe, Poule, & Bland, 2009).

Further research on 34 junior elite female soccer players determined that 45% of the athletes were hypo-hydrated upon arriving at practice at the same urine specific gravity cut off value (1.020) of the subjects in the study by Volpe et al. (2009). Urine samples, weight, and sweat sodium content were collected at two different training sessions. Changes in body weight suggested that mild exercise induced dehydration occurred. Despite having fluids available at practice fluid consumption was low (Gibson et al., 2012).

The previous two studies demonstrate that both sexes have tendencies to be hypo-hydrated prior to exercise. However, subjects in both studies were not given specific instructions or protocols on how to hydrate prior to testing, as is the case in the present study. This may have contributed to the large percentage of athletes who presented in the hypo-hydrated condition due to inadequate fluid consumption prior to practice. It is still worth noting that in general males present in a hypo-hydrated state more often than females. Furthermore, as demonstrated by Costill et al. (1976), ICF will potentially increase or not change during exercise. Therefore, if athletes are hypo-hydrated prior to exercise there may already have been a retraction of ICF into the interstitial and vascular space. This would mean that athletes who presented in a hypo-hydrated state would have lower levels of ICF prior to the start of exercise. This could lead to potential increases of ICF immediately following exercise due to metabolic water production to be more pronounced. Because UV measures both the intracellular and extracellular space, it possible that athletes who present in a hypo-hydrated state may have reduced changes in UV immediately following exercise compared to pre exercise baseline. Thus changes in UV in these athletes may need to be confirmed by additional hydration assessment (U_{sg}, P_{osm}, and U_{osm}), as is the case with the present study.

Considerations of menstrual cycle phase. In the study by Volpe et al. (2009), no differences between menstrual cycle phases were observed. This suggests that menstrual cycle phase did not affect pre-exercise TBW content. Additionally, an earlier study of five subjects was conducted to determine the effect of menstruation on acute dehydration and rehydration. Dehydration was induced through cycle ergometer exercise two days prior to the onset of menses and then again at five and 19 days after the onset. Dehydration was asses as a decrease in body mass and rehydration by the volume of fluids ingested. No differences in dehydration and rehydration were observed at the three different time points. These studies provide evidence that phase differences in the menstrual cycle do not affect TBW as it relates to dehydration and rehydration. Therefore, menstrual cycle phase was not recorded in the present study.

Exercise and Dehydration

Anaerobic exercise and dehydration. In a recent study by Maxwell, Mackenzie, & Bishop (2009), eight unacclimated cyclists rode a cycle ergometer for 90 min on day one and then followed one of three hydration strategies designed to induce euhydration, mild hypo-hydration, and moderate hypo-hydration. Hypo-hydration was determined as a percent of body mass lost and for mild and moderate conditions mean values were 0.99% and 3.88%, respectfully. On day two, all subjects performed an intermittent sprint test on a cycle ergometer. Peak power and total work were measured on the last sprint following the initial intermittent sprints. Heart Rate and rectal temperature were also collected and used to calculate a physiological strain index value. Urine samples and blood samples were also collected during both days to determine plasma volume, via hematocrit and hemoglobin concentration, along with changes in urine specific gravity and osmolality. All testing was

performed in a 35ºC environment. The authors concluded that there was increased

physiological strain in the moderate hypo-hydrated condition when compared to the

euhydrated condition. Total work and peak power were also reduced in the moderate hypo-

hydrated condition when compared to the other conditions. There were increases in

hematocrit and hemoglobin on day one from pre to post along with body mass reductions

while no changes were found in U_{sg} or U_{osm}. Urine specific gravity and U_{osm} increased from

day 1 to day 2 for the hydrated conditions (Maxwell, Mackenzie, & Bishop, 2009).

In a study by Kraft et al. (2011), ten males served as their own controls and

performed exercise in euhydrated, hypo-hydrated, or dehydrated conditions. Both hypo-

hydration and dehydration were achieved via hot water bath submersion until a 3% decrease

in body mass was observed. In the hypo-hydrated, condition subjects consumed fluid to

replace the losses incurred during the bath. Subjects then performed six 15 s sprints on a

cycle ergometer with 30 second active recoveries. Peak and mean power was recorded for all

groups. Initial hematocrit was determined by pre and post blood draws following all sprinting

performances. The authors found a reduction in mean power output for the dehydrated

condition that also approached significance for the hypo-hydrated trial. Peak power was

significantly reduced compared to the control trial in the hopo-hydrated condition and

approached significance for the dehydrated condition. Dehydration impaired peak and mean

power the most when compared to the control condition with hypo-hydration as an

intermediate.

In further work by Kraft et al. (2010), ten males performed two bouts of total body

resistance exercises. Subjects served as their own controls and were heat exposed to incur

either hypo-hydrated or dehydrated conditions and heat exposure methods were the same as

the previous study discussed. During hypo-hydrated trials subjects were permitted to replace

fluids lost with water. Total repetitions, heart rate, and RPE were recorded during testing

sessions. The subjects performed three sets of resistance exercise to failure for bench press,

lat pull down, overhead press, barbell curl, triceps press, and leg press. The authors found

that total repetitions for all sets and exercises combined were significantly lower for the

dehydrated condition when compared to the hypo-hydrated condition. RPE values

approached significance between dehydrated and hypo-hydrated conditions. Post exercise

heart rates were higher in the dehydrated condition than the hypo-hydrated condition (Kraft

et al., 2010). This study demonstrates that when 3% of body mass is lost as body water,

decreases in anaerobic exercise performance in the form of weight training are observed.

Another study by Jones, Cleary, Lopez, Zuri, & Lopez (2008) examined seven

subjects serving as their own controls. All subjects performed upper and lower body

anaerobic Wingate tests in both euhydrated and dehydrated conditions. Dehydration was

achieved via treadmill exercise in a warm 33.1°C environment to a point of 3% reduction in

body mass. Dehydration was confirmed by urine color and specific gravity methods. The

authors found that self-reported fatigue severity was significantly increased in the dehydrated

verses euhydrated condition. Mean and peak power output were significantly reduced in the

upper and lower body for the dehydrated condition as compared to euhydrated condition

(Jones et al., 2008).

The works of Maxwell et al. (2009) and Kraft et al. (2011) suggest that regardless of

how dehydration is induced, reduction in peak and mean power along with total work occur

around a loss of 3% of body weight, which is the desired change in the present study. The

work by Maxwell et al. (2009) is more comparable to the present study because exercise

induced dehydration was used to elicit the percent changes in body weight. Additionally, it should be mentioned that the authors found only a weak positive correlation between body weight reductions and several of the performance variables. However, when connecting the sum findings of each study in this section, it appears that a decrease of 3% body weight due to dehydration or hypo-hydration will reduced anaerobic exercise performance regardless of the mode of exercise. Additionally, in the studies where perceived exertion was measured, Jones et al. (2008) and Kraft et al. (2010), perceived effort increased with dehydration further confirming that exercise increases both the actual and perceived effort to sustain exercise.

Dehydration and Aerobic Exercise Performance

  Considerations of methodology. In his meta-analysis of literature regarding endurance exercise performance and dehydration, Goulet (2013) determined that a 2% decrease in percent body weight impairs endurance performance when a fixed intensity model is used. However during exercise a decrease in body weight less than 4% was unlikely to impair aerobic exercise performance under time trial conditions. This suggests under "real world" conditions, when fluids are available and consumed, dehydration at the level currently being studied may not impair exercise performance. However, the author restricted his analysis to that of studies where individuals began exercise in a euhydrated state. This means that Goulet, (2013) is not considering the possibility that athletes in real world settings may not be well hydrated prior to starting exercise. This seems to be a likely situation as determined by the work of Volpe et al. (2009) and Gibson et al. (2012) who demonstrated that almost half of athletes in a population similar to those in the present study, were dehydrated upon starting exercise.

Pre exercise hypo-hydration and aerobic exercise. In a recent study by Lopez et al. (2011) of 14 subjects, seven males and seven females were randomly assigned to either euhydrated or hypo-hydrated groups. Euhydrated subjects consumed water prior to and during testing, while dehydrated subject were not allowed to consume water for the 22 hr prior to or during testing. All subjects ran three 4 km laps on a trail in a warm environment. All runners ran at the same relative intensity as indicated by heart rates. Hypo-hydrated runners exhibited increased ratings of perceived exertion, muscle pain, and slower run times when compared to the euhydrated runners. Hypo-hydrated runners also exhibited a 2% in body mass (Lopez et al., 2011). This study highlights a common theme in the literature of a 2% reduction in body weight due to dehydration eliciting a decrease in performance during aerobic exercise. It also demonstrates that when intensity is fixed hypo-hydration can impair exercise performance in a field setting, which is line with a recent meta-analytic comparison of the literature by Goulet (2013). Additionally, subjects in this study were exposed to pre exercise fluid intake restriction to achieve dehydration which, as previously noted, maybe similar to actual hydration statuses of athletes during practice and competition.

Another study of eight rugby players serving as their own controls followed a pre-exercise dehydration protocol. Subjects performed two 30 min aerobic exercise sessions on a cycle ergometer. During each trial subjects were either dehydrated or euhydrated as was achieved by fluid intake manipulation in the 12 hr preceding testing. U_{osm} values were also measured to quantify hydration status. U_{osm} values were 385 ± 184 mOsmol/kg for the euhydrated trials and 815 ± 110 mOsmol/kg for Hypo-hydrated trials. The authors found significant increases in heart rate, RPE, and VO_2 in the hypo-hydrated condition when compared the euhydrated condition (Aldridge et al., 2005). It is worth noting that subjects in

this study had a controlled meal prior to exercise and the euyhdrated group had clear

instructions to consume 500 ml of fluid the morning prior to testing. This is similar to the

procedure described by (Mora-Rodríguez et al., 2014). The researchers only used one

method for the identification of hydration status which is unusual for work in this field and

may present a potential limitation to the results. However, this study still provides further

evidence that the negative effects of hypo-hydration on aerobic exercise performance are a

function of impaired cardiovascular function resulting from decreased plasma volume lost

during exercise. In the present study instructions were given to the subjects to present in a

euyhdrated state but no specific food or water intakes were recommended and thus may

present an additional limitation to the results.

Other research examining six trained and untrained cyclists serving as their own

controls found that heart rate and rectal temperature were increased in the hypo-hydrated

condition for untrained but not trained individuals. Subjects performed two 40 min exercise

bouts in either a euyhydrated or hypo-hydrate state. Hypo-hydrated states were identified as

a body mass reduction of 1.5% to 2%. Urine specific gravity, rectal temp, heart rate, sweat

rate and volumes, and hematocrit and hemoglobin were recorded. The subjects were allowed

to drink ad libitum during their exercise trial in the hypo-hydrated state (Merry, Ainslie, &

Cotter, 2010). The findings of this study suggest that hypo-hydration impairs the body's

ability to maintain thermoregulation due to a reduced ability to dissipate metabolic heat only

untrained individuals. Furthermore, a hypo-hydration level of up 2% is not enough to impair

aerobic performance in trained individuals and thus a larger threshold of body weight

reduction due to dehydration maybe needed to elicit negative effects of training. This is in

contrast to many other studies and recent review papers that have identified a 2% reduction

in body mass due to dehydration as the point at which aerobic exercise performance decreases (Cheuvront et al., 2010; Goulet, 2012).

Based on current evidence, it appears that both anaerobic and aerobic exercise performance are impaired by a reduction in total body water as indicated by a reduction in body mass. In most cases this was achieved by fluid restriction prior to exercise which confirms that starting exercise in a hypo-hydrated state is deleterious to exercise performance. Additionally, it appears that when subjects start exercise in a euhydrated state, fluid losses incurred during exercise may or may not reduce exercise performance. In the present study, athletes were asked to decrease TBW by 3% of body weight. This was done with the intention that it would cover the range of dehydration that causes impairment in both aerobic and anaerobic exercise.

Rehydration Protocols

In a recent study, eight volunteers dehydrated via intermittent exercise in the heat to an average level of 1.94% reduction of body weight. All subjects served as their on controls. Following dehydration subjects ingested one of four different beverages designed to induce rehydration. Body weight, sweat content, blood plasma, electrolytes, and urine output were recorded for each trial at six time points. These time points were pre-exercise, post-exercise, one hour, two hours, three hours, and four hours post exercise. The Authors found that blood plasma osmolality only returned to baseline during trials where a carbohydrate beverage (Gatorade) was ingested following exercise (Shirreffs, Aragon-Vargas, Keil, Love, & Phillips, 2007). The findings from this study demonstrate that carbohydrate beverages are advantageous to other fluids for eliciting rehydration following exercise induced dehydration.

In a similar study by Osterberg, Pallardy, Johnson, & Horswill (2010), 15 heat-acclimatized subjects reduced their body weight by 2% while dehydrating during a 90 min exercise bout and then ingested one of five beverages. The beverages were placebo (water), placebo with electrolytes, as well as 3%, 6%, and 12 % carbohydrate solutions. Urine specific gravity, U_{osm}, and P_{osm}, as well as, a number of other blood based markers were examined for all time points. Urine and blood samples along with weight measures were collected at pre, post, 60 min, 90 min, 180 min, and 240 min time points. There were no differences in the markers for dehydration between trials. Fluid retention for all carbohydrate beverages was greater than placebo or placebo with electrolytes at all-time points and no significant differences were observed between carbohydrate beverages (Osterberg et al., 2010). This study suggests that differences between carbohydrate content in beverages may not affect the ability of a fluid to elicit hydration as long as it contains at least 3% carbohydrate. In the present study, Gatorade®, Barrington, IL is being used to elicit rehydration following exercise induced dehydration which is representative of the 6% carbohydrate beverage reported on in this study.

Recent research by Clayton, Evans, & James (2014) examined eight healthy males using 2% and 10% carbohydrate beverages and measured the gastric emptying rate of the each beverage at 15, 30, 45, 60, 90, and 120 min post ingestion. At the end of the gastric sampling period, .3% of the 2% beverage and 54% of the 10% beverage remained in the stomach, respectfully. The authors found that the ingestion of a hypertonic carbohydrate beverage at 10% may reduce gastric emptying. The authors also determined that the 2% beverage had been retained more effectively at 60 and 120 minutes than the 10% beverage

(Clayton et al., 2014). This study suggests that with increases in carbohydrate content a delay in gastric emptying may occur and fluid retention may decrease.

In an recent study by Shi, Bartoli, Horn, & Murray (2000) eight healthy subjects ingested four different 6% carbohydrate beverages of varying osmolality's and compositions that were chilled to a temperature of 10°C. Gastric emptying rate and temperature was measured repeatedly using a nasogastric tube. There were no differences observed between beverages in gastric emptying times (Shi, Bartoli, Horn, & Murray, 2000). Another study using a similar protocol as above examined eight subjects who ingested two different beverages at two different temperatures. The subjects either ingested water at 10°C or 26°C or a sports drink (Gatorade®) at the same temperatures. Heart rates, exercise duration, rectal temperatures, plasma volume changes, plasma osmolality, and percent body weight reductions were comparable in all pre and post trials. These measurements were taken at pre, post, 30 min, 60 min, and 90 min time points. Subjects were allowed to drink ad libitum after exercise induced dehydration through the 90 min time point. Beverage consumption volumes were higher for the sports drink compared to water at both temperature conditions. Weight retention was highest for the 10°C sports drink condition at the 90 min time point (Park, Bae, Lee, & Kim, 2012). It should be noted that in the present study subjects had to ingest fluids equal to the difference in body weight lost between pre and post exercise weight collections.

In the present study, the beverages consumed by subjects are roughly 6% carbohydrate in content. Based on the findings of Clayton et al. (2014), Osterberg et al. (2010), and Shirreffs et al. (2007), it seems that the role of carbohydrate content on gastric emptying rate may or may not play a role in a beverages ability to elicit rehydration with in

60 and 120 min following exercise induced dehydration. Furthermore, the beverage chosen in this study is effective at eliciting fluid retention and hydration within the 120 min time period as is the time period used in this study. Moreover, the findings of Park, Bae, Lee, & Kim (2012) and Shi et al. (2000) suggest that lower beverage temperatures may increase beverage retention which might be due to a decreased gastric emptying time. Collectively, the literature seems to support the decision in the present study to provide beverages of 6% carbohydrate content. The literature also suggests that beverages ingested at different temperatures, as is the case in the present study, could affect retention rates and may present a potential limitation to the current study.

Body Mass as a Measure of Dehydration Status

In a recent work, 65 men were studied retrospectively by compiling results from earlier research examining hydration habits in soldiers undergoing exercise in the heat. The subjects nude body mass was recorded each morning for four to 15 days. The authors found that BM varied by 0.5 kg daily and that three consecutive days of measurements were able to accurately assess daily body mass variability. The authors also concluded that daily body mass variability is less than 1% for individuals who are replacing 100% of TBW losses incurred through exercise. This value was not significantly correlated with absolute body mass or the number of measurements taken (Cheuvront, Carter, Montain, & Sawka, 2004). This study provides evidence that body mass is relatively stable in individuals who are well hydrated even when engaging in exercising in the heat. The authors also suggested that any daily changes in body mass of less than 1% could not be ruled out as daily fluctuations.

An additional study of eight subjects serving as their own controls performed interval training session on separate days. All subjects performed tests under two conditions.

Condition 1 allowed for fluid consumption throughout testing while condition 2 did not.

Change in body weights and TBW were recorded pre and post exercise. Body weight

reductions during dehydration were between 2% and 4% of body weight. The authors found

the average change in body weight was not significantly different than the change in TBW.

The slope intercept relationship between BM and TBW did not approach zero or one, and the

intra-class correlation had a Pearson product correlation value of 0.76. Additionally, the

authors correlated values of U_{sg}, U_{osm}, and P_{osm} to changes in TBW and found correlation

values of 0.68, 0.61, and 0.63. Finally, the others found that the change in BM and TBW

during dehydration was not significantly different ($p = 0.29$; Baker, Lang, & Kenny, 2009).

The findings of this study suggest that exercise induced dehydration equivalent to the

decrease in body mass as in the present study is moderately correlated with decreases in

TBW. Furthermore, body weight was more highly correlated to changes in TBW than the

three other hydration markers used in the present study. This finding along with those of

Cheuvront et al. (2004) suggest that day to day body weight fluctuations are minimal and that

body weight losses incurred during exercise are more highly correlated with TBW than the

other hydration markers available for use in the current study. Together, these findings

support the use of body mass as the independent variable by which loss in body water due to

exercise induced dehydration was determined in the present study.

Plasma Osmolality and Urinary Measures of Hydration Status

       Considerations of storage. An early study of 28 neurological patients was

performed to determine the effects of freeze thawing procedures and storage temperature of

blood plasma samples on plasma osmolality values. Samples were collected from each

patient at different time points. Two samples were collected from each subject. One sample

was analyzed immediately and the other was stored in either a 4°C or ambient air temperature

environments. Frozen samples were thawed and analyzed alongside their ambient

counterparts at 0, 3, 6, and 24 hr time points. The authors found that at all time points P_{osm}

values decreased from the values of the samples that were analyzed immediately. However,

the mean values of the samples that were frozen did not decrease more than two mOsmol/kg

from the initial value (Bohnen, Terwel, Markerink, Ten Haaf, & Jolles, 1992). Because

blood samples in this study were frozen, it appears that there may be some variation induced

by the freeze-thaw method. Additional research examined the effects of freeze-thaw on 100

urine samples using a portable refractory index or freezing point depression osmometer.

Subjects either reported in a well hydrated state or following an overnight fast. All samples

were analyzed immediately and then 81 samples were then frozen at -80°C for two hours.

After freezing, samples thawed for 1 hr at 18°C. The authors found that freeze-thawing the

urine samples decreased mean mOsm values for freezing point depression by 50 mOsm

(Sparks & Close, 2013).

In both of the previous studies, freeze-thaw effects decreased osmolality values from

that of baseline. It should be noted that in the study by Sparks & Close (2013) the standard

variations at each time point were larger than the change exhibited by freeze thaw conditions.

This suggests that in the case of urine osmolality, freeze thawing effects only cause minimal

changes when compared to those created by individual variability. In the case of blood

plasma, the decreases exhibited by freeze thawing also had a minimal effect on P_{osm} values.

Urine and blood samples in the present study will be stored at -80°C and will be thawed and

analyzed on the same days in order to further reduce any error that may also occur due to

freeze thaw cycling.

Cross-validation of plasma osmolality, urine specific gravity, and urine osmolality. The combination of P_{osm}, U_{sg}, and U_{osm} in the following review material reflects the apparent inter-connectedness of the topics in the literature. In a study 12 subjects were dehydrated to a loss of 5% body weight by exercising in a $40^{o}C$ environment. Urine and blood samples were taken at 1%, 3%, and 5% reductions in body mass for the determination of U_{sg}, U_{osm}, and P_{osm}. The authors found that P_{osm} was significantly different from baseline at all three measurement points, while U_{sg} was only significant at the 3% and 5% measurement points and U_{osm} only at the 5% measurement points. Additionally, the authors reported values of false positive and false negative tests for U_{sg} and P_{osm} as percentages. Cut offs of ≤ 1.20 and > 290 mOsm·L for U_{sg} and P_{osm}, respectively, were chosen to determine a false negative test. Values of > 1.20 and < 290 mOsm·L were chosen to determine a false positive test. At these cut off values there was a 58% chance of finding a false positive test at the 3% dehydration measurement point. This percentage was not improved by lowering the value to 1.15 for specific gravity (Popowski et al., 2001). A later study by this same research group used the same methodology with a larger sample size of 51 subjects. Additionally, the authors determined false positive, false negative, true positive, and true negative tests for U_{sg} and U_{osm} by comparing them to P_{osm}. The same cutoffs as the previous study of 1.20 and 1.15 were used for U_{sg} while 700 mOsmol/kg and 800 mOsmol/kg were chosen for urine osmolality. The authors found that only 65% and 63% of subjects were correctly identified by U_{sg} and U_{som} respectively (Oppliger et al., 2005).

A more recent study by Cheuvront et al. (2010) consisted of two phases and was designed to determine biological variation and accuracy of U_{sg}, U_{osm}, and P_{osm}. Phase 1 consisted of subjects following a standardized fluid ingestion protocol with limited activity

and lasted three days. Testing occurred on each day during which urine samples, blood

samples, and body weights were collected. Phase 2 consisted of the same protocol as the

protocol for the morning of day one. Individuals then returned in the evening to complete an

exercise session to induce dehydration spanning a range of a 2% to 7% body weight

reduction. Following dehydration, blood samples, urine samples, and weights were collected

on the next morning. The authors determined that P_{osm} was the only useful marker for

determining hydration status from one measurement, while P_{osm}, U_{sg}, and U_{osm} were found to

be useful in settings where multiple measurement were taken. Additionally, the authors

reported the diagnostic cut off values to identify the euhydrated state for P_{osm}, U_{sg}, and $U_{osm,}$

as 297 mOsmol, 1.25, and 831 mOsmol, respectively. The sensitivity of these cut offs was

determined to be 90%, 89%, and 91%. The specificity was determined as 100%, 91%, and

91%, respectively. These values represent the lowest values that also fall outside the ranges

the authors determined as normal biological fluctuations as a result of phase one of the study.

The authors also determined that when greater than two hydration markers were measured it

is more likely that dehydration will be accurately detected. When changes in P_{osm}, U_{sg}, and

BM are $\geq$ 9 mOsmol, 0.010, and 2.5 kg a 95% probability of detecting dehydration was

observed. When these values were increased to 13 mOsmol, 0.014, 3.5 kg the probability

increased to 99 % (Cheuvront et al., 2010).

Another study conducted by Hamouti, Del Coso, Andrea, Mora- Rodriguez (2010)

found that athletes with large amounts of muscle mass had a 45 % greater chance of being

incorrectly identified as being hypo-hydrated than endurance athletes. The authors studied

nine rugby players and nine distance runners whose muscle mass differed by 10 kg. Urine

samples were collected each morning for six days and a fasted blood sample was collected on

the sixth morning. The authors found a positive correlation value of $r = 0.47$ for urine

protein metabolites and muscle mass. The findings of this study suggest that athletes with

large amounts of muscle mass such as wrestlers may be incorrectly classified as hypo-

hydrated by U_{sg}. It should be noted that the cutoff value for U_{sg} used to determine hypo-

hydration in this study was 1.20 (Hamouti et al., 2010). Further work by Hamouti, Del Coso,

& Mora-Rodriguez (2013) examined 18 well trained athletes. The subjects reported in the

morning to have blood and urine samples collected. Subjects then returned in the evening to

undergo exercise induced dehydration. Blood and urine samples were collected prior to the

start of the exercise protocol and at 1%, 2%, and 3% of body mass lost. The authors

determined that U_{sg} was as sensitive as P_{osm} at detecting low levels of dehydration.

The Findings of the preceding studies suggest that U_{sg} and U_{osm} have limited accuracy

in detecting euyhydration and dehydration in single measurement settings as well as with

serial measurements during exercise induced dehydration when the cut off value of 1.20 is

used, as is the case in the current study. Additionally, according to the work by Hamouti et

al. (2010) some of the inaccuracies associated with U_{sg}'s ability to assess hydration status

may be a result of large amounts of muscle mass as is the case for one population in this

present study. Additionally, work by Cheuvront et al. (2010) demonstrated that there is a

large amount of individual biological variability in U_{sg} and U_{osm} for the euyhydrated state

which further complicates the interpretation of U_{sg} and U_{osm} values. However, it appears that

when combined with P_{osm} and BW, as is the case in the current study, changes U_{sg} and U_{osm}

can provide an accurate interpretation of hydration status, as is true when changes from

baseline values suggested by Cheuvront et al. (2010) are observed. These findings support

the decision to include U_{sg} and U_{osm} in the study as additional hydration markers alongside

body weight and P_{osm}, as well as provide evidence that the current field based hydration

methods are far from being the most accurate and precise methods available.

Ultrasound and Hydration

   In vitro animal tissue water content. A study by Sarvazyan et al. (2005) examined

the speed of sound through ex vivo animal muscle tissue. Samples were linearly dehydrated

by a manual dehydration device. An ultrasound wave was passed through the tissue at a

fixed distance and then velocity was then calculated via the change in time over the distance

covered. The authors found a linear relationship between decreases in tissue water content

and increases in UV. A decrease of 1% in tissue water content resulted in an UV increase of

3 m/s. Fat and protein content remained constant in all samples throughout the dehydration

process. Additionally, a temperature dependence was found to exist in the animal tissue with

higher tissue temperatures resulting in an increase in ultrasound velocity. A 1° change in

temperature corresponding to a one meter per second increase in ultrasound velocity. The

measurement error of the ultrasound apparatus in this study was determined as 2 m/s

(Sarvazyan et al., 2005). Another later study of ultrasound velocity in ex vivo bovine

corneas also demonstrated the dependence of speed of sound on tissue water content.

   Each cornea was placed in four different mediums of decreasing tonicity and then

distilled water. The authors found that corneal thickness increased progressively due to the

influx of fluid into the cornea from decreasing medium tonicity. UV decreased with

increasing corneal thickness (Silverman et al., 2009). The two previous studies suggest that

in ex vivo animal tissues changes in water content where detectable with ultrasound. The

study by (Sarvazyan et al., 2005) suggests that the relationship between muscle tissue water

content, and the speed of sound through that tissue, is both inversely and linearly related. In

the present study, the ability of ultrasound to detect changes in total body water content is

dependent on water losses from the ECF generating similar increases in UV in a similar

manner (Sarvazyan et al., 2005).

In vivo canine study. Further work published in the same year examined 10 canines

of different breeds and sex. An ultrasound device was placed on the animal's skin to assess

in vivo hydration status before and after intravenously receiving and isotonic solution at 30

ml/kg/hr for 30 min. This was done by examining images obtained after the day of testing.

Cutaneous skin thickness and ultrasound images were taken at the frontal, sacral, flank, and

metatarsal regions, respectively. After receiving the solution, mean skin thickness increased

while mean tissue water content, as detected by ultrasound, decreased in all regions tested

(Diana et al., 2008).

In vivo human study. A study of 16 astronauts examined the effects of hyper

gravity and hypo-hydration induced shifts of interstitial fluid in the skin using ultrasound.

The astronauts were exposed to 2Gz in a human centrifuge following either a 12 hour nil-by-

mouth period or a 30 min ingestion of 250 ml dose of water prior to entering the centrifuge.

Ultrasound measurements were taken at the forehead and tibia prior to and immediately after

the centrifuge protocol. UV was determined as the time it took the sound wave to contact the

bone and echo back to the transducer. All subjects were kept in the same positions for each

measurement. Following hypo-hydration UV increased in the tibia site but not at the

forehead. After hyper gravity exposure UV did not change in the tibia site but decreased at

the forehead for both conditions (Eichler, Frank, Nehring, Welsch, & Klotz, 2004).

Although different types of ultrasound were used, this study along with the work of Diana et

al., (2008) demonstrates that UV was able to detect changes in tissue water content in vivo in

both humans and canines. Additionally, the work of Eichler et al. (2004) demonstrated that ultrasound could be used to detect changes in tissue water content due to hypo-hydration.

Pulse burst transmission. Further in vivo human work studied 127 individuals with and without lower leg edema. The subject population consisted of 60 males and 67 females. Of these individuals, 51 were healthy individuals, 16 with chronic renal failure, and 61 with cardiovascular disorders. Additionally, 15 subjects presented with edema and 112 presented without edema. Age ranged from 15 to 70 years. UV was determined using a prototype caliper that transmitted a burst of ultrasound through the soleus muscle and caught it on the other side. Acoustic gel was used as the conduction medium between the transducers on the tips of each caliper head. The caliper was placed on the soleus muscle at two-fifths the distance between the tibia-femur joint and the middle of the ankle. Three to four measurements were made at each time point and the caliper was taken off and placed back onto the measurement site between each individual measurement. The authors found that UV values ranged from 1530-1615 m/s amongst subjects. There was no significant difference between age groups. Males exhibited an average velocity of 1583 m/s while females had a lower mean value of 1561 m/s. Edema patients exhibited a mean ultrasound value of 1554 ± 20 m/s and non-edema patients a mean of 1574 ± 28 m/s. The ultrasound velocities in renal patient's pre and post dialysis were 1587.3 m/s and 1592.4 m/s, respectively. No standard deviation was given for the dialysis UV measurements. The dialysis patients lost an average of 5% of their body mass during dialysis. UV was also correlated with BMI and the authors stated that those with higher BMI values had lower UV values, but no Pearson correlation value was given (Topchyan, Tatarinov, Sarvazyan, & Sarvazyan, 2006).

The findings of Topchyan et al. (2006) suggest that UV, as assessed by an earlier version of the equipment used in the present study, maybe capable of detecting changes in TBW in healthy individuals as well as those with chronic renal failure and cardiovascular disorders. It should be noted that the change in UV seen in the renal failure group after dialysis was only 5 m/s after a loss of 5% body mass. This is in contrast with the earlier findings by their colleagues Sarvazyan et al. (2005) who found a 3 m/s increase in UV for a 1% decrease in muscle water content which is equivalent to roughly a 0.63% change in body mass for an individual who weighs 150 lb and has 10% body fat. Theoretically, based on the in vitro study in 2005, a 3% change in body mass should elicit a 15 m/s increase in UV using the weight and body fat percentage listed above. The authors did not give a direct explanation for the low change in UV. The authors did suggest that based upon the indirect relationship of BMI and body fat that dispersions of results could be a result of difference in lower limb adiposity. This seems a reasonable conclusion, given that the reported value for UV in adipose tissue (1400-1450 m/s) is lower than that of muscle tissue due to lesser water content. However, without reporting a Pearson correlation value and no inclusion of a measure of body density to obtain a body fat percentage, it is presumptuous to limit the lack of increase in UV solely to difference in adiposity. Still, in the present study three site skinfold measurements will be performed in order to obtain information on the possible relationship between UV and body density as this has yet to be examined.

The most recent study of UV and human hydration status was completed in collaboration with Artann Laboratories and the lab group in the present study. In this study, the version of the hydration monitor used by Topchyan et al. (2006) was used for ultrasound measurements. Fifty-six National Collegiate Athletics Association Division 1 (NCAA D1)

wrestlers were studied. Urine and blood samples along with UV and body fat were measured at pre, post, 1 hr, and 2 hr points. Body weight was assessed at the pre, post, and 2 hr post exercise points. Dehydration was induced via exercise supervised by the coaching staff between the pre and post time points. Athletes were allowed to shower and redress between practice and the post-exercise measurement. All weights were obtained in underwear only. Between the post and 1 hour measurement points athletes ingested an amount of carbohydrate beverage (Gatorade) equivalent to the amount of body weight lost. All fluids were ingested within 40 min following the post exercise time point. The authors found that changes in hydration status were positively correlated with changes in P_{osm} between the 1 hour and 2 hour time points ($r = 0.27$, $p < 0.05$). Additionally, UV was significantly different from baseline measurements at the ($p < 0.001$) level during dehydration. Ultrasound Velocity in the dehydrated state was also significantly different at the ($p < 0.001$) level then in the rehydrated state. Urine specific gravity and U_{osm} exhibited similar main effect findings of UV. Urine osmolality increased from baseline to post exercise then returned to below baseline at the two hour time point. The same trend was also observed with U_{sg}. Plasma osmolality values increased incrementally through the 1 hour post exercise time point then dropped to below baseline between the one and 2 hour time points (Utter et al., 2010).

This study was the precursor and basis for the present study. The work of Utter et al. (2010) provided evidence that UV is a potentially useful tool for assessing changes in total body water due to exercise induced dehydration and rehydration with a common carbohydrate beverage. However, it was noted that there was a large amount of individual variability in response to the UV measurements amongst subjects. This seems likely, given

that Topchyan et al. (2006) had a range of values between 1530-1615 m/s and standard

deviations of 20 and 28 in edema and non-edema patients alike with an earlier version of the

hydration monitoring device. Additionally, in the first in vitro study by Sarvazyan et al.

(2005), the authors concluded that tissue temperature, tissue composition (intramuscular fat

content), internal changes to muscle structure following exercise (muscle damage), individual

anatomical structure of the musculature, changes in intramuscular blood flow, and adequate

probe contact were all sources of potential error that have not been addressed in any of the

later in vivo studies. While the present study does not address concerns of muscle

temperature, blood flow, or structural changes, a measurement protocol was developed in

order to reduce possible error associated with probe placement and transducer contact.

Furthermore, in each of the previous studies only the soleus muscle was examined.

Conversely, in the present study, both the soleus and biceps brachii muscles will be used to

assess TBW with UV. It is hoped that by studying two separate sites along with using an

improved prototype of the hydration monitor that there will be a reduction in the individual

variability in response to UV measurements during dehydration and rehydration.

Summary

Ultrasound velocities ability to assess changes in TBW during exercise induced

dehydration and rehydration are subject to a large amount of daily and weekly variability in

TBW levels. Additionally, differences in TBW content and lean tissue content between races

and sexes may also contribute to individual differences observed in UV measurements.

Considerations to postural shifts in the compartmentalization of TBW may also create a

chance for Type I and Type II errors in UV measurements at all-time points as obtained in

the present study. Furthermore, the relative stability and potential increase of intramuscular

water immediately following exercise may increase the chances of finding a Type I error

when assessing dehydration induced by exercise. Differences in body mass, sweat rates, and

pre exercise hydration status between sexes suggest that potential differences in response to

UV between sexes exist. Although, it appears that menstrual cycle phase should not affect

UV measurements. Additionally, it seems that the amount of body weight selected as the cut

off point for exercise induced dehydration in the present study is associated with decreases in

both aerobic and anaerobic exercise performance. It also seems likely that changes in body

weight are well correlated with water losses incurred during exercise induced dehydration

and should serve well as the primary independent variable in the present study. Moreover,

while error incurred from freeze-thaw is being minimized in the present protocol, it appears

that urine and plasma osmolality values will be subject to some error due to freeze-thaw

effects. Current urinary and blood based measures of TBW are fairly accurate and precise

and will serve to confirm the ability of the hydration monitor being tested to detect changes

in TBW. However, these measures still have the limitation of needing a urine or blood

sample in order to provide information on TBW content. Previous research regarding UV

and TBW provided evidence of an inversely linear relationship between a tissues water

content and UV. This relationship was tested in both in vitro and in vivo animal and human

studies. In both human studies completed to date, large individual variability in response to

UV was observed.

In light of previous research, it is unclear whether UV can accurately detect changes

in human hydration status due to dehydration and rehydration. It has also been noted that no

standardized, non-invasive, reproducible tool to accurately assess hydration status exists

(Armstrong, 2005). Thus, additional investigation into the ability of UV to detect changes in

human hydration status is clearly needed. The purpose of the present study was twofold. First, is to confirm UV as a valid form of hydration assessment for both male and female collegiate athletes in the field setting. The second purpose is to determine if individual subject variability can be reduced using the soleus and biceps muscles as measurement sites. I hypothesize that UV will be able to detect states of euhydration and dehydration in both subject populations with decreased individual variability due to the two site measurement approach. Validation of UV to detect acute changes in hydration status due to exercise will have direct applications to coaches and athletic trainers in the field because it will allow them to rapidly determine an athlete's hydration status throughout practice and competition.

Method

Experimental Approach to the Problem

The purpose of this study is to determine if UV can detect changes in TBW amongst male and female Division 1 collegiate athletes using a two-site measurement approach after undergoing an acute dehydration and two hour rehydration protocol. TBW was assessed using body weight, P_{osm}, U_{sg}, and U_{osm}. The experiment will follow a repeated measures design with all subjects serving as their controls. Subjects came to the Appalachian State University (ASU) Human Performance Lab for orientation on a day prior to the subsequent dehydration/rehydration trial.

Subjects

Fifty-six NCAA D1, male wrestlers and 26 Division #1 Collegiate, female soccer athletes served as subjects for this study. All participants were screened using the American College of Sports Medicine (ACSM) Screening Questionnaire for risk assessment and were classified as "low risk." Exclusion criteria for male subjects were an inability to perform exercise or give an adequate amount of tissue samples. Exclusion criteria for female subjects included the same parameters as male subjects along with pregnancy. Subject characteristics are presented in the Table 1.

Experimental Design

Subjects were instructed to report to the laboratory in a euhydrated state at 8:00 a.m. Subjects were screened for proper hydration status by obtaining a baseline urine specimen for measurement of U_{sg}. U_{sg} was assessed using a handheld refractometer (NVG Precision Cells

Inc., Farming-Dale, NY, USA). Upon arrival to the laboratory, body weight, height, body composition (skinfold thickness), urine specific gravity, UV, limb circumference (Bicep and Soleus), and urine osmolality were obtained. A blood sample (plasma osmolality) and ultrasound velocity (UV) was also obtained. Body composition was assessed with a three-site skinfold measurement in males (Triceps, subscapular, and abdominal) and females (Triceps, suprailiac, and abdominal) using a Lange skinfold caliper (Cambridge Scientific Industries, Inc., Cambridge MD, USA). In males and females body density was determined using the prediction equation D_b = [1.0982 – (sum skinfolds) x 0.000815] + [(sum skinfolds) ^{2} x 0.00000084] as validated by (Lohman, 1981). Plasma Osmolality and U_{osm} were assessed using a freezing point depression osmometer (Model 3250, Advanced Instruments Inc., Norwood, MA, USA). All body mass measurements were made on the same digital scale.

Upon arriving to the lab subjects followed a series of stations in the following order: urine samples, body weights, skinfolds, and each subject had an option to have ultrasound or blood drawn in either order after the other measures and samples were obtained. This order was followed at baseline, post dehydration, one hour rehydration, and two hour rehydration time points. Four blood samples were obtained by licensed phlebotomists from the median cubital veins in both the right and left forearms of participants for each trial.

After completing baseline measurements participants were assigned a weight loss goal of 3% of their body weight. The participants then underwent a supervised exercise regimen to induce the prescribed weight loss. Scales were available at all times for the subjects so that after their vigorous exercise session they could check if they had dehydrated to the extent of 3% of their body weight and thus attained their weight-loss goal. Upon exercise completion, subjects were permitted to shower and then return to the performance lab. Once

in the lab, subjects followed the same measurement order as baseline. Once post-exercise

measures were completed subjects were given an electrolyte-carbohydrate beverage (6% or

60 g·L^{-1}; Gatorade®, Barrington, IL, USA). The electrolyte-Carbohydrate beverage

contained 20 mmol·L^{-1} sodium and 3.2 mmol·L^{-1} of potassium. The amount of beverage

assigned was equal to the body mass lost by each subject during exercise. All fluids were

measured out and provided to subjects by research assistants. Each subject was required to

consume fluids equivalent to half of the body mass loss within the first 20 min of the

rehydration protocol and then consume the second half within 20 to 40 min. After

completing the rehydration protocol, the third UV, U_{sg}, U_{osm}, and P_{osm} measurements were

completed at 60 and 120 min after fluid consumption commenced.

Ultrasound Measurements

Ultrasound velocity was measured using a hand-held and self-contained hydration

monitor (Artann laboratories, Trenton, NJ, USA). The monitor was comprised of a handle

from which the device was controlled and four smart probes. Each probe stored subject

information and were sizes of 60, 80, 100, and 120 mm. The probe tips were lubricated with

water by dipping them into a water bath and then placed on each side of the subject's soleus

or bicep muscle. The measurement site for the biceps is halfway between the acromion

process of the scapula and the inferior part of the olecranon process. The measurement site

for the soleus is located in the upper part of the calf, at 2/5 of its length from the tibia-femur

joint to the middle of the ankle and in the middle of the posterior part of the calf cross-

section. The measurement sites were determined using a standard body measurement tape

and then marked with a surgical skin marker (XL prep resistance ink, Viscot Medical, LLC,

East Hanover, New Jersey, 07936). Marks were made to insure the probe was placed on the

limb in as close to the same orientation as possible at every time point. The marks were made after a test measurement was taken to ensure that there was proper conduction between the probe tips and the skin and that the ultrasound path was free of the tibia and humerus. Triplicate measurements were made for each time point without removing the probe between measurements and took approximately five seconds to complete. Measurements were displayed as velocity in m/s $^{-1}$. Ultrasound was emitted as a 1-Mhz 1.5-µs tone burst that was modulated by a gauss envelope. The signals were digitized at a sampling rate of 0.05 microseconds. Each measurement was taken while the subjects were standing.

Statistical Analysis

Dependent variables were analyzed using a one-way repeated-measures analysis of variance (ANOVA). All ANOVA's were assessed for sphericity using Mauchley's W and adjusted when necessary using the Greenhouse-Geisser and Huynh-Feldt methods. Significant main effects were assessed with paired t-tests using a bonferroni adjustment, with statistical significance set at $p \leq 0.016$. Independent t-tests were used to examine differences between genders for baseline subject characteristics. All values are presented as mean $\pm$ standard error of measurement.

Table1

Differences between Male and Female Athlete Descriptive Statistics

	Males n = 56	Females n = 26
Height (m)	1.75 (±0.01) *	1.64 (±0.01) *
Age (yrs)	19.3 (±0.2)	19.8 (±0.3)
Years of experience (yrs)	9.6 (±0.5) *	15.1 (±0.3) *
Weight (kg)	78.1 (±1.8) *	62.2 (±1.2) *
Percent fat (%)	13.9 (±0.6) *	20.3 (±0.7) *

Note. * Denotes statistically significant difference at $p < 0.001$

Table 2

Male Ultrasound Velocity and Measures of Hydration Status throughout the Trial

Variable name	Pre-dehydration	Post-Dehydration	1-hour rehydration	2- hour rehydration
Soleus ultrasound velocity (m·s^{-1})	1554.3 ($\pm$1.1)	1557.6 ($\pm$1.1)*	1555.4 ($\pm$0.9)	1556.4 ($\pm$0.9)
Biceps ultrasound velocity (m·s^{-1})	1558.5 ($\pm$2.2)	1560.2 ($\pm$2.3)	1559.4 ($\pm$2.3)	1560.4 ($\pm$2.4)
Urine osmolarity (mOsm·L^{-1})	966.8 ($\pm$25.9)	882.3 ($\pm$30.5)	846.7 ($\pm$26.8)*	675.0 ($\pm$48.11)*
Urine specific gravity (g·mL^{-1})	1.027 ($\pm$.001)	1.028 ($\pm$.001)	1.027 ($\pm$.001)	1.021 ($\pm$.001)*
Plasma osmolarity (mOsm·L^{-1})	280.4 ($\pm$2.2)	288.1 ($\pm$2.4)*	282.0 ($\pm$2.3)	278.0 ($\pm$2.4)*

Note. *Denotes Statistically Significant difference from pre-dehydration, $p < 0.016$.

Table 3

Female Ultrasound Velocity and Measures of Hydration Status throughout the Trial

Variable name	Pre-hydration	Post-Dehydration	1-hour rehydration	2- hour rehydration
Soleus ultrsound velocity (m·s^{-1})	1546.1 (±1.3)	1548.4 (±1.4)	1547.9 (±1.4)	1547.1 (±1.3)
Biceps ultrasound velocity (m·s^{-1})	1518.2 (±3.2)	1519.1 (±3.1)	1518.7 (±2.3)	1522.2 (±2.4)
Urine osmolarity (mOsm·L^{-1})	737.4 (±50.5)	617.6 (±60.1)	700 (±52.3)	407.2 (±67.3)*
Urine specific gravity (g·mL^{-1})	1.021 (±.001)	1.021 (±.002)	1.022 (±.002)	1.011 (±.001)*
Plasma osmolarity (mOsm·L^{-1})	281.2 (±2.2)	282.1 (±2.4)	280.9 (±2.5)	282.4 (±2.9)

Note. *Denotes Statistically Significant difference from pre-dehydration, $p < 0.016$.

Pre-dehydration   Post-dehydration   1-hr rehydration   2-hr rehydration
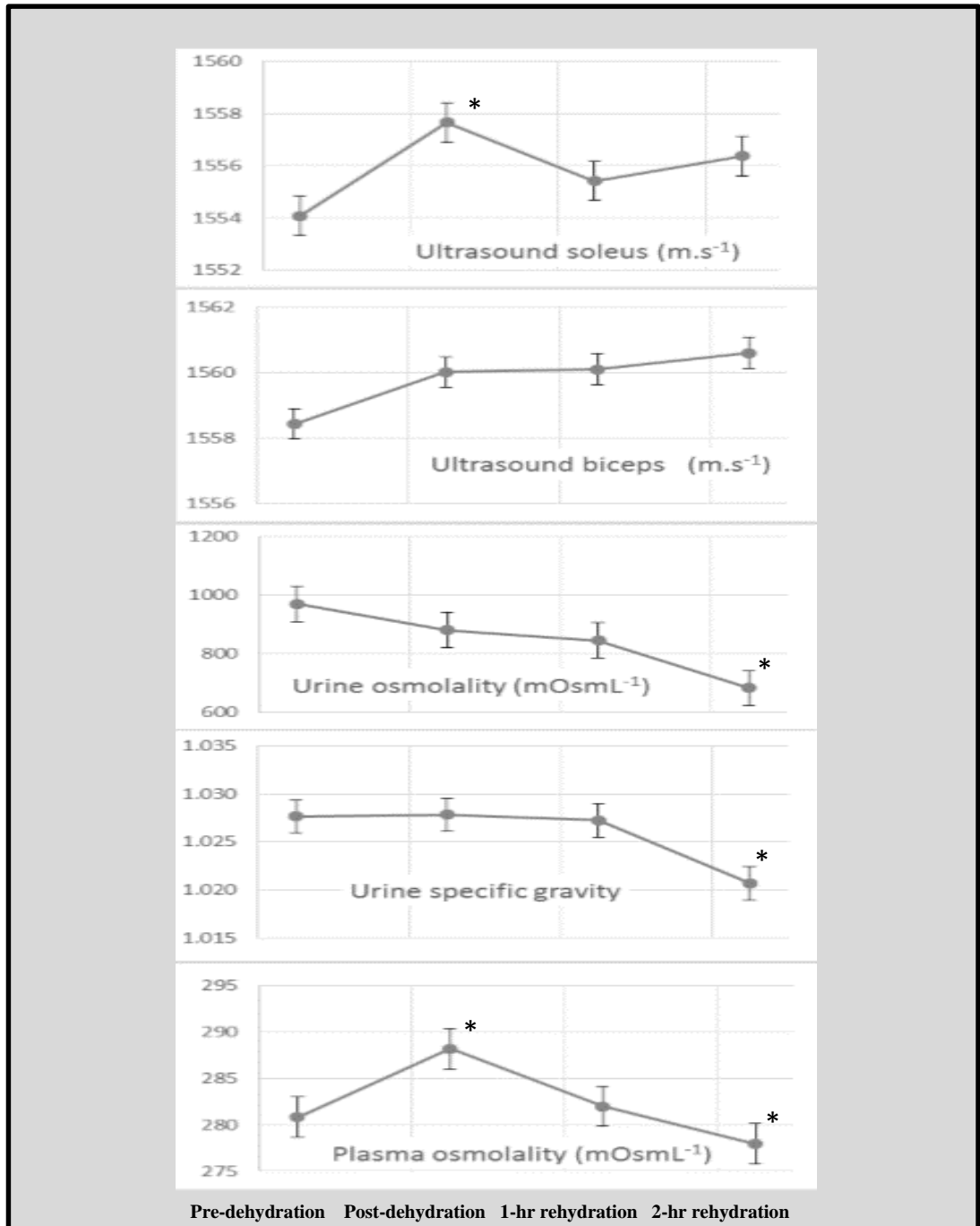
Figure 1. Male ultrasound velocity, urine osmolality, urine specific gravity, and plasma osmolality measurements at pre-hydration, post-dehydration, and 1 and 2 hr post-rehydration in 56 Male athletes.
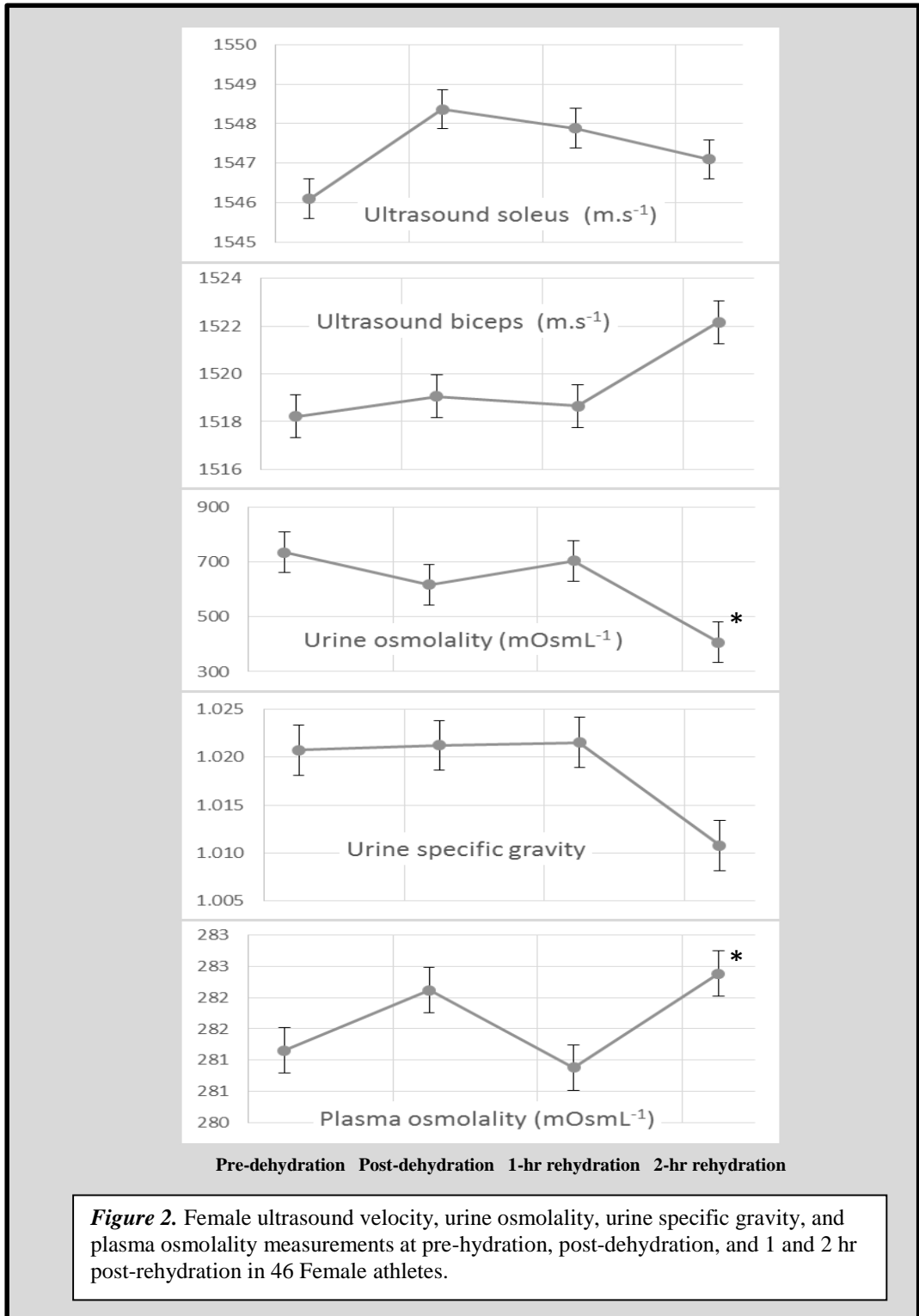
Figure 2. Female ultrasound velocity, urine osmolality, urine specific gravity, and plasma osmolality measurements at pre-hydration, post-dehydration, and 1 and 2 hr post-rehydration in 46 Female athletes.
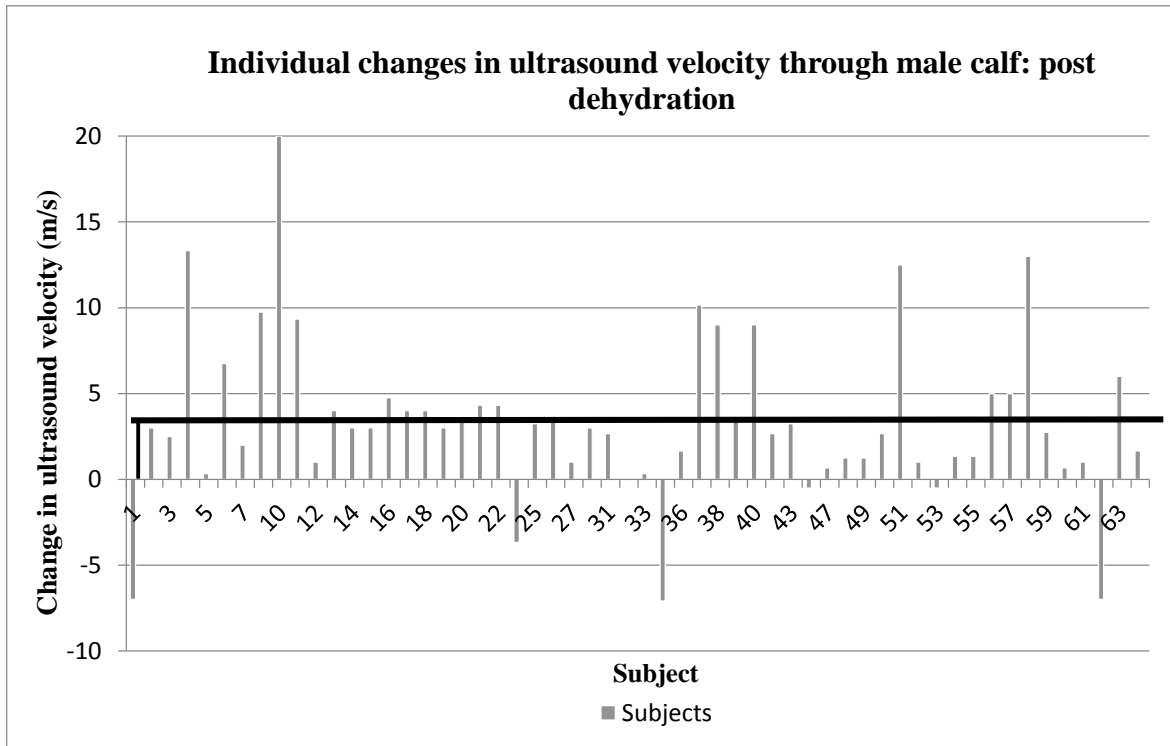
Figure 3. Shows the individual differences in UV between pre-dehydration and post-dehydration measures at the soleus for each male subject.
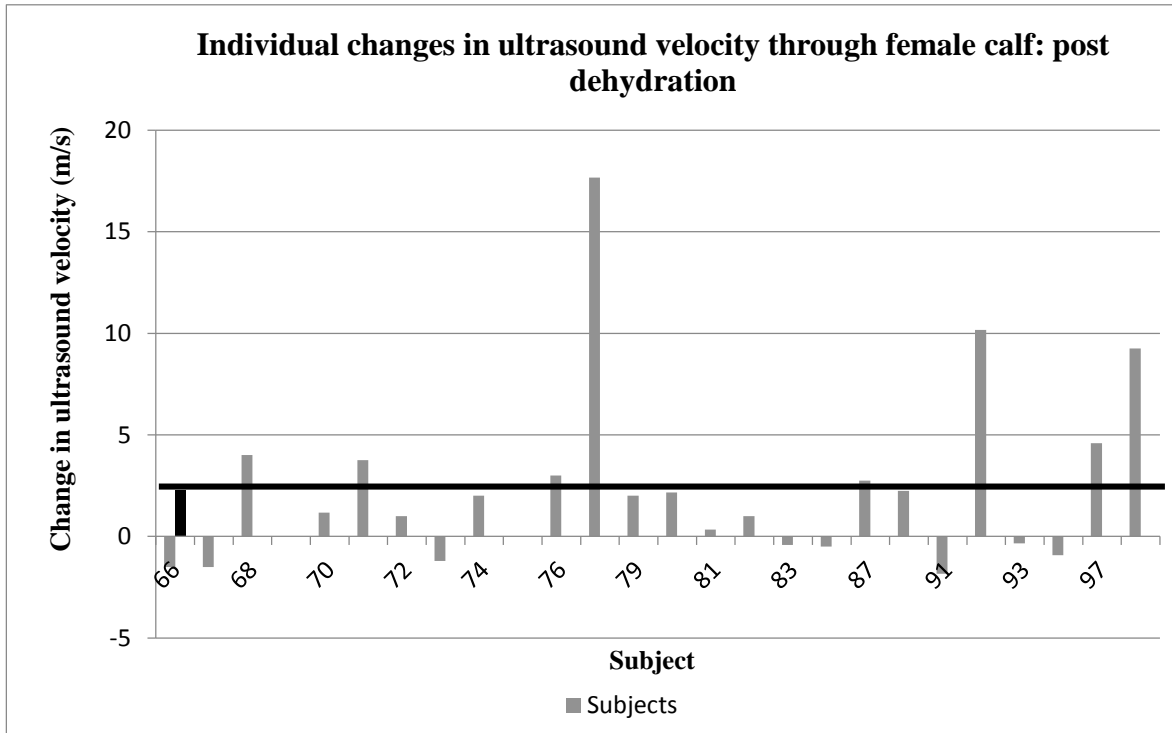
Figure 4. Shows the individual differences in UV between pre-dehydration and post-dehydration measures at the soleus for each female subject.

Results

Male subjects dehydrated to achieve an average BM loss of $2.9 \pm 0.09\%$. Body mass changes (kg) for males throughout the study were as follows: Pre-dehydration (baseline) = 78.1 ± 1.8, Post-dehydration = 75.8 ± 1.8, and 2 hr rehydration = 77.8 ± 1.8. Female subjects dehydrated to achieve an average BM loss of $1.9 \pm 0.03\%$. Body mass changes (kg) for females throughout the study were as follows: Pre-dehydration (baseline) = 62.2 ± 1.2, Post-dehydration = 61.0 ± 1.2, and 2 hr rehydration = 62.0 ± 1.3. For rehydration, subjects were provided with beverage amounts equal to 100% of their BM loss. Male subjects were able to regain $2.6 \pm 0.09\%$ of the BM loss, while females were able to regain $1.6 \pm 0.06\%$ of the BM loss during the 2 hr rehydration period.

Ultrasound velocity significantly increased ($p < 0.016$) from pre-dehydration (1554.3 ± 1.1 $m \cdot s^{-1}$) to post-dehydration (1557.6 ± 1.1 $m \cdot s^{-1}$) for the male biceps measurement see Table 2 and Figure 1. No significant main effects for time were found at any other time point for the biceps or soleus ultrasound measurements for both sexes. Additionally, no significant correlations were found between P_{osm}, U_{sg}, U_{osm} and change in ultrasound velocity for male athletes. The individual responses for the change in ultrasound velocity from pre-dehydration to post-dehydration are depicted Figures 3 and 4.

Significant main effects ($p < 0.001$) were found for $P_{osm,}$ U_{osm}, and U_{sg} for males and U_{osm} and U_{sg} for females. P_{osm} for the males significantly increased ($p < 0.0025$) from baseline to post-dehydration (280.4 ± 2.2 to 288.1 ± 2.4 $mOsm \cdot L^{-1}$), decreased at the 1 hr rehydration period (282.0 ± 2.3 $mOsm \cdot L^{-1}$) and returned to below baseline at the 2 hr

rehydration period (278.0 ± 2.1 mOsm·L^{-1}) see Table 2 and Figure 3. A non-significant change was found for P_{osm} in females from baseline to post-dehydration (281.2 ± 2.2 to 282.1 ± 2.4 mOsm·L^{-1}) and a decrease at the 1hr rehydration period (280.9 ± 2.5 mOsm·L^{-1}) see Table 3 and Figure 2. For males, a non-significant decreased for U_{osm} from baseline to post-dehydration was found (966.8 ± 25.95 mOsm·L^{-1}) with a statistically significant differences at all three time points thereafter (882.3 ± 30.5 to 846.7 ± 26.8 to 675.0 ± 48.1 5 mOsm·L^{-1}). There was a significant decrease in U_{osm} from baseline to the 2 hr rehydration period (407.2 ± 67.3 5 mOsm·L^{-1}) for females. The U_{sg} for the males increased from baseline to post-dehydration (1.027 ± 0.001 to 1.028 ± 0.001 g·mL^{-1}), and significantly decreased ($p < 0.0025$) below baseline at the 2 hr rehydration period (1.021 ± 0.001 g·mL^{-1}). For females U_{sg} remained constant from baseline to post-dehydration (1.021 ± 0.001 to 1.021 ± 0.002 g·mL^{-1}), and significantly decreased below baseline at the 2 hr rehydration period (1.011 ± 0.002 g·mL^{-1}). No significant correlations were found between UV, U_{sg}, U_{osm} P_{osm}, during either the hydrated or dehydrated state for males or females.

Discussion

The results of the present study indicate that UV is able to track changes in TBW during periods of acute dehydration and rehydration in the soleus muscle of male collegiate aged wrestlers and might be able to track similar changes in the soleus muscle of female soccer players. To my knowledge this is the first study to investigate ultrasound velocity as a potential non-invasive marker of hydration status in female athletes undergoing acute hypertonic hypovolemic dehydration and rehydration. Furthermore, this investigation sought to expand upon the findings of an earlier investigation by Utter et al. (2010) that examined male wrestlers only. Results from the present investigation demonstrated that significant

acute hypertonic hypovolemic dehydration did occur in both athlete groups. However, it

should be noted that fluid and food intake prior to the baseline weight was not controlled and

that no specific fluid intake instructions were provided other than a word of mouth

instruction to come in well hydrated on the day of testing. Therefore, the lack of specific

hydration instruction may present a limitation to the accuracy of the established baseline

weight. The losses in percent body mass observed, if incurred prior to exercise, are

comparable to those that should elicit reductions in aerobic and anaerobic exercise

performance as suggested by guidelines from the NATA and the ACSM (Casa et al., &,

2000; Sawka et al., 2007). Therefore, any marker or test for whole body hydration status

must be able to identify losses of TBW at a minimal level of 2% of body mass. As identified

by Sarvazyan et al. (2005), tissue temperature, tissue composition (intramuscular fat content),

internal changes to muscle structure following exercise (muscle damage), individual

anatomical structure of musculature, changes in intramuscular blood flow, and adequate

probe contact are sources of potential error when using UV as a measure of whole body

hydration status. These areas of potential error will provide the basis of discussion along

with exploration of the possible contribution of sex based differences. Additionally, changes

in blood markers and urinary markers and their potential contributions to the results of the

present study will be addressed.

Differences between male and female athlete's ability to shed and then reabsorb fluid

during periods of acute dehydration appear evident. Males having lost a greater percentage

of body mass ($2.9 \pm 0.09\%$) when compared to females ($1.9 \pm 0.03\%$) undergoing similar

periods of acute exercise induced dehydration, and having regained a 1% greater amount of

the mass lost during exercise compared to females. Additionally, the body mass losses

incurred in this study for male athletes ($3.6 \pm .14\%$) were lower than that of the athletes in the previous study by Utter et al. (2010). Although not measured in this study, the difference between sweat rates in males and females may account for some of the differences in body mass losses between sexes. As suggested by Ichinose-Kuwahara et al. (2010), females may need to exercise at a higher intensity than their male counterparts in order to elicit a similar reduction in body mass by sweating. Also, the male athletes have had greater experience with rapid weight reduction when compared to the female athletes and therefore may have established positive adaptations to elicit greater sweat rates. Additionally, it appears that there are differences between athletes of different sexes regarding the regulation of water prior to and during exercise as identified by baseline and subsequent changes in P_{osm}, U_{osm}, and U_{sg}.

Significant changes in ultrasound velocity (UV change = 3.4 ± 4.8 m/s) during periods of acute dehydration (BM change = $-2.9 \pm 0.09\%$) were observed at the soleus measurement site in male collegiate wrestlers. The female soccer players achieved a significant net loss of (BM change = $-1.9 \pm 0.03\%$) and exhibited a non-significant change in ultrasound velocity during a similar period of acute dehydration (UV = 2.3 ± 4.3 m·s^{-1}) at the soleus measurement site. Neither group exhibited significant changes in UV at the biceps measurement site. The mean ultrasound velocity for male athletes in the euhydrated state at the soleus measurement site was (1554.3 ± 1.1 m·s^{-1}). This is lower than the mean value for wrestlers in the previous study by Utter et al. (2010; 1583 ± 5.3 m·s^{-1}). The mean values for UV in all states for male athletes fell within in the known range for in vivo human muscle tissue as provided by Mol & Breddels (1982), Sarvazyan et al. (2005), and Topp & O'Brien (2000). However, the female biceps values are below the lower bound for the known range

of UV for in vivo muscle tissue and appear to be much closer to that of water at body

temperature. One possible explanation for this is that females generally have higher amounts

of total body fat and had a loss of only 1.9% of baseline BM as compared to the 2.9 % loss

by the males.

Indeed, the mean body fat percentage for females in this study was 6.4 % higher than

that of the males. Additionally, the nature of the activity undertaken by the female athletes

would suggest that it is likely that they have a greater proportion of muscle tissue in their

lower limbs as compared to their upper limbs. This rationale is consistent with reports that

identify females as having a larger proportion of muscle mass in their legs as compared to

their arms Nindl, Scoville, Sheehan, Leone, & Mello (2002). Nindl et al. (2002) further

reported that females have a larger proportion of fat mass in their entire body when compared

to males. Understanding, that the water content of a tissue is one of the main determinants of

UV through that tissue, it is likely that differences in regional muscle and adipose tissue

amounts are contributing factors to the differences seen between sexes and between

measurement sites in the present study. Furthermore, individual differences in regional

muscle and adipose tissue distribution may be a contributing factor to a large amount of

individual variability seen amongst all of the study participants for the pre-dehydration

measurement point. Body fat ranges for male subjects at 8.4% to 29.9% and females at

12.6% to 28.2%.

Another possible contributor to the consistent increase in the UV for the biceps

muscle over all four time points maybe a result of the exercise used during the dehydration

period. While full body exercise was used in the present study perhaps previous adaptations

from chronic exercise stimulus resulted in a non-uniform loss of BW from different muscle

groups in the body. Recently, Hackney, Cook, Fairchild, & Ploutz-Snyder (2012) found that during cycling exercise to the point of 3% dehydration muscle volume decreases were exhibited in the more active lower body musculature but not the less active upper body musculature. This suggests that water may not be uniformly lost from all tissues during exercise. With uniform 3% reduction of total body water resulting in a uniform 3% loss of intramuscular water from all tissues being a chief assumption for the use of UV, it may present an additional source of error in that this shift is only exhibited in the exercising tissue.

Further sources of variability across all four time points seen in UV may have to do with the internal structure of the muscle sites in question. In their paper, Sarvazyan et al. (2005) suggested that the contribution to error of measurement of muscle macro structure was equivalent to an increase in UV of 0.4 cm/s to 4 m/s as determined by measuring UV in ex vivo muscle tissue before and after homogenization. This implies that when the macro structure of the muscle fiber is compromised (such as exercised induced damage) decreases in UV can occur. The largest value is roughly equal to 1.5 % of the water lost from the tissue and represents full tissue homogenization. However, with mean changes of 3 m/s even a one meter per second discrepancy could add significantly to the overall change in speed of sound between pre and post exercise measurements.

Moreover, Mol & Breddels (1982) described the relationship between ultrasound signal frequency and attenuation as approximately linearly proportional. Meaning increasing frequency results in an increased attenuation of the signal in muscle tissue. Noting that as an ultrasound signal is passed through a tissue it will scatter resulting in a lower mean frequency upon arriving at the receiver. Therefore, it is possible that differences in individual muscle

structure could cause a greater or lesser attenuation between individuals and muscle states (pre exercise verses post exercise). This could then lead to a forward or backward shift in the zero time point wave detection methods utilized by the hydration monitor in this study. A forward shift in detection would decrease the time for signal conduction and increase the calculated velocity, while a backward shift would do the opposite. However because we were unable to determine whether the internal structure of the muscle tissue was significantly altered due to the exercise undertaken, or the differences in internal structure of muscle compartments between each person at rest, structural discrepancies between individuals and sexes present a limitation to the results of the present study.

Similarly, changes in regional blood flow and the amount of vasculature found in the muscles measured present another possible source of error. Changes in blood flow and vasculature would not only alter the internal structure of the muscle compartment but may also change the water content held in that compartment. Following exercise, it is possible in the present study that changes in regional blood flow and thus blood content inside of the muscle being measured may have contributed to a dampened change in the UV recorded. Because post-dehydration measurements were taken within an hour post exercise, blood may have not been fully redirected away from the previously working muscle, meaning increased intravascular pressure was present. An increased intravascular pressure could lead to increased water content in the muscle compartment due to high hydrostatic forces in the intravascular space. Therefore, if water is still being pushed into the intracellular space along with metabolic water being trapped in the intracellular space it is likely that net changes in muscle water content could be too small to be accurately detected by UV. This may partially explain why significant increases in UV at three of the four measurement sites were not

found. However, because we did not measure blood flow to the working muscle nor were we able to measure metabolic water production, it presents an additional limitation to the present study.

Likewise, I did not measure temperature which also may play a role in understanding the UV values recorded. In a paper by Kenny et al. (2003), it was determined that intramuscular temperature stayed elevated at an hour post exercise and that tissue closer to the surface was warmer than deep muscle tissue. Intermuscular temperatures of the vastus lateralis stayed elevated from resting conditions on the order of 0.92, 1.05, and 1.77°C with progressively decreasing depth from the femur. According to the work of Sarvazyan et al. (2005) a 1°C increase in intramuscular temperature was equivalent to a 3 m/s increase in UV which again corresponds to roughly a 1% decrease in tissue water content. Therefore, it is possible that changes in intramuscular temperature could have caused a false increase or decrease of UV at the level of $\pm 1.0 - 1.5$ m/s in some individuals at the post-dehydration, 1 hr rehydration and 2 hr rehydration time points. However, because we did not measure intra-muscular temperature it presents an additional limitation to the present study.

Moreover, probe placement and difficulties associated with the probes used in this study can offer further insight into the UV measured. A standardized measurement protocol was developed for the standing conditions and was similar to the protocol described by Utter et al. (2010). This was used to establish the site of probe placement and to determine the probe size that fit the site diameter. There were some instances when the subject's calf musculature was disproportionally located on the proximal third of the tibia and thus our measurement system placed the starting site on the lower section of the calf-soleus complex. At this starting location, there may have been a larger proportion of connective tissue present

which could have caused an inaccurate increase in UV at baseline. However, relative

changes should not have been effected for that subject, albeit that at all time points UV

would be higher than that of subjects on whom the probe was originally placed in the middle

of the soleus.

Furthermore, the hydration monitor used in the present study had four available probe

sizes. In several cases exceptions on probe placement had to be made in order to

accommodate exceptionally wide, narrow, or muscle widths that fell in-between probe sizes.

Often athletes with muscles in-between two probe sizes were measured with the lower probe

size or with modified positioning. This meant that although a standardized measurement

protocol was developed, there were instances when deviations were made by rotating,

lowering, or impressing the probe on the calf or bicep in order to obtain a reading. Again,

this adjusted location was marked during the baseline reading so relative changes should not

have been effected, but it may have led to an inaccurate baseline UV value.

In instances where the probe was compressed into the muscle, which occurred with

several female and male athletes, it was difficult to apply the same amount of pressure and

therefore compression of the tissue may have been heterogonous throughout the study.

These modifications were made to ensure adequate contact between the ultrasound

transducers in either probe tip. However, as noted in the paper by Topp & O'Brien (2000),

UV for an ultrasound burst through muscle in an orientation of 45º as opposed to 90º

decreased average velocity by 6 m/s in rat soleus tissue. This was also discussed by Mol &

Breddels (1982) who suggested that the difference in UV with changing muscle fiber

orientation was due to an increased attenuation factor. During probe repositioning every

effort was made to keep the probe tips level and in perpendicular orientation to the majority

of muscle fibers. However, in some cases the compression of the muscle tissue or use of a

smaller probe on a lesser portion of a muscle may have led to small changes in fiber

orientation, which then led to potentially lower UV values then if optimal probe positioning

was achieved.

Plasma osmolality for the males significantly increased from baseline to post

dehydration (280.4 ± 2.2 to 288.1 ± 2.4 mOsm·L^{-1}), decreased at the 1 hr rehydration period

(282.0 ± 2.3 mOsm·L^{-1}) and returned to below baseline at the 2 hr rehydration period (278.0

± 2.1 mOsm·L^{-1}) see Table 2 and Figure 3. This represents the expected changes in P_{osm}

during a period of acute dehydration and rehydration and is similar to what was demonstrated

by Utter et al. (2010). However, the P_{osm} value at the point of dehydration still falls below

the typically accepted diagnostic dehydration cut-off point of 290 mOsm·L^{-1} which is in

contrast to the previous study by Utter et al. (2010). Additionally, females exhibited a

dampened P_{osm} response to dehydrating exercise from baseline to post-dehydration ($281.2 \pm$

2.2 to 282.1 ± 2.4 mOsm·L^{-1}). This finding may be a result of female's generally lower

sweat rates contributing to a reduced ability dehydrate rapidly (i.g., 1.9% BM loss) and thus a

lower P_{osm} response. It may also suggest that P_{osm} was not sensitive enough to detect changes

in TBW at the threshold of a 2% decrease in body mass. This is in contrast to the general

findings in the literature that suggest that P_{osm} is the most sensitive measure of hydration

status during periods of acute dehydration (Cheuvront et al., 2010; Utter et al., 2010;

Popowski et al., 2001; Hamouti et al., 2013; Opplinger et al., 2005).

The male P_{osm} response is in contrast with that of U_{osm} which did not significantly

change from baseline (966.8 ± 25.95 mOsm·L^{-1}) but did at all three time points thereafter

(882.3 ± 30.5 to 846.7 ± 26.8 to 675.0 ± 48.1 5 mOsm·L^{-1}). This finding suggests that the

male athletes were dehydrated until the two hour rehydration time point according to the 700-800 mOsm·L^{-1}cut off described in the literature (Cheuvront et al., 2010; Utter et al., 2010; Popowski et al., 2001; Hamouti et al., 2013; Opplinger et al., 2005). These results differ from what was found for the female athletes who exhibited a significant decrease in U_{osm} from baseline to the two hour rehydration. For females, U_{osm} indicated dehydration at the pre-dehydration time point (737.4 ± 50.5 mOsm·L^{-1}), then decreased following the dehydration protocol (617.6 ± 60.1 mOsm·L^{-1}) finally decreasing to (407.2 ± 67.3 5 mOsm·L^{-1}) at the 2 hr rehydration time point.

Similar trends were also found in U_{sg} for both male and female athletes. The U_{sg} for the males increased from baseline to post-dehydration (1.027 ± 0.001 to 1.028 ± 0.001), and significantly decreased below baseline at the 2 hr rehydration period (1.021 ± 0.001). However, the U_{sg} values for males throughout the study are above the diagnostic cutoff of 1.021 that indicates dehydration as described in the literature (Cheuvront et al., 2010; Utter et al., 2010; Popowski et al., 2001; Hamouti et al., 2013; Opplinger et al., 2005). For females U_{sg} remained constant from baseline to post-dehydration (1.021 ± 0.001 to 1.021 ± 0.002 g·mL^{-1}), and significantly decreased below baseline at the 2 hr rehydration period (1.011 ± 0.002 g·mL^{-1}). This suggests that in females U_{sg} was not able to detect changes in acute TBW changes or that the body mass change incurred during exercise was not enough to elicit a change in U_{sg}.

Additionally, athletes of both sexes were classified as euhydrated by P_{osm} but not by U_{osm} or U_{sg}. With females and males exhibiting reduced concentrations at the 2 hr rehydration time point compared to baseline. Based upon these results it appears that at the current levels of dehydration P_{osm} is the only viable index of TBW. Similarly, Hamouti et al.

(2010), reported in their study that 50% of the athletes classified as having a large muscle mass were also identified as being dehydrated by U_{sg} when P_{osm} indicated that they were euhydrated. The author's hypothesized that athletes with large amounts of muscle mass would have an increase in protein metabolites in their urine and therefore higher U_{osm}. This would seem to fit the male athletes being studied at present and may offer an explanation as to why there was such a large discrepancy between U_{osm}, U_{sg}, and P_{osm}. Conversely, the average body fat percentage for the female athletes in the present study ($20.3 \pm 0.7\%$) is only slightly lower than the average for women of the same age as the athletes in the present study (21.0%). Therefore, the females in the present study may have a somewhat larger amount of muscle mass and may be subject to the same inconsistencies as the male athletes when compared to more sedentary controls.

These findings along with the results of the present study may provide the basis for an argument that athletes accustomed to regular elongated periods of water restriction (wrestlers) may have altered basal set points for their TBW levels. Perhaps this is being accomplished by an altered set point in the hypothalamus or kidney allowing for in increased release of anti-diuretic hormone at even lower osmolality's in order to maintain plasma volume and reduce the thirst reflex in the hypothalamus. However, because we did not measure any hormonal or ionic concentrations in the blood or urine this is a purely speculative statement. Still, if athletes generally require higher osmolality values in blood and urine to trigger thirst, a new set of standards may need to be developed to more accurately identify dehydration in this group. Future research may be needed to develop a new set of standards for blood and to greater extent urinary measures of hydration status in athletes. The current results suggest that the methods of field based hydration assessment

may be limited and the need for a non-invasive method of hydration assessment that does not

rely on bodily fluid is warranted.

Conclusion

 The results of the present study appear to confirm that UV is able to track changes in

TBW during periods of acute dehydration and rehydration in the soleus muscle of male

collegiate aged wrestlers. While significance was not found for UV in the soleus of the

female athletes it did exhibit a trend towards what would be expected during the

experimental protocol. The lack of significance found in both of the urinary measures of

hydration status may indicate that athletes with large proportions of muscle mass maybe

incorrectly classified as dehydrated when using U_{sg}. The limited changes in plasma

osmolality in females and the relatively low P_{osm} values exhibited through all four time points

may suggest that the athletes in this study may have had altered TBW regulatory set points

for P_{osm} and $U_{osm,}$ or that these tests are not sensitive enough to detect dehydration in this

particular group of individuals. Again, there is inter and intra-individual variability for UV.

It appears that damage to the muscle tissue, differences in tissue temperature, and proper

probe positioning alone could account for as much as 12 m/s of difference from measurement

to measurement. This amount of change is on the order of what is expected with a 3%

change in tissue water content and thus it becomes vital to identify ways to minimize the

contribution of these sources of error.

 Therefore, future studies should attempt to reduce these sources of error in order to

improve the accuracy of UV to asses' human hydration status. One such way to accomplish

this is to combine additional sensors with that of the ultrasound transducers. A possibility is

the use of a temperature sensor and a measure of blood flow to the limb in order to quantify

how these two factors change during acute dehydration and rehydration alongside of UV. The issue of individual differences in body composition may be accounted for by utilizing a more comprehensive measure of body composition such as underwater weighing or DEXA scanning. Further animal studies may be needed to determine if structural damage incurred during exercise effects the propagation of the ultrasound burst through the tissue. The issue of inadequate probe contact maybe most easily solved with the addition of four more probe sizes allowing for 10mm increments instead of 20mm. However, an adjustable single probe may provide the greatest degree of individual customization regarding probe fit. Finally, a more comprehensive approach to identifying and marking the site for initial probe place on each individual maybe warranted to ensure that each subject is being measured in the same area of the soleus muscle.

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Vita

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