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Hydration Status, Association of Body Composition and Bone Mass in Children and Adolescents

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HYDRATION STATUS, ASSOCIATION OF BODY COMPOSTIION AND BONE MASS IN CHILDREN AND ADOLESCENTS

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Priscilla Clayton

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To: Dean Tomás Guilarte
Robert Stempel College of Public Health and Social Work

This dissertation, written by Priscilla Clayton, and entitled Hydration Status, Association of Body Composition and Bone Mass in Children and Adolescents, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this dissertation and recommend that it be approved.

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The dissertation of Priscilla Clayton is approved.

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DEDICATION

I dedicate this dissertation to my mother and father, my best friends who instilled the principles of hard work, who have fueled my ambitiousness and drive, and who never failed to stand by me during this journey. Through my parents I learned to appreciate even the smallest of victories, to never give up, and to be the best version of myself. This work is also dedicated to my aunt Velvett, who showed me that the world is filled with so many possibilities if you only apply yourself and have faith. This work is also dedicated to all of those in my community that never ceased to provide support, inspiration, and share their mutual love for science and nutrition. Without each of you, I would not be the individual I am today.

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ABSTRACT OF THE DISSERTATION

HYDRATION STATUS, ASSOCIATION OF BODY COMPOSITION AND BONE MASS IN CHILDREN AND ADOLESCENTS

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The objective of this study was 1) to evaluate which foods or beverages are significantly associated with an objective marker of hydration status (USG), to examine the association between hydration status or total fluid intake with body fat % and fat-free mass (FFM) (2) and bone mineral density (BMD) and bone mineral content (BMC) (3) in children (10-13y) and adolescents (18-20y). Intake was assessed from three 24-hour dietary recalls and analyzed using the Nutrition Data System for Research (NDSR). Hydration status was objectively measured using USG via 24-hour urine collection and body composition and all bone parameters were measured using the Dual-X-Ray Absorptiometry Scan (DEXA). Descriptive statistics, independent t-test, Shapiro-wilk test, and multiple linear and logistic regressions were conducted to analyze the data.

A total of 52 children (n=18) and adolescents (n=34) were recruited (50% females). Mean age was 17.0 ± 3.72 years, most participants were white (57.7%), and reported being Hispanic (73.1%). Intake of fruit juice, water, all beverages, and total water intake from all sources was significantly ($p<0.05$) and inversely correlated with USG scores in the overall sample. Also, intake of water, fruit juice, all beverages, and total water intake from all sources was significantly associated with a higher odd of being euhydrated ($p<0.05$). Total water intake from all sources was a significant predictor of FFM ($p<0.05$), lean mass ($p<0.05$), and body fat% ($p<0.05$) in the overall and children group. For FFM and lean mass, those with a lower total water intake from all sources had 4.40 times higher odds of being below the median than those with higher total water intake from all sources (OR 4.40, 95% CI: 1.05, 18.5, $p=0.043$). For BMC, those with lower total water intake from all sources had 3.57 times higher odds of being below the median than those with higher total water intake from all sources (OR 3.57, 95% CI: 1.38, 11.19, $p=0.029$).

Our data suggested that 1) water, fruit juice, and total water intake from all sources is significantly associated with hydration status; 2) total water intake from all sources is associated with FFM and lean mass; and 3) total water intake from all sources is associated with BMC. Future research should include a larger and more generalizable sample. The study was approved by the Institutional Review Board of Florida International University (IRB-22-0045).

TABLE OF CONTENTS

LIST OF TABLES

ABBREVIATIONS AND ACRONYMS

DRI	Dietary Reference Intake
NHANES	National Health and Nutrition Examination Survey
USG	Urine Specific Gravity
FWR	Free Water Reserve
TBW	Total Body Water
SFT	Skin Fold Thickness
BIA	Bioelectrical Impedance Analysis
WBQ	Water Balance Questionnaire
HSQ-AY	Hydration Status Questionnaire
BEVQ	Beverage Intake Questionnaire
DEXA	Dual X-Ray Absorptiometry Scan
BMI	Body Mass Index
FFM	Fat Free Mass
ADH or AVP	Anti-Diuretic Hormone or Arginine Vasopressin
ANG	Angiotensin
AT	Adipose Tissue
BM	Bone Mass
BMD	Bone Mineral Density
BMC	Bone Mineral Content
MM	Muscle Mass
ssNMR	State Nuclear Magnetic Resonance Spectroscopy

CHAPTER I: INTRODUCTION

Water is a key component of hydration and plays a variety of important physiological roles in the body as it is essential to cells, tissues, and organs.[1] At infancy, total body water (TBW) represents approximately 75% of an infant's body weight, and in children/adolescents, it decreases to 65%.[1,2] To maintain this level of water in the body in children, the Dietary Reference Intakes (DRI) for water in the US was established at 2,100 mL/d for girls and 2,400 mL/d for boys ages 9-13 years, 2300 mL/d for girls and 3300 mL/d for boys ages 14-18 years, 2700 mL/d for women and 3700 mL/d for men ages 19-30 years.[3] DRIs were set based on median intakes of total water from drinking water, beverages, and foods from NHANES data.[3] However, data from NHANES 2005-2010 among 4,766 children 4-13 years in the US showed that 75% of children 4-8y, 87% of girls 9-13y, and 85% of boys 9-13y did not meet DRIs for total water intake.[4] Adolescents ages 14-20 years failed to meet the adequate water intake as those ages 12-19 years met only 2400 mL/d and those ages 19-20 years met only 1050 mL/d.[4] The study found the main contributor of total fluid intake was water and other beverages (70-75%).[4]Another study using data from NHANES 2009-2012 among 4,134 participants ages 6 to 19 years using an objective marker of hydration (urine osmolality) showed that 55% had inadequate hydration (urine osmolality > 800 mOsm/kg).[5,6] They also found that boys (OR = 1.76; 95% CI = 1.49, 2.07) and non-Hispanic Blacks (OR = 1.34; 95% CI = 1.04, 1.74) were at significantly higher risk for inadequate hydration.[5]After adjusting for age, race/ethnicity, sex, income, time of examination, and BMI, they found that an increase in an 8-fluid ounce serving of plain water was associated with a -8.0 mOsm/kg decrease in urine osmolality (95% CI = -11.7, -4.2).[5,6] Another study among Spanish adolescents ages 12-18 years found a high percentage not meeting the water intake recommendations (72% males and 69% females) and that 71% of boys and 65% of girls had inadequate hydration (urine osmolality > 800 mOsm/kg).[7] Among Greek children ages 8-14, the prevalence of hypohydration (assessed from urine osmolality ⩾800 mmol/kg) was 44% in boys and 23% in girls.[8] Among Belgium children ages 7-13, the prevalence of hypohydration (assessed from urine osmolality ⩾800 mmol/kg) was 54%.[9]

Low water intake or mild hypohydration can result in a decrease in exercise performance, mood and fatigue, glucose regulation, blood vessel function, thermoregulation, and impaired cognitive function.[10] Hypohydration is also associated with chronic diseases such as hypertension, obesity, stroke, coronary heart disease, and venous thromboembolism.[10,11] This has been demonstrated as a result of enhanced serum- and glucocorticoid-inducible kinase and plasma sodium, which play a role in the pathophysiology of each.[10]

Assessment of hydration

The studies reviewed have demonstrated the need to adopt adequate hydration habits early in life, along with the need to evaluate adequate water intake in children and adolescents.[4,12,13] Understanding the different markers to assess hydration in these groups is essential, considering that each of these markers can be used in other laboratory conditions, clinical practices, and sports.[13] Therefore, studies have utilized several different objective markers to assess hydration in children and adolescents, such as urine osmolality, urine specific gravity (USG), urine color, and free water reserve (FWR).

Objective markers of hydration

1. **Urine osmolality** is based on the number of osmotic solutes present in the urine and the volume of water with very high sensitivity (91%).[13] Urine osmolality reflects how the kidney responds to the variations in the body's water balance, and results can range from 50 to 1400 mOsm/kg.[13] Among healthy individuals, dehydration results in a small volume of highly concentrated urine, increasing osmolality.[13] This can be detected by using either a freezing-point or vapour pressure-depression osmometer and with the use of a random or 24-hour urine collection.[13]
2. **Free water reserve (FWR)** is the difference between the measured urine volume (ml/24-h) and the ideal urine volume (ml/24-h) required to excrete the necessary 24-hour urine solutes (mOsm/24-h).[14] The obligatory urine volume (mL/24 h) derives from the excreted 24-h urine solutes (mOsm/24 h; mainly determined by urinary concentrations of nitrogen, sodium, potassium, and phosphorus).[14] FWR is calculated by subtracting the 24-hour urine volume from the obligatory urine volume. These results are then divided by the 97th percentile of urine osmolality for healthy children and adolescents with an adequate water intake of 830 mOsm/kg; this indicates the upper hydration limit for children with a typical western diet.[15] Positive values indicate euhydration, and negative values indicate risk of hypohydration.[11,15] This measure, however, has no results reported for sensitivity or specificity.
3. **Urine specific gravity** (USG) is considered the weight of urine with an equal volume of distilled water.[13]According to the National Collegiate Athletic Association, the range used to identify dehydration is defined as a value over 1.020-1.025, with a normal urine sample ranging from 1.013-1.020.[13,16]The gold standard measurement

for USG is using refractometry.[13]USG has similar specificity as urine osmolality and, therefore is the recommended test for hydration status in relatively large population studies.[13]Also, USG and urine color can be easily performed and can be carried out quickly compared to urine osmolality which requires technical expertise.[13] Therefore, this study will use USG as a measure of hydration.

4. **Total body water content** (TBW) represents about 60% of an individual's body weight, in which any changes in body water can be determined by body weight changes.[13] Many approaches are available to measure TBW: skinfold-thickness (SFT), bioelectrical impedance analysis (BIA), differential X-ray absorptiometry, computerized tomography scanning, underwater weight, or air displacement.[17] The most direct and accurate method to assess TBW has been the use of isotope dilution.[17] Though changes in body composition can make this specific measure unstable in studies of long duration and are best used in studies involving sports activities or intense exercising.[17]
5. **Urine color**, a common marker used for assessing hydration, was developed to evaluate the urine of healthy individuals.[13] A standardized color scale was developed and ranged from 1 (pale yellow, diluted urine) to 8 (dark brown, concentrated urine).[13]Advantages of using urine color as a tool to measure hydration are that it is both cheap and non-invasive. However, it has a lower sensitivity score (81%) than USG and urine osmolality. In addition, it may be challenging to apply to large-sample studies.[13]

Non-objective markers of hydration

There are different ways to assess hydration from questionnaires: beverage intake, water from solid and fluid food sources, sweating (physical activity or sedentary conditions), and frequency of urination and defecation. There are several questionnaires specifically developed to assess this. For example, the Water Balance Questionnaire (WBQ) in adults[18] and the Hydration Status Questionnaire (HSQ-AY) in children ages 12-18 years.[19] Other studies have also used 24-h recalls and records to evaluate hydration, although errors have been linked to recalling and estimating the portions of intake.[20] The next section discusses the evaluation of these non-objective markers of hydration against objective markers.

Evaluation of questionnaires to estimate hydration against objective markers of hydration

There are limited studies available that have evaluated questionnaires to evaluate hydration status in children and adolescents against an objective marker. Most studies have explored this in adults. [18,21] The Water Balance Questionnaire (WBQ), which takes into account consumption of intake of foods and beverages, physical activity, sweating, urination, and defecation, found moderate agreement when tested against urine volume, urine color, and urine osmolality in adults.[18] The beverage intake questionnaire (BEVQ) showed a negative correlation between USG and total daily beverage consumption at different times in adults (r range -0.202, -0.238; $p < 0.05$).[21] However, there are only a few studies with children and adolescents. The BEVQ (used in adults) was modified for children and adolescents (BEVQ-15), but it was not evaluated against an objective marker.[22] Similarly, the Beverage Frequency Questionnaire and the Block Kids Food

Screener (BKFS) have been used to assess beverage intake in children but neither has been evaluated against an objective marker. [20,22]

To date, there are only 3 studies that have associated intake of food and beverages from the questionnaire with an objective marker. [7,19,23] The first study correlated the Hydration Status Questionnaire (HSQ-AY), a modified version of HSQ developed in adults, with the results from USG, urine color, and TBW in Spanish adolescents 12-17 years old.[19] Moderate agreement between water balance and total water intake was found with USG ($r = -0.202$, $p = 0.023$ and $r = -0.184$, $p = 0.03$, respectively).[19] Total water intake was also correlated with TBW ($r = 0.263$, $p = 0.003$).[19]However, this study did not use a 24-hour urine sample and the measurement of USG was conducted with the use of a reagent strip, which is not the gold standard for measuring USG.[19] The second study correlated food and beverage intake from 24-h dietary recalls to urine osmolality among 194 Spanish adolescents ages 12-18 years.[7] They found that consumption of fruits and urine osmolality were significant in both girls ($\beta = -0.41$; 95% CI -0.73,-0.10, $p = 0.01$) and boys ($\beta = -0.41$; 95% CI -0.63,-0.19, $p = 0.00$).[7] Additional significant association were found for intake of vegetables (excluding potatoes) in boys ($\beta = -0.60$; 95% CI -1.02, -0.18, $p = 0.01$) and dairy drinks in girls ($\beta = -0.39$; 95% CI -0.76, -0.02, $p = 0.04$).[7] However, the study only collected first-morning urine which is not representative of the participant's 24-hour hydration.[7] The third study associated the results from 3-day food records with FWR among 424 German children ages 4-10 years.[23]Results showed a significant association between intake of fruits and vegetables, juice, water, and milk with FWR in both boys and girls ($p<0.001$).[23]The highest association with FWR was found for intake of fruits and vegetables in boys ($\beta = 0.56$; 95% CI 0.33, 0.79) and for water intake in girls ($\beta = 0.55$; 95% CI 0.48, 0.63).[23] Though significant association was found in water and fruit and vegetable intake with hydration status and they collected 24-hour urine, the study only included children ages 4-10 years and hydration status was calculated directly to measure hydration status.

In summary, the three studies available to date evaluating hydration status from questionnaires against an objective marker, have all shown some limitations. The studies have used different questionnaires and objective markers of hydration, not all studies collected a 24-hour urine collection, and they have been conducted in different populations of European children and adolescents. The current study evaluated which particular food or beverage intake from 24-h dietary recalls is significantly associated with an objective marker of hydration (USG from 24-h urine collection) in a sample of children and adolescents ages 10-20 years in the US. By determining which component of diet better predicts hydration status in children and adolescents, can help future studies to focus on the type of beverage and foods that may contribute to hydration.

Hydration and weight status or obesity

There is some evidence that hydration may be associated with weight status. This is important as childhood obesity continues to be a major public health concern in the US, with 19.1% of boys and 17.8% of girls ages 2-19 years in the US being obese.[13,24,25] This, in turn, increases other conditions, such as diabetes mellitus, hypertension, coronary artery disease, cancer, and sleep apnea.[13,24] In adults, a study using data from NHANES 2009-2012 showed that those inadequately hydrated, which was measured by urine osmolality, had significantly higher BMI ($P<0.001$) and had higher odds of being obese (OR=1.59; 95% CI, 1.35-1.88, $P<0.001$) compared to those adequately hydrated.[26]

Similar results were found in 1500 Portuguese adults, with the greatest odds of obesity (OR = 1.97, 95% CI, 1.06-3.66) in men in the highest osmolality tertile (≥ 602.1 mOsm/kg) compared to the lowest tertile.[27] Furthermore, in 260 Spanish adults, higher odds of obesity was found among those with higher urine osmolality (OR 1.00; 95% CI 1.00-1.01, P = 0.05) and USG (OR 1.02; 95% CI 1.00-1.04, p = 0.05).[28] This evidence suggests an association between hydration and obesity.

In children and adolescents, there is less evidence available associating an objective marker of hydration with weight or obesity. A study among 86 obese and 89 normal weight Italian children ages 7-11 years found that hypohydration, assessed using FWR, was found in 34% of obese and 20% of normal-weight children ($p<0.05$).[7]They also found that fat mass, assessed from bioimpedance, ($r = -0.17$, $p < 0.05$), BMI z-score ($r=-0.18$, $P< 0.05$), and waist-to-hip ratio ($r = -0.17$, $p < 0.05$), were all significantly associated with the hydration status in these children.[7]Another study among 371 Belgian children ages 7-13 years found that BMI was a significant predictor of total fluid intake ($\beta = 0.110$, $p = 0.038$), although no significant association was found between fluid intake and osmolality during the day ($p = 0.283$).[9]The authors explained the lack of association related to the lack of representativeness of the pooled urine samples for the whole school day.[9]

There are also a few studies associating hydration status (total water intake) assessed from questionnaires with weight status. One study among 366 Spanish children ages 9-11 years found a positive association between water intake, as assessed from 24-h dietary recalls, with BMI ($p<0.001$), waist circumference ($p<0.001$), and body fat ($p<0.004$) fat-free mass ($p<0.001$), assessed via bioimpedance analysis.[29] Another study among 372 healthy Spanish adolescents ages 12-18 years old found that water intake, assessed from the HSQ-AY questionnaire, was inversely correlated with BMI in boys ($r = -0.548$, $p=0.000$) and girls ($r = -0.376$, $p<0.001$).[30] Water intake, adjusted for body weight, was inversely associated with fat ($\beta = -1.04$; 95 CI, -1.43 to - 0.66, $p<0.001$) and dry lean body mass ($\beta = 0.27$; 95 CI, -5.51 to – 2.17, $p<0.001$).[30]However, none of the studies used dual-energy X-ray absorptiometry (DEXA), which is considered the gold standard for measuring body composition.[31] In addition, the few studies to date were done in European populations.

The current study evaluated the association between hydration using USG as an objective marker from a 24-hour urine collection and also from three 24-h dietary recalls with body composition as measured using DEXA.

Mechanism explaining association between hydration and weight status

Several mechanisms may explain how hydration is associated with weight or obesity. One of the mechanisms may be related to the anti-diuretic hormone (ADH) or also known as arginine vasopressin (AVP), the principal hormone for the regulation of body fluid by stimulating thirst.[24,32,33] Studies in rats show that, as intracellular and extracellular dehydration occurs, ADH is released from the neurohypophysis and results in a decrease in blood volume and an increase in plasma osmolality, resulting in regulation of the body and cellular hydration.[24]An elevated ADH response results in an enzymatic release of renin in the kidneys to produce angiotensinogen in the blood to produce angiotensin I (AngI) which is responsible for producing AngII. Once AngII has been produced, this aids in the stimulation of thirst to allow blood volume to return to normal levels.[24]However, due to ADH being a relatively short-lived peptide, the stable analog copeptin, a derivative precursor of ADH, is considered a reliable surrogate marker of fluid intake in plasma.[32,33] Elevation of copeptin in rats showed to be associated with metabolic syndrome and obesity as higher fluid intake results in a lower serum copeptin and lower body weight, body fat percentage, and fat mass.[33]

Another mechanism to explain this association is through changes in lipolysis.[24] This process occurs in all triglyceride storing tissues, although adipose tissue (AT) reflects the majority of lipolytic activity.[34] Adipose tissue (AT), a major organ for energy storage, secretes adipokines to regulate lipid metabolism by lipogenesis and lipolysis.[35] During energy demands, through fasting or physical exercise, adipocytes in the AT mobilize stored fat, in which triglycerides are then broken down to fulfill energy needs by supplying it throughout the body,[35]however the metabolism of an adipocyte may be dictated based on the cell's hydration status. When the adipocyte is euhydrated, (sufficient water intake) both glucose and free fatty acids produce triglycerides, in which the rate of lipolysis is dependent on the need for ATP in the cell. However, during hyperosmolality (insufficient water intake), the adipocyte becomes dehydrated, and free fatty acids are unable to be metabolized in the mitochondria as an excess of triglycerides began to form; this may occur when the urine osmolality threshold is approximately 280 mOsm/L and elevated urine osmolality above 800 mOsm/L.[24,36] The underlying cause of the excess production of triglycerides that occur during dehydration is due to the increase in the glycerol transporter, aquaporin 9 (AQP9) that produces an excess amount of glycerol.[24] Furthermore, glucose uptake continues in the adipocyte that is stimulated by insulin, thus also increasing the synthesis of triglycerides.[24] Therefore, hydration may lead to a reduction in the upregulation of glycerol and triglycerides and increase cell volume, this resulting in an increase in insulin sensitivity while enhancing mitochondrial function and metabolism.[24]

This mechanism has been explored in one study in adults. The study was conducted with 10 healthy young males, ages 25 years old, to examine whole-body lipolysis and lipid oxidation and whether this was affected by hypo- and hyper-osmolality conditions.[34,37] The young males had an average BMI of 23.0 ± 0.8 kg/m^2.[34,37] Lipolysis was measured by glycerol rate of appearance (glycerol R_a) via stable isotope method and indirect calorimetry. Results showed after a 14h fast, glycerol R_a was higher during hypo-osmolality than during iso-osmolality (sufficient fluid balance) (2.35 ± 0.40 vs 1.68 ± 0.21 mmol/kg/min, P=0.03). The study showed that high fluid consumption leading to hypo-osmolality suggested a reduction in body fat, therefore, promoting lipolysis.[34,37] Therefore, a relationship between hydration and weight status is evident, although the limitations of this study were that it did not include children and adolescents and the study required a strict intervention (infusions and strict fasting) that may be too invasive for this group.

Exploration of the association between hydration and bone

This study explored if hydration is associated with bone mass, although less evidence is available. This is important to evaluate as low bone mass is highly prevalent in the US (43.9% in adults).[38] One of the main determinants of low bone mass in older adults is behavior during puberty, as a large proportion of bone mass is acquired during this stage.[38] The onset of bone mineral accretion is at approximately 12.5 ± 0.90 years in girls and 14.1 ± 0.95 years in boys ages 8-14 years and during a 3- to 4-year period, in which 40% of the total lifetime bone mass is achieved.[39] Therefore, the importance of proper dietary practices along with the lifestyle factors of the child, impact the prevalence of low bone mass in later adulthood.

There are 2 studies in the literature that have explored this association in adults. The first study was conducted on older adults to evaluate bone mass and muscle mass. Results showed that those that were adequately hydrated, which was measured by total body water (TBW), had a lower risk of osteoporosis (OR = 0.76, 95% CI, 0.66-0.88, $P < 0.0001$).[40] The second study in 30 healthy male students administered a fluid and salt supplementation found a significant effect of the supplementation on bone mineral density as measured by DEXA ($r = 0.93$, $p < 0.05$).[41] This same study also found a significant increase in several parts of the bone (i.e. trabecular bone volume, cortical thickness, trabecular plate thickness, and density) on bone mineralization with the fluid and salt supplementation.[42] To date, there are no studies evaluating this in children and adolescents.

The potential mechanisms explaining the association between hydration and bone are related to the composition of bone, as water contributes approximately 20% of its wet weight and is a third major component of bone.[42] There is water in the microscopic pores of bone (known as mobile water or the free water content of bone) and also in the extracellular matrix of bone tissue (known as bound water), where the residues of collagen reside.[43] Collagen is an important protein for the strength of the bone and water has been shown to affect its shock-absorbing capacity.[42] An *in vitro* study using the state nuclear magnetic resonance spectroscopy (ssNMR) found a decline in native collagen with dehydration, specifically low content of the mobile water.[44] This dehydration resulted in an exchange of hydrogen to deuterium, a water-mediated interaction, which appeared to affect bone mechanical properties and it also decreased bone quality.[44] Also using NMR, a study of 18 specimens of human femurs collected from 18 middle-ages and elderly male donors showed significant changes in bound water by the toughness and strength of the bones.[43] Based on this limited evidence, both mobile and bound water could affect bone. However, there are no *in vivo* studies providing physiological evidence on whether hydration directly affects bone mass in children or adults.

Therefore, the current study explored the potential association between hydration (measured using USG from a 24-hour urine collection and estimated from three 24-h dietary recalls) and bone mass (measured using DEXA) in children and adolescents.

Specific Aims and Hypotheses

To address the gaps on hydration, body fat percentage, fat-free mass, and bone mass, the aims of the study were:

Specific Aim 1: To evaluate which particular food or beverage intake (assessed from three 24-h dietary recalls) is significantly associated with an objective marker of hydration (measured using USG from a 24-hour urine collection) in children and adolescents. It is hypothesized that USG will be associated with the type and quantity of the liquid consumed and foods consumed.

Specific Aim 2: To examine the association between hydration status (measured from USG in a 24-h urine sample and assessed from three 24-h dietary recalls) and body fat percentage and fat-free mass (assessed from DEXA) in children and adolescents. It is hypothesized that a higher hydration status will be associated with a lower body fat percentage and higher fat-free mass (FFM) (after controlling for important covariates).

Exploratory aim: To explore the association between hydration status (measured from USG in a 24-h urine sample and assessed from three 24-h dietary recalls) and bone mass (assessed from DEXA) in children and adolescents. It is hypothesized that a higher hydration status will be associated with a higher bone mass.

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CHAPTER II: THE ASSOCIATION BETWEEN HYDRATION STATUS AND TOTAL FLUID INTAKE IN HEALTHY CHILDREN AND ADOLESCENTS.

Introduction

Water is a key component of hydration and plays a variety of important physiological roles in the body as it is essential to cells, tissues, and organs.[1] Hydration is important for overall mental, physical, and emotional health that are associated with inadequate hydration.[2] Hydration may also have an impact on body composition as studies in children show that BMI was significantly associated with hydration status and that water intake was inversely associated with fat.[3,4] Yet, evidence from the National Health and Examination Survey (NHANES) shows that children in the US ages 9-13 years have suboptimal hydration status, with an average water intake of 577 mL/d[5] and those ages 14-18 years met only 866 mL/d and those ages 19-30 years consumed 1,305 mL/d.[5] This is well below the US Dietary Reference Intakes (DRI) of 2,100 mL/d for girls and 2,400 mL/d for boys ages 9-13 years,[5,6] of 2,300 mL/d for girls and 3,300 mL/d for boys ages 14-18 years, and of 2,700 mL/d for women and 3,700 mL/d for men ages 19-30 years.[6] Data from NHANES in children showed that 55% had inadequate hydration, as measured by urine osmolality.[7]

Hydration is usually best assessed using objective markers such as urine osmolality, urine specific gravity (USG), urine color, and free water reserve (FWR).[8] USG measures the concentration of all chemical particles in the urine and it is considered a non-invasive marker of hydration in children and adolescents, recommended for larger population studies, which has similar specificity (91%) and sensitivity (89%) as urine osmolality.[8] However, it is not always feasible in epidemiological studies to assess hydration using these markers. Therefore, questionnaires are often used to estimate hydration status but there are limited studies available that used questionnaires to assess hydration status in children and adolescents against an objective marker. Most studies have explored objective markers in adults. The limited available studies have explored an association between an objective marker of hydration status with an estimation of hydration assessed from food and beverage questionnaires. Though the studies measured with an objective maker from a 24-h urine collection which is more precise, the studies available have been conducted primarily in European populations. Therefore, the present study aimed to evaluate which foods or beverages are significantly associated with an objective marker of hydration (USG) assessed from a 24-h urine collection of US children and adolescents. It is hypothesized that USG will be associated with the intake of certain foods and beverages, which may be different from studies in adults and studies in European children.

Methods

Study Design

This was a cross-sectional analysis to evaluate which foods or beverages are significantly associated with an objective marker of hydration status (USG) assessed from a 24-h urine collection and three 24-hour dietary recalls in a sample of children and adolescents.

Study Population

The data for children were used from the baseline visit of the MetA-Bone Trial, a trial to determine the effects of soluble corn fiber (SCF) supplementation for one year on bone metabolism in healthy children.[9] Inclusion criteria for children of the MetA-Bone

Trial were ages 10-13 years, had low calcium intake (2 or fewer servings of dairy products/day), and had adequate vitamin D levels. Children were excluded if they had chronic illnesses requiring medication use or if using regular calcium (>200 mg/d) or vitamin D supplements (> 400 IU/d). Children were recruited throughout Miami-Dade schools, clinics, after-school programs, emails, online, among other strategies in South Florida. Interested parents completed a short pre-screening questionnaire to verify the eligibility of their child and if eligible, parents were asked to sign the consent form, and children were asked to sign the assent form. The use of the protocols was approved by the Institutional Review Board at Florida International University (IRB-21-0429).

The data for adolescents was collected directly from college students ages 18-20 years at Florida International University. Any adolescents with the presence of chronic illness requiring medication use were excluded. Adolescents were recruited using flyers distributed in classes or by email through faculty and staff. Those interested were explained the study and were asked to answer a brief pre-screening questionnaire with the inclusion criteria. If eligible, participants were asked to sign the consent form. This study was approved by the Institutional Review Board at Florida International University (IRB-22-0045).

Measurements

General questionnaire. Participants were administered a questionnaire to determine their age, sex, race, and ethnicity.

Anthropometric measurements. Weight and height were obtained from the participants wearing light clothing, utilizing a standardized scale and a wall-mounted stadiometer. Height was measured with the participant's back and heels touching the vertical board of the stadiometer. Both weight and height were measured to the nearest 0.1 kg and 0.1 cm, respectively.[10] BMI percentiles for sex and age were calculated using standardized growth charts.[11]

Physical activity. Participants were asked to complete a short version of the International Physical Activity Questionnaire (IPAQ) to assess physical activity based on the intensity of the activities during the previous seven days (e.g., moderate versus vigorous).[12]

24-h dietary recalls. Participants were asked to complete three 24-hour dietary recalls; the first one was completed in-person, and the two others were completed in the next few days to represent 2 weekdays and 1 weekend day. The participant's food description was reviewed with the participant to ensure completeness and correctness. The 24-hour dietary recalls were analyzed using the dietary computer-based analysis software application 'Nutrition Data System for Research (NDSR)' developed at the University of Minnesota Nutrition Coordinating Center (NCC). The data were analyzed as food group serving count estimates (i.e., servings of dark green vegetables, sugar-sweetened beverages, etc.) derived by the US Dietary Guidelines. The average of the three 24-hour recalls was first calculated and then the following categories were grouped for beverages: water (bottled or tap), fruit juice (citrus juice, fruit juice without citrus, and other juices), milk (unflavored and flavored whole milk, reduced-fat milk, and low-fat milk, and also milk substitutes), sugar-sweetened beverages and soda (regular and diet), tea and coffee (sweetened and unsweetened), and total liquid from all beverages (all of these beverages combined). The following foods were grouped: other dairy products (cheese, yogurt, ice cream, dairy substitutes), fruits, vegetables, grains (flour and dry mixes, bread, tortillas, muffins, crackers, pasta, cereals), protein (chicken, pork, beef, fish, sausage, eggs), oils and fats, sauces and condiments (salad dressing, barbeque sauce, hollandaise sauce, gravy, syrup and jams), candies and desserts (chocolate, non-chocolate candy, pastries, danish, doughnuts, cakes), nuts and seeds (nut and seed butters included), and salty snacks (bars, chips, popcorn). Finally, NDSR also calculated total water intake from all sources.

Objective marker of hydration status**.** Participants were asked to collect urine for 24-h to determine hydration status using Urine Specific Gravity (USG). For this, participants were provided with a urine hat to initially collect the urine and then transfer it to sterile urine containers. Participants were instructed to store the urine in the refrigerator throughout their collection until they were brought back to the lab at FIU. For total volume, the urine from the different containers was combined in a volumetric cylinder. From the total volume, a sample of 0.3 ml was transferred into the ATAGO PAL 10S digital pocket refractometer that utilizes a refractive index method to measure USG. The urine sample was placed on the refractometer's prism and analyzed to reveal the measurement value within 3 seconds. Removal of the sample was cleaned using a Kim wipe along with the removal of excess moisture on the prism before the next sample. Zero testing was performed after each participant's testing by placing 3 drops of deionized water with a plastic dropper on the prism service. The refractometer has a urinary USG range of 1.000 to 1.060 with a resolution of 0.001.[13] USG measures the concentration of all chemical particles in the urine and compares the density of urine to density of water; therefore, the higher the number, the higher the concentration of the urine and the lower the hydration status. Testing was conducted three times for each participant to verify stable USG values and, if results varied, an additional measurement was made to provide consistent results. The average of the three measurements was calculated. USG values were also categorized as euhydrated (USG < 1.020 μG), dehydrated (USG 1.020-1.030 μG),[14,15] or severely dehydrated (USG > 1.030 μG).[16]

Statistical Analysis

For descriptive statistics, categorical variables were presented as frequencies and percentages, while continuous variables were presented as mean ± standard deviation. Two-sample t-tests were used to compare mean values between age groups and chi-square and Fisher's exact tests were used to compare differences in proportions between groups. Spearman's rho correlation coefficient tests were conducted to assess correlations between hydration status, type of beverage, and type of food consumed. Mean values of total water intake, other beverages, and foods were used to compare to DRIs for total water intake from all sources by age group and sex. To evaluate which particular food or beverage intake was significantly associated with the objective marker of hydration in the sample, a series of logistic regressions were used, adjusting for age and sex. All analyses were performed using SPSS software version 26.0 and significance was evaluated at an alpha level of 0.05.

Results

A total of 52 participants were included in the overall study consisting of 18 children and 34 adolescents. Table 1 shows the characteristics of these groups; sex distribution was similar between age groups, and most were Hispanic or Latino (73.1%), which was similar between age groups. The overall group was classified as normal weight based on their BMI percentile with no difference by age group. Statistically significant differences were found for age, race, and physical activity.

Table 1. Characteristics of study population (N=52)

Variable	Overall (n=52)	10-13 (n=18)	18-20 (n=34)	P-Value
	Mean ± SD or N (%)			
Age, y	17.0 ± 3.72	12.0 ± 1.37	19.1 ± 0.64	**0.000***
Sex				
Female	26 (50.0)	8 (44.4)	18 (52.9)	0.560
Male	26 (50.0)	10 (55.6)	16 (47.1)	
Race				
White	30 (57.7)	2 (11.1)	28 (82.4)	**0.000***
African American	4 (7.7)	3 (16.7)	1 (2.9)	
Asian	2 (3.8)		2 (5.9)	
Native Hawaiian or other Pacific Islander	2 (3.8)		2 (5.9)	
Other	14 (26.9)	13 (72.2)	1 (2.9)	
Hispanic or Latino	38 (73.1)	13 (72.2)	25 (73.5)	0.919
BMI percentile	64.0 ± 24.8	58.0 ± 27.8	67.0 ± 24.7	0.278
IPAQ score (MET)	3639 ± 3714	1729 ± 1328	4540 ± 4194	**0.001***

**Significant difference between age groups at p<0.05 by independent sample t-test or Chi-square. Abbreviations: IPAQ, International Physical Activity Questionnaire.*

Intake of foods and beverages in the sample is shown in Table 2. Children had a significantly higher intake of milk and candy and desserts compared to adolescents ($p<0.05$). However, adolescents had a significantly higher intake of water, tea and coffee, total liquid from beverages, vegetables, and total water intake from all sources ($p<0.05$). Differences in fluid intake among age groups in relation to BMI was observed (data not shown) and results showed adolescents had a significantly higher intake of water, tea and coffee, and milk ($p<0.05$).

Table 2. Intake of foods and beverages in children (10-13y) and adolescents (18-20y)

Food group intake	Overall (n = 52)	10-13 (n = 18)	18-20 (n = 34)	P-Value*
	Mean ± SD			
Beverages (mL/d)				
Water	924 ± 715	409 ± 409	1197 ± 695	**0.000**
Fruit juice	55.7 ± 80.6	79.2 ± 98.3	43.3 ± 67.8	0.178
Milk	181 ± 237	325 ± 252	105 ± 192	**0.003**
Sugar sweetened beverages	57.6 ± 96.8	78.2 ± 123	46.7 ± 79.7	0.269
Tea and coffee	46.7 ± 90.7	5.64 ± 16.5	68.5 ± 106	**0.002**
Total liquid from beverages	1265 ± 722.9	897 ± 428	1460 ± 774.7	**0.001**
Foods (servings/d)[1]				
Other dairy products	2.73 ± 2.38	2.99 ± 2.19	2.60 ± 2.50	0.585
Fruit	0.46 ± 0.66	0.67 ± 0.85	0.34 ± 0.51	0.090
Vegetables	2.49 ± 2.09	1.31 ± 0.91	3.11 ± 2.27	**0.000**
Grains	5.45 ± 2.83	5.98 ± 2.02	5.16 ± 3.17	0.327
Protein	6.74 ± 3.77	5.69 ± 2.84	7.29 ± 4.11	0.146
Oil and fats	2.48 ± 4.34	1.54 ± 1.64	2.97 ± 5.19	0.146
Sauces and condiments	1.63 ± 2.83	0.82 ± 0.99	2.05 ± 3.37	0.139
Candy and dessert	0.63 ± 0.64	1.08 ± 0.72	0.40 ± 0.44	**0.001**
Nuts and seeds	0.54 ± 1.08	0.31 ± 0.64	0.66 ± 1.24	0.268
Salty snacks	0.46 ± 0.74	0.64 ± 0.94	0.36 ± 0.61	0.261
Total water from all sources (g/d)	1831± 841.4	1307 ± 543.8	2109 ± 843.9	**0.001**

*Difference between age groups was tested by two-sample t-test.

[1]A serving of other dairy is 1 cup of yogurt, 1½ ounces of natural cheese, or 2 ounces of processed cheese; a serving of fruits is 1 medium apple, banana, orange, or pear, ½ cup of chopped, cooked, or canned fruit, ¼ cup of dried fruit or ½ cup of fruit juice; a serving of vegetable is 1 cup of raw leafy vegetables, ½ cup of other cooked or raw vegetables, ½ cup of vegetable juice, or 15-30g of pickled foods; a serving of grains is 1 slice of bread, 1 ounce of ready-to-eat cereal, or ½ cup of cooked cereal, rice, or pasta; a serving of protein is one-ounce equivalent; a serving of oils and fats is 1 teaspoon; a serving of sauces and condiments is ¼ cup for toppings, 1 teaspoon for minor condiments, 1 tablespoon for major condiments, and 2 tablespoon for BBQ, hollandaise, or tartar sauce; a serving of nuts and seeds is ½ cup or 1 tablespoon of nuts and seed butters; a serving of candy and dessert is 30 g for cookies, 40 g for chocolate candy and non-chocolate candy, 40 g for brownie, 55-125 g for cake, 55 g for donuts and sweet rolls, or 125 g for pies and pastries; a serving of snacks is 1 ounce.

Hydration status using USG from 24-h urine samples are shown in Table 3. No significant difference between groups was found in USG. When categorized, 51.9% of children and adolescents were euhydrated and 48.1% were dehydrated, with no differences by age group.

Table 3. Hydration status using an objective marker of hydration status (USG) in children (10-13y) and adolescents (18-20y)

Hydration Status	Overall (n = 52)	10-13 (n = 18)	18-20 (n = 34)	P-Value*
	Mean ± SD			
USG (μG)[1]	1.018 ± 0.005	1.020 ± 0.006	1.017 ± 0.005	0.072
Hydration categories				
Euhydrated	27 (51.9)	9 (50.0)	18 (52.9)	0.840
Dehydrated	25 (48.1)	9 (50.0)	16 (47.1)	

*Statistical difference between groups was tested using an independent sample t-test and chi-square
[1]A high USG is indicative of lower hydration.

Table 4 displays the total water intake from all sources (all beverages, and moisture in foods) and the comparison with the DRIs. Overall, total water intake from all sources was similar by sex but adolescents (both females and males) ages 18-20 years had a significantly higher intake compared to children ages 10-13 years ($p<0.05$). When observing among age groups the differences in total water intake from all sources in females and males in relation to BMI (data not shown), no statistically significant difference was observed. Overall, 86.5% failed to meet the recommendations for total fluid intake, with a similar proportion by age and sex.

Table 4. Total water intake from all sources in children (10-13y) and adolescents (18-20y) by sex and comparison with the dietary reference intakes (DRIs) for total fluid intake

Hydration Status	**Overall (n = 52)**	**10-13y (n = 18)**	**18-20y (n = 34)**	**P-Value***
	Mean ± SD or N (%)			
Total water from all sources	1831± 841.4	1307 ± 543.8	2109 ± 843.9	**0.001**
Male	2002 ± 980.4	1384 ± 545.1	2388 ± 1005	**0.003**
Female	1661 ± 650.1	1210 ± 563.0	1861 ± 594.1	**0.018**
P-value (difference between sex)	0.146	0.518	0.068	
Met the DRI for total fluid intake recommendation	7 (13.5)	2 (11.1)	5 (14.7)	0.539
Male	3 (11.5)	1 (10.0)	2 (12.5)	0.608
Female	4 (15.4)	1 (12.5)	3 (16.7)	
Fail to meet the DRI for total fluid intake recommendation	45 (86.5)	16 (88.9)	29 (85.3)	
Male	23 (88.5)	9 (90.0)	14 (87.5)	1.000
Female	22 (84.6)	7 (87.5)	15 (83.3)	

*Statistical difference between groups was tested using an independent sample t-test and chi-square.

Table 5 shows the Spearman's rho correlation results between intake type and hydration status. Overall, there was a significant negative correlation between USG and the intake of fruit juice (r_s = -0.35; $p<0.05$), water (r_s = -0.34; $p<0.05$), total liquid from all beverages (r_s = -0.32; $p<0.05$), vegetables (r_s = -0.29; $p<0.05$), and total water from all sources (r_s = -0.43; $p<0.05$).

Table 5. Spearman correlations between intake type and hydration status in children (10-13y) and adolescents (18-20y)

Beverage or food intake	**Overall**		**10-13y (n = 18)**		**18-20y (n = 34)**	
	r_s	P value	r_s	P value	r_s	P value
Beverages						
Water	**-0.34**	**0.013**	0.01	0.984	**-0.43**	**0.012**
Fruit juice	**-0.35**	**0.012**	**-0.72**	**0.001**	-0.17	0.333
Milk	0.09	0.538	-0.03	0.900	-0.02	0.910
Sugar sweetened beverages	0.06	0.659	0.14	0.582	-0.02	0.905
Tea and coffee	-0.13	0.352	-0.37	0.130	0.06	0.722
Total liquid from beverages	**-0.32**	**0.021**	-0.16	0.523	**-0.33**	**0.057**
Foods						
Other dairy products	-0.05	0.747	0.11	0.660	-0.06	0.759
Fruit	0.01	0.935	-0.07	0.782	-0.14	0.430
Vegetables	**-0.29**	**0.036**	-0.19	0.455	-0.23	0.199
Grains	0.03	0.839	-0.16	0.526	0.05	0.776
Protein	-0.20	0.163	-0.19	0.463	-0.15	0.410
Oil and fats	-0.21	0.133	-0.12	0.642	-0.24	0.164
Sauces and condiments	-0.09	0.513	-0.26	0.293	0.11	0.524
Candy and dessert	0.11	0.452	0.22	0.383	-0.13	0.453
Nuts and seeds	-0.12	0.384	0.00	0.988	-0.15	0.397
Salty snacks	-0.10	0.483	-0.29	0.260	-0.06	0.733
Total water from all sources	**-0.41**	**0.003**	-0.08	0.760	**-0.43**	**0.012**

Table 6 shows the binary logistic regressions between intake type and hydration status in the sample. Hydration status was categorized as "euhydrated" and "dehydrated," while all beverages and foods remained continuous. Due to the small sample size, all analyses were conducted for the overall sample only. In the unadjusted model, intake of fruit juice was significantly associated with a higher odds of being euhydrated (OR 1.009, 95% CI: 1.001,1002). After adjusting for age and sex, the intake of water (OR 1.001; 95% CI:

1.000, 1.002), fruit juice (1.011, 95% CI: 0.018), all beverages (OR 1.001, 95% CI: 1.000, 1.002), and total water intake from all sources (OR 1.001, 95% CI: 1.000, 1002) was significantly associated with higher odds of being euhydrated.

Table 6. Binary logistic regression between intake type and hydration status in children (10-13y) and adolescents (18-20y)

Food group intake (servings)	Unadjusted			Adjusted[2]		
	OR[1]	95% CI	P-value*	OR[1]	95% CI	P-value*
Beverages						
Water	1.001	1.000-1.002	0.101	**1.001**	**1.000-1.002**	**0.047**
Fruit juice	**1.009**	**1.001-1.018**	**0.037**	**1.011**	1.002-1.020	**0.018**
Milk	0.999	0.997-1.002	0.652	1.001	0.998-1.003	0.729
Sugar sweetened beverages	0.999	0.993-1.005	0.692	1.000	0.994-1.007	0.898
Tea and coffee	1.000	0.994-1.006	0.971	0.999	0.993-1.006	0.801
All beverages	1.001	1.000-1.002	0.099	**1.001**	**1.000-1.002**	**0.032**
Foods						
Other dairy products	1.064	0.841-1.345	0.606	1.066	0.837-1.358	0.603
Fruit	1.095	0.472-2.544	0.832	0.963	0.386-2.402	0.936
Vegetables	1.189	0.891-1.587	0.238	1.190	0.865-1.636	0.286
Grains	1.064	0.871-1.300	0.545	1.139	0.914-1.421	0.247
Protein	1.092	0.934-1.275	0.269	1.124	0.951-1.329	0.172
Oil and fats	1.060	0.915-1.227	0.439	1.067	0.926-1.231	0.370
Sauces and condiments	0.882	0.682-1.140	0.338	0.875	0.686-1.116	0.281
Candy and dessert	1.059	0.445-2.521	0.897	1.278	0.444-3.678	0.650
Nuts and seeds	0.876	0.523-1.468	0.615	0.855	0.494-1.480	0.576
Salty snacks	0.964	0.460-2.020	0.923	1.080	0.500-2.335	0.844
Total water from all sources	1.001	1.000-1.001	0.132	**1.001**	**1.000-1.002**	**0.049**

[1]Reference is Euhydrated. [2]Adjusted for age and sex*Statistically significant values (p<0.05) are in bold.

Discussion

To our knowledge, this is the first study that examined which type of beverages and foods are associated with USG, an objective marker of hydration status, in US children and adolescents. It was found that intake of fruit juice ($r_s = -0.35$; $p<0.05$), water

(r_s = -0.34; $p<0.05$), total liquid from all beverages (r_s = -0.32; $p<0.05$), vegetables (r_s = -0.29; $p<0.05$), and total water from all sources (r_s = -0.43; $p<0.05$) was significantly and inversely correlated with USG scores in the overall sample. The logistic regression showed that intake of water (OR 1.001; 95% CI: 1.000, 1.002), fruit juice (1.011, 95% CI: 1.002, 1.020), all beverages (OR 1.001, 95% CI: 1.000, 1.002), and total water intake from all sources (OR 1.001, 95% CI: 1.000, 1002) was significantly associated with a higher odd of being euhydrated.

There are only a few published studies assessing these associations in children. A study in Portugal with 831 children ages 3-17 years found no significant associations between hydration and intake of foods or beverages, although this study did not measure hydration using USG.[17] Another study using data from NHANES 2009-2012 with 4,134 children and adolescents ages 6-19 years, found a significant association between hydration status and water intake but not with intake of milk, 100% juice, diet beverages, unsweetened tea, or moisture from foods.[7] However, this study only reported one 24-hour recall prior to the day of urine collection to assess beverage intake, and one urine void was used to assess hydration. [7] The study failed to collect a 24-hour urine collection to best assess daily hydration. Another study conducted with 548 children ages 9-11 years from Los Angeles and New York City found a significant association between elevated urine osmolality and beverages, except water.[18] This is somewhat similar to our study, although the researchers did not collect a 24-hour urine or use multiple 24-hour dietary recalls.[18] Another study conducted with 210 children ages 8-14 years from Greece observed an inverse association with milk and water intake added together in the model with hydration ($p<0.01$); this study assessed hydration from 24-hour urine osmolality and fluid intake from a two-day fluid diary.[16] Also, a study conducted in 1,286 German children ages 4-10 years found that fruits and vegetables ($p<0.01$) and fruit and vegetable juices were significant predictors of hydration as measured by FWR ($p<0.01$),[19] which is somewhat similar to the present study, as intake of fruit juice and vegetables were correlated with hydration status in our study. Another study conducted in China with 68 college students ages 18-25 years found a similar association between daily fluid intake ($r = -0.56$, $p<0.05$), daily fluid intake from foods ($r = -0.47$, $p<0.05$), and daily total drinking fluids ($r= -0.54$, $p<0.05$) and USG.[20] Therefore, our results are consistent with most of the studies cited in the literature, in which intake of several types of beverages and overall water from all sources, foods and beverages, are associated with hydration status.

In general, we found that 86.5% of the sample were considered dehydrated based on US Dietary Reference Intakes (DRI), although average overall score for USG was 1.018 ± 0.005 uG, which is categorized as euhydrated. These findings are similar to a study conducted with 210 Greek children ages 8-14 years, in which the average USG score was 1.018 ± 0.006 uG.[16] Another study conducted with 29 males and females ages 16-24 years old in the US reported an average overall score of 1.013 ± 0.006 in non-Hispanic white and 1.020 ± 0.007 in non-Hispanic blacks, with 29.7% overall dehydration.[21] Also, in our sample, 100% were below the DRIs for total water intake from all sources, as those ages 10-14 years had an average consumption of only 897 mL/d and those ages 18-20 years had an average consumption of 1460 mL/d, which is similar to the data from NHANES.[5]

The strength of the present study is the utilization of a 24-hour urine sample to assess hydration and the use of three 24-hour dietary recalls to assess intake of beverages and foods. Among the limitations is the small convenience sample size of children and adolescent from south Florida, which is predominately Hispanic. Data from this study may not be generalizable to others from differing geographical, cultural, racial, and ethnic backgrounds. A larger sample size may have provided further insight to other beverage types and foods that may not have been explored.

In conclusion, our findings showed that the main predictors of hydration were water and fruit juice intake in children and water intake in adolescents. Overall, based on USG, 48.1% of the sample was dehydrated while by total fluid intake, 86.5% of the participants fail to meet the recommended total fluid intake. Future research should explore the differences in dietary patterns in a larger, more generalizable sample and discover other potential predictors that may be associated with hydration status when using USG.

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CHAPTER III: THE ASSOCIATION BETWEEN HYDRATION STATUS AND BODY COMPOSITION IN HEALTHY CHILDREN AND ADOLESCENTS.

Introduction

Childhood obesity continues to be a major public health concern in the US, with 19.1% of boys and 17.8% of girls ages 2-19 years in the US being obese.[1–3] As rates increase, children are more susceptible to conditions, such as diabetes mellitus, hypertension, coronary artery disease, cancer, and sleep apnea.[1,2] Emerging evidence also shows an increased prevalence of children and adolescents with suboptimal hydration across the globe.[4] Evidence from the National Health and Examination Survey (NHANES) of 7,453 children ages 4-18 years and 3,248 adolescents ages 19-30 years in the US was examined. Average water intake was 577 mL/d in those ages 9-13 years, 866 mL/d in those ages 14-18 years, and 1,305 mL/d in those ages 19-30 years.[5] These levels are below the US Dietary Reference Intakes (DRI) of 2,100 mL/d for girls and 2,400 mL/d for boys ages 9-13[6] years, 2,300 mL/d for girls and 3,300 mL/d for boys ages 14-18 years, and 2,700 mL/d for women and 3,700 mL/d for men ages 19-30 years.[5,6] Furthermore, the study using data from NHANES showed that 60% did not meet DRIs for total water intake.[5]

Studies have shown an association between hydration and weight status,[7–12] mainly in adults using various markers to assess hydration status, such as urine osmolality, urine specific gravity, total body water (TBW), free water reserve (FWR), urine color, and thirst perception. A study using data from NHANES 2009-2012 of adults showed that those inadequately hydrated, which was measured by urine osmolality, had significantly higher BMI ($P<0.001$) and had higher odds of being obese (OR=1.59; 95%

CI, 1.35-1.88, P<0.001) compared to those adequately hydrated.[13] Similar results were found in 1,500 Portuguese adults, with the greatest odds of obesity (OR = 1.97, 95% CI, 1.06-3.66) in men in the highest osmolality tertile (≥ 602.1 mOsm/kg) compared to the lowest tertile.[14] For children and adolescents, less evidence is available associating an objective marker of hydration with weight or obesity. A study among 86 obese and 89 normal weight Italian children ages 7-11 years found that hypohydration, assessed using free water reserve (FWR), was significantly higher in obese children (34%) compared to children with healthy weights (20%).[15] Another study of 371 Belgian children ages 7-13 years reported that BMI was a significant predictor of total fluid intake, assessed using urine osmolality, ($\beta = 0.110$, $p = 0.038$), although no significant association was found between fluid intake and hydration status measured using urine osmolality.[16,17] Another study among 358 Spanish adolescents ages 18-39 years found an inverse association between water intake, assessed from questionnaires, and BMI ($r = -0.18$, $p<0.01$) and body fat percentage (%) ($r = -0.14$, $p<0.05$).[17] Although some studies associated hydration status assessed from questionnaires with weight status, to our knowledge, only two studies assessed hydration using an objective marker.[17–19] In addition, the studies did not evaluate body mass using Dual-energy X-ray absorptiometry (DEXA) which is considered the gold standard for measuring body composition.[20] Most of the studies evaluating the association between hydration and body composition were done primarily in European children and adolescents.

To date, there are no studies that have explored the relationship between hydration status via an objective marker and body composition using DEXA scan in children and adolescents in the US. Therefore, this study aimed to examine the association between hydration status (measured from USG in a 24-h urine sample and assessed from three 24-h dietary recalls) and body fat % and fat-free mass (FFM) (assessed from a DEXA scan) in children and adolescents.

Methods

Study Design

This was a cross-sectional analysis to evaluate the association between hydration status (measured from USG in a 24-h urine sample and assessed from three 24-h dietary recalls) and body-fat % and FFM using DEXA in a sample of children (ages 10-13 years) and adolescents (ages 18-20 years).

Study Population

The data for children (ages 10-13 years) were taken from the baseline visit of the MetA-Bone Trial, a trial to determine the effects of soluble corn fiber (SCF) supplementation for one year on bone metabolism in healthy children.[21] Inclusion criteria for children of the MetA-Bone Trial were age 10-13 years, had low calcium intake (2 or fewer servings of dairy products/day), and had adequate vitamin D levels. Children were excluded if they had chronic illnesses requiring medication use or if using regular calcium (>200 mg/d) or vitamin D supplements (> 400 IU/d). Children were recruited throughout Miami-Dade schools, clinics, after-school programs, emails, online, among other strategies in South Florida. Parents interested completed a short pre-screening questionnaire to verify the eligibility of their child and if eligible, parents were asked to sign the consent form, and children were asked to sign the assent form. The use of this data was approved by the Institutional Review Board of Florida International University (IRB-21-0429).

The data for adolescents (ages 18-20 years) was collected directly from college students at Florida International University. Any adolescents with the presence of chronic illness requiring medication use were excluded. Adolescents were recruited using flyers distributed in classes or by email through faculty and staff. Those interested were explained the study and were asked a brief pre-screening questionnaire with the inclusion criteria. If eligible, participants were asked to sign the consent form. This study was approved by the Institutional Review Board of Florida International University (IRB-22-0045).

Measurements

General questionnaire. Participants were administered a questionnaire to determine their age, sex, race, and ethnicity.

Anthropometric measurements. Weight and height were obtained by the participant wearing light clothing, utilizing a standardized scale and a wall-mounted stadiometer. Both weight and height were measured to the nearest 0.1 kg and 0.1 cm, respectively.[22] Height was measured with the participant's back and heels touching the vertical board of the stadiometer. The moveable headboard was adjusted based on the participant's height and brought to the most superior point with sufficient pressure to compress the hair.[22] BMI percentiles for sex and age were calculated using the CDC standardized growth charts.[23]

Physical activity. Participants were asked to complete a short version of the International Physical Activity Questionnaire (IPAQ) to assess physical activity based on the intensity of the activities during the last seven days (e.g., moderate versus vigorous).[24]

24-h dietary recalls. Participants were asked to complete three 24-hour dietary recalls; the first one was completed in-person, and the two others were completed in the next few days to represent 2 weekdays and 1 weekend day. The participant's food description was reviewed with the participant to ensure completeness and correctness. The 24-hour dietary recalls were analyzed using the dietary computer-based analysis software application 'Nutrition Data System for Research (NDSR)' developed at the University of Minnesota Nutrition Coordinating Center (NCC). Data for total water intake from all sources (all beverages and foods) was used in the analyses.

Body fat % and FFM. This was measured through a whole-body scan using the Hologic-Dual-Energy-X-Ray Absorptiometry (DEXA). Participants were asked to lie down for 10 minutes in the DEXA scan bed while wearing no shoes and light clothing. This was conducted by a trained and certified personnel. The analysis provided data for total fat mass in grams, %, and fat mass index (fat mass/height^2), and lean mass in grams.[25] Body fat % was used to calculate fat-free mass (FFM) with the following equation: FFM = weight [kg] * (1 - (body fat %/ 100)).[26] Binary variables were created for these measures and was coded 0 as "below the median" and 1 as "above the median."

Objective marker of hydration status**.** Participants were asked to collect urine for 24-h to assess hydration status using Urine Specific Gravity (USG). For this, participants were provided with a urine hat to initially collect the urine and then transfer it to sterile urine containers. Participants were instructed to store the urine in the refrigerator throughout their collection until they were brought back to the lab. The first sample was collected at the study visit and the rest after the study visit. The total volume and USG were measured in the laboratory at FIU. For total volume, the urine from the different containers was combined in a volumetric cylinder. The sample of 0.3 ml was transferred into the ATAGO PAL 10S digital pocket refractometer that utilizes a refractive index method to measure USG. The urine sample was placed on the refractometer's prism and analyzed to reveal the measurement value within 3 seconds. Removal of the sample was cleaned using a Kim wipe along with the removal of excess moisture on the prism before the next sample. Zero testing was performed after each participant's testing by placing three drops of deionized water with a plastic dropper on the prism service. The refractometer has a urinary USG range of 1.000 to 1.060 with a resolution of 0.001.[27] USG refractometry score results are based on the number, mass, and chemical structure of the dissolved particles in the urine, so the higher the number, the higher the concentration. Testing was conducted three times for each participant to verify stable USG values. If results varied, an additional measurement was made to provide consistent results. The average of the three measurements was used as the final level. Participants with USG values < 1.020 μG were considered euhydrated, and those with USG values > 1.020 μG were dehydrated.[28–30] USG values above > 1.030 μG indicated participants were severely dehydrated.[30]

Statistical Analysis

For descriptive statistics, categorical variables were presented as frequencies and percentages, while continuous variables were presented as mean ± standard deviation. Two-sample t-tests were used to compare mean values between age groups and chi-square and Fisher's exact tests were used to compare differences in proportions between groups. To examine the association between hydration status (assessed from USG in a 24-h urine sample) and body fat %, FFM, and lean mass in the sample (assessed from DEXA), a series of linear and logistic regressions were used, adjusting for age, sex, and physical activity. To assess the overall fit of the regression equation and/or model to the observed data, fit indices (e.g. R^2) were examined. Normality was tested using the Shapiro-Wilk test. For non-normally distributed variables, log-transformations were used to test linear association. Regression coefficients were evaluated to further examine the individual contribution of each predictor variable to the equation and/or model. Medians or respective distributions were used for logistic regression to categorize body fat%, fat-free mass outcome measures as below or above the median. Linear regression was also used to explore the association between total water intake from all sources assessed from 24-h food recalls and body fat%, FFM, and lean mass (assessed from DEXA). All analyses were performed using SPSS software Version 26.0 and significance was evaluated at an alpha level of 0.05.

Results

A total of 52 participants were included in the study consisting of 18 children and 34 adolescents. Table 1 shows the characteristics of these groups; sex distribution was similar between age groups, and most were Hispanic or Latino (73.1%), which was similar between age groups. There was a significant difference in the race distribution by age group. The overall group was classified as normal weight based on their BMI percentile with no difference by age group. Total water intake from all sources, physical activity, lean mass, and fat-free mass were significantly higher in adolescents (18-20 years) compared to children (10-13 years; $p<0.05$).

Table 1. Characteristics of the study population (N=52)

Variable	**Overall (n=52)**	**10-13 (n=18)**	**18-20 (n=34)**	**P-Value**
		Mean ± SD or N (%)		
Age, y	17.0 ± 3.72	12.0 ± 1.37	19.1 ± 0.64	**0.000***

Sex				
Female	26 (50.0)	8 (44.4)	18 (52.9)	0.560
Male	26 (50.0)	10 (55.6)	16 (47.1)	
Race				
White	30 (57.7)	2 (11.1)	28 (82.4)	**0.000***
African American	4 (7.7)	3 (16.7)	1 (2.9)	
Asian	2 (3.8)		2 (5.9)	
Native Hawaiian or other Pacific Islander	2 (3.8)		2 (5.9)	
Other	14 (26.9)	13 (72.2)	1 (2.9)	
Hispanic or Latino	38 (73.1)	13 (72.2)	25 (73.5)	0.919
IPAQ score (MET)	3639 ± 3714	1729 ± 1328	4540 ± 4194	**0.001***
BMI percentile	64.0 ± 24.8	58.0 ± 27.8	67.0 ± 24.7	0.278
Body fat %	32.2 ± 7.31	30.1 ± 7.91	31.8 ± 7.02	0.423
Lean mass, kg	38.7 ± 10.8	29.1 ± 9.28	43.8 ± 7.68	**0.000***
FFM, kg	41.7 ± 11.10	30.9 ± 6.92	47.5 ± 8.16	**0.000***
Total water intake	1831± 841.4	1307 ± 543.8	2109 ± 843.9	**0.001**
USG (μG)	1.018 ± 0.005	1.020 ± 0.006	1.017 ± 0.005	0.072

**Significant difference between age groups at p<0.05 by independent sample t-test or Chi-square. Abbreviations: IPAQ, International Physical Activity Questionnaire, USG, urine specific gravity, FFM, fat-free mass.*

Table 2 shows the linear regression between body composition with total water intake from all sources (assessed from 24-h food recalls). After adjusting for age, sex, and physical activity, there was a significant negative association between total water intake from all sources and body fat % for the overall group (B = -12.0, p<0.05). However, for FFM, there was a significant positive association with total water intake from all sources for the overall group after adjusting for age, sex, and physical activity (B = 18.2, p<0.05) and in children (B = 21.5, p<0.05) after adjusting for sex and physical activity. For lean mass, a similar result was found, whereas a significant positive association was found with total water intake from all sources in children (B = 28.0, $p<0.05$) after adjusting for covariates.

Table 3 displays the linear regressions between body composition with hydration status (assessed from USG in 24-h urine samples). After adjusting age, sex, and physical activity, no significant association was found between body fat %, FFM, or lean mass and hydration status, overall or by age groups.

Table 4 displays the logistic regression between hydration status or total water intake from all sources with body composition. Hydration status was categorized as "euhydrated" and "dehydrated", while total water intake from all sources, body fat %, FFM, and lean mass were categorized as "below the median" or "above the median." The analyses were done only for the overall sample, due to the small sample size. After adjusting for age, sex, and physical activity, no significant association was found between body fat % and hydration status or total water intake from all sources. Those with lower total water intake from all sources had 3.57 times higher odds of being below the median for FFM and lean mass than those with higher total water intake from all sources (OR 3.57, 95% CI: 1.14, 11.9). After adjusting for age, sex, and physical activity, lower total water intake from all sources was 4.40 times higher odds of being below the median for FFM and lean mass (OR 4.40, 95% CI: 1.05, 18.5).

Table 2. Linear regression between total water intake (assessed from 24-h food recalls) and body composition (assessed from DEXA scan) in children (10-13y) and adolescents (18-20y).

	Simple linear regression									Adjusted linear regression[1]								
	Overall			Children (10-13 years) (n = 18)			Adolescents (18-20 years) (n = 34)			Overall			Children (10-13 years) (n = 18)			Adolescents (18-20 years) (n = 34)		
	B	SE	P-value	B	SE	P-value	B	SE	P-value	B	SE	P-value	B	SE	P-value	B	SE	P-value
Body fat %	-10.2	5.22	0.057	-20.0	10.3	0.072	-16.0	7.33	**0.037**	-12.0	5.40	**0.035**	-13.4	9.09	0.161	-10.1	6.76	0.144
FFM	36.5	6.39	**0.000**	18.2	8.53	**0.041**	23.0	8.26	**0.015**	18.2	8.55	**0.041**	21.5	8.98	**0.031**	6.00	5.87	0.348
Lean mass	40.0	6.28	**0.000**	28.4	11.4	**0.024**	17.1	8.03	**0.042**	11.4	5.81	0.056	28.0	12.4	**0.042**	5.24	5.47	0.346

[1]Adjusted for age, sex, and physical activity overall and sex and physical activity among age groups;*Statistically significant values ($p<0.05$) are bold.

Table 3. Linear regression between hydration status (assessed from USG in 24-h urine samples) and body composition (assessed from DEXA scan) in children (10-13y) and adolescents (18-20y).

	Simple linear regression									Adjusted linear regression[1]								
	Overall			Children (10-13 years) (n = 18)			Adolescents (18-20 years) (n = 34)			Overall			Children (10-13 years) (n = 18)			Adolescents (18-20 years) (n = 34)		
	B	SE	P-value	B	SE	P-value	B	SE	P-value	B	SE	P-value	B	SE	P-value	B	SE	P-value
Body fat %	-1.56	1.80	0.390	-1.67	3.06	0.593	-0.95	2.43	0.698	0.54	1.61	0.738	0.50	2.64	0.853	0.63	2.08	0.763
FFM	-4.38	2.68	0.109	-2.96	2.60	0.272	0.78	2.83	0.784	-1.78	1.63	0.280	-3.76	2.70	0.185	-1.57	1.75	0.378
Lean mass	-4.20	2.61	0.114	-4.61	2.52	0.346	-1.57	2.66	0.801	-2.06	1.70	0.230	-2.06	3.72	0.236	-1.57	1.63	0.343

[1]Adjusted for age, sex, and physical activity overall and sex and physical activity among age groups. [2]Variable was rescaled by dividing by 100.
*Statistically significant values ($p<0.05$) are bold.

Table 4. Logistic regression between hydration categories and total water intake categories and body composition categories in the sample

Body composition[2]	Overall			
	OR (95% CI)	p-value	Adjusted OR[1] (95% CI)	p-value
Body fat % and hydration status				
Euhydrated (USG < 1.020 μG)	1		1	
Dehydrated (USG ≥ 1.020 μG)	1.59 (0.53, 4.76)	0.406	1.08 (0.28, 4.12)	0.912
FFM and hydration status				
Euhydrated (USG < 1.020 μG)	1		1	
Dehydrated (USG ≥ 1.020 μG)	1.17 (0.39, 3.47)	0.781	5.68 (0.97, 33.3)	0.055
Lean mass and hydration status				
Euhydrated (USG < 1.020 μG)	1		1	
Dehydrated (USG ≥ 1.020 μG)	1.17 (0.39, 3.47)	0.781	5.68 (0.97, 33.3)	0.055
Body fat % and total water intake				
Above the median	1	0.099	1	0.230
Below the median	0.39 (0.13, 1.19)		0.46 (0.13, 1.64)	
FFM and total water intake				
Above the median	1	**0.029**	1	**0.043**
Below the median	3.57 (1.14, 11.2)		4.40 (1.05, 18.5)	
Lean mass and total water intake				
Above the median	1		1	
Below the median	3.57 (1.14, 11.9)	**0.029**	4.40 (1.05, 18.5)	**0.043**

[1]Logistic regressions were adjusted for age, sex, and physical activity. **[2]**Reference category is above the median.
*Statistically significant values ($p<0.05$) are in bold.

Discussion

To our knowledge, this is the first study to examine the association between hydration status (measured from USG in a 24-h urine sample and assessed from three 24-h dietary recalls) and body composition in a sample of children and adolescents in south Florida. Total water intake from all sources was negatively associated with body fat % (B = -12.0, $p<0.05$) while it was positively associated with FFM (B = 18.2, $p<0.05$) overall, after adjusting for sex and physical activity. In children, total water intake from all sources was positively associated with both FFM (B = 21.5, $p<0.05$) and lean mass (B = 28.0, $p<0.05$) after adjusting for covariates. In addition, those with a lower total water intake from all sources had 4.40 times higher odds of lower FFM and lean mass than those with higher total water intake from all sources (OR 4.40, 95% CI: 1.05, 18.5) in the overall group. However, USG or hydration status was not associated with body fat %, FFM, or lean mass in the linear or in the logistic regression models.

There are only a few studies that have found an association between body composition and an objective marker of hydration status in children and adolescents. A study in Poland with 264 children ages 7-15 years showed an increase in the odds of dehydration due to excess body fat % measured by urine osmolality (OR 2.39, 95% CI 1.15, 4.94), although this study used a Tanita analyzer to assess body composition and a 24-hour urine collection was not used.[31] Another study conducted in the United Kingdom with 936 children and adolescents ages 4-22 years showed a significant association between hydration (measured by total body water; TBW) and FFM ($\beta = 0.59$, $p<0.01$).[32] The study in Italy with 175 children ages 7-11 years found similar results with BMI and hydration status using the free water reserve (FWR) equation and two 24-hour urine collections ($p<0.05$), although did not use DEXA for analysis of body composition.[15]

Similar to the present study, other studies have found significant associations between total water intake from all sources assessed from questionnaires with body composition. For example, a study in Spain with 372 children and adolescents ages 12-18 years found a significant inverse association between water intake assessed from the hydration status questionnaire (HSQ) and body fat % ($\beta = -0.284$, $p<0.01$).[18,33] Another study conducted in Spain with 366 children ages 9-11 years found a positive association between water intake assessed from 24-h dietary recalls with BMI ($p<0.001$), body fat ($p<0.01$), and fat-free mass ($p<0.01$), assessed via bioimpedance analysis.[19] However, neither study assessed body composition using DEXA and they were conducted primarily in European children and adolescents.

There are several mechanisms that may explain how hydration status is associated with weight or obesity. One mechanism is in relation to the anti-diuretic hormone (ADH) that is released as a result to an increase of plasma osmolality during dehydration, in which ADH is responsible for stimulating thirst to help regulate body fluid. During increased plasma osmolality (insufficient water intake), the precursor of ADH, known as serum copeptin, is elevated and results in an increase in fat accumulation. Another mechanism is the process of lipolysis that occurs in all triglyceride storing tissues, although adipose tissue is known to reflect a majority of lipolytic activity. During hyperosmolality (insufficient water intake), adipocytes become dehydrated, resulting in excess triglycerides due to an inability to metabolize free fatty acids in the mitochondria. Therefore, a relationship between hydration and weight status may be evident.

The strength of the present study is the utilization of a DEXA scan to analyze body composition, the use of an objective marker of hydration, and three 24-hour dietary recalls to assess total water intake from all sources. Furthermore, the use of accurate measures such as FFM provides a more detailed indicator of obesity in children.[26] Body fat % has been commonly used in practice, however, cannot be evaluated independently from FFM due to its dependence on height.[34] Body fat indices such as FFMI and FMI have been used to evaluate changes in body composition in FFM in recent studies, although no significant association or trend was shown in the present study between FMI and FFMI and hydration status (not shown) when tested.[34]Among the limitations is the small convenience sample size of children and adolescents from south Florida. A larger sample may be needed to find significant associations between body fat % or FFM and hydration status with USG. USG was chosen as the objective marker for the present study instead of urine osmolality, urine color, FWR, and TBW due to its ability to adapt to pediatric populations and use without technical expertise, suitable to studies with a larger sample size and limited resources.[2] However, other measures may impact the association with USG as protein, glucose, ketones, bilirubin, and urobilinogen may alter the results if present in the urine. [39] During proteinuria, each 10g/1 protein may increase USG by 0.003 when measured by refractometry. Similar is shown for every 10 g/1 of glucose may increase USG by approximately 0.002. [39] Several markers should be used together to explore these associations in this population to account for limitations in objective measures.

In conclusion, our findings showed that total water intake from all sources was significantly associated with FFM and lean mass in both the linear and logistic regression models, in which may provide insight to the importance of children and adolescent's water intake to have a greater FFM and lean mass. Future research should be conducted to explore other objective markers of hydration that may serve as a better predictor to body fat % along with a larger sample to explore the associations between hydration and body composition in this population

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CHAPTER IV: EXPLORING THE ASSOCIATION BETWEEN HYDRATION STATUS AND BONE MASS IN HEALTHY CHILDREN AND ADOLESCENTS.

Introduction

Low bone mass is highly prevalent in the US with about 32-46% of adults having low bone mass.[1] Low bone mass in children and adolescents has attracted much attention in recent years as low bone mass in older adults starts at puberty, considering the large proportion of bone mass is acquired during this stage[2]. The peak of bone mineral accretion is at approximately ages 12.5 ± 0.90 years in girls and 14.1 ± 0.95 years in boys and spans for 3- to 4-years and during this period, 40% of the total lifetime bone mass is achieved.[2] Peak bone mineral accretion is determined by several factors, including diet along with other lifestyle behaviors, in childhood and adolescence.[2] Therefore, dietary patterns in this period will impact the prevalence of low bone mass in adulthood.

Several studies have determined the impact of diet on bone. It is known that consumption of fruits and vegetables, fish, dietary fiber, and dairy products leads to a higher bone mass while the intake of processed, refined, or high energy dense foods results in low bone mass.[3–8] However, there is little evidence exploring fluid intake and hydration status on bone. This could impact bone mass as water contributes to approximately 20% of the bone's wet weight being the third major component of bone.[9] Recent literature has shown when water is mechanically removed from bone, it significantly affects the strength, toughness, and stiffness of bone, although there are no *in vivo* studies providing physiological evidence on whether hydration directly affects bone mass in children or adults.[10,11] In humans, there are two studies within the literature that have explored the associations between hydration status and bone mass in adults. The first study was conducted in older adults and showed that those that were adequately hydrated, which was measured by total body water (TBW), had a lower risk of osteoporosis (OR = 0.76, 95% CI, 0.66-0.88, P < 0.0001).[12] The second study tested the effects of fluid and salt supplementation during hypokinesia in 30 healthy male students.[13] This study found that a higher bone mineralization when supplemented with fluids and salt than without the supplementation. They also found a significant correlation between bone electrolyte density and bone mineral density (measured by dual-energy X-ray absorptiometry (DEXA) scan) (r = 0.93, p < 0.05). To our knowledge, there are no reported studies that have evaluated total fluid intake via questionnaires and bone mass in children and adolescents.

Therefore, the present study aimed to explore the potential association between hydration status, assessed using an objective marker of hydration (urine specific gravity, USG) from a 24-hour urine collection) and from total water intake from all sources from 3 24-h dietary recalls, and bone mass, measured using a DEXA scan, in a sample of children and adolescents.

Methods

Study Design

This was a cross-sectional analysis to evaluate the association between hydration status assessed from 24-hour urine collection using USG and bone mineral density and bone mineral content measured from a DEXA scan in a sample of children (ages 10-13 years) and adolescents (ages 18-20 years). It also assessed the association between total water intake from all sources assessed from 24-h recalls with bone mass.

Study Population

The data for children (ages 10-13 years) were from the baseline visit of the MetA-Bone Trial, a trial to determine the effects of soluble corn fiber (SCF) supplementation for one year on bone metabolism in healthy children.[14] Inclusion criteria for children of the MetA-Bone Trial were ages 10-13 years, with low calcium intake (2 or fewer servings of dairy products/day), and adequate vitamin D levels. Children were excluded if they had chronic illnesses requiring medication use and regular use of calcium (>200 mg/d) or vitamin D supplements (> 400 IU/d). Children were recruited throughout Miami-Dade Schools, clinics, after-school programs, emails, online, among other strategies in South Florida. Interested parents completed a short pre-screening questionnaire to verify the eligibility of their child and if eligible, parents were asked to sign the consent form, and children were asked to sign the assent form. The use of the protocols was approved by the Institutional Review Board at Florida International University (IRB-21-0429).

The data for adolescents (ages 18-20 years) was collected directly from college students at Florida International University. Any adolescents with the presence of chronic illness requiring medication use were excluded. Adolescents were recruited using flyers distributed in classes or by email through faculty and staff. Those interested were explained the study and were asked to answer a brief pre-screening questionnaire with the inclusion criteria. If eligible, participants were asked to sign the consent form. This study was approved by the Institutional Review Board of Florida International University (IRB-22-0045).

Measurements

General questionnaire. Participants were administered a questionnaire to record participant's age, sex, race, and ethnicity.

Anthropometric measurements. Weight and height were obtained from the participants wearing light clothing, utilizing a standardized scale and a wall-mounted stadiometer. Both weight and height were measured to the nearest 0.1 kg and 0.1 cm, respectively.[15] Height was measured with the participant's back and heels touching the vertical board of the stadiometer. The moveable headboard was adjusted based on the participant's height and brought to the most superior point with sufficient pressure to compress the hair.[15] BMI percentiles for sex and age were calculated using the CDC standardized growth charts.[16]

Physical activity. Participants were asked to complete a short version of the International Physical Activity Questionnaire (IPAQ) to assess physical activity based on the intensity of the activities during the previous seven days (e.g., moderate versus vigorous) as part of the study visit.[17]

24-h dietary recalls. Participants were asked to complete three 24-hour dietary recalls; the first one was completed in-person, and the two others were completed in the next few days to represent 2 weekdays and 1 weekend day. The participant's food description was reviewed with the participant to ensure completeness and correctness. The 24-hour dietary recalls were analyzed using the dietary computer-based analysis software application 'Nutrition Data System for Research (NDSR)' developed at the University of Minnesota Nutrition Coordinating Center (NCC). Data for total water intake from all sources (all beverages and foods) was used in the analyses.

Bone mass. This was measured through a whole-body scan using the Hologic-Dual-Energy-X-Ray Absorptiometry (DEXA). Participants were asked to lie down for 10 minutes for the DEXA scan while wearing no shoes and light clothing. DEXA was operated by a trained and certified personnel. The analysis provided data for total bone mineral content (BMC) in grams and bone mineral density (BMD) in grams/cm^2.[18] Binary variables were created for these measures and was coded 0 as "below the median" and 1 as "above the median."

Objective marker of hydration status**.** Participants were asked to collect urine for 24-h to assess hydration status using Urine Specific Gravity (USG). For this, participants were provided with a urine hat to initially collect the urine and then transfer it to the urine containers. Participants were instructed to store the urine in the refrigerator throughout their collection until they were brought back to the lab. The first sample was collected at the study visit and the rest after the study visit. The total volume and USG were measured in the laboratory at Florida International University. For total volume, the urine from the different containers was combined in a volumetric cylinder. The sample of 0.3 ml was transferred into the ATAGO PAL 10S digital pocket refractometer that utilizes a refractive index method to measure USG. The urine sample was placed on the refractometer's prism and analyzed to reveal the measurement value within 3 seconds. Removal of the sample was cleaned using a Kim wipe along with the removal of excess moisture on the prism before the next sample. Zero testing was performed after each participant's testing by placing three drops of deionized water with a plastic dropper on the prism service. The refractometer has a urinary USG range of 1.000 to 1.060 with a resolution of 0.001.[19] USG refractometry score results are based on the number, mass, and chemical structure of the dissolved particles in the urine, so the higher the number, the higher the concentration. Testing was conducted three times for each participant to verify stable USG values. If results varied, an additional measurement was made to provide consistent results. The average of the three measurements was used as the final level. Participants with USG values < 1.020 μG were considered euhydrated, and those with USG values > 1.020 μG were dehydrated.[20–22] USG values above > 1.030 μG indicated participants were severely dehydrated.[22]

Statistical Analysis

For descriptive statistics, categorical variables were presented as frequencies and percentages, while continuous variables were presented as mean ± standard deviation. Two-sample t-tests were used to compare mean values between age groups and chi-square and Fisher's exact tests were used to compare differences in proportions between groups. To examine the association between hydration status (assessed from USG in a 24-h urine sample) and bone mass and bone mineral content in the sample, a series of linear and logistic regressions were used, adjusting for age, sex, and physical activity. To assess the overall fit of the regression equation and or/model to the observed data, fit indices (e.g. R^2) were examined. Normality was tested using the Shapiro-Wilk test. For non-normally distributed variables, log-transformations were used to test linear association. Regression coefficients were evaluated to further examine the individual contribution of each predictor variable to the equation and/or model. Medians or respective distributions were used for logistic regression to categorize bone parameters as below or above the median. Linear regression was also used to explore the association between total water intake from all sources assessed from 24-h food recalls and bone parameters (assessed from DEXA). All analyses were performed using SPSS software Version 26.0 and significance was evaluated at an alpha level of 0.05.

Results

A total of 52 participants were included in the study consisting of 18 children and 34 adolescents. Table 1 shows the characteristics of these groups; sex distribution was similar between age groups, and most were Hispanic or Latino (73.1%), which was similar between age groups. The overall group was classified as normal weight based on their BMI percentile with no difference by age group. Total water intake from all sources, physical activity, BMD and BMC were significantly higher in adolescents (18-20 years) compared to children (10-13 years; p<0.05).

Table 1. Characteristics of the study population (N=52)

Variable	Overall (n=52)	10-13 (n=18)	18-20 (n=34)	P-Value
	Mean ± SD or N (%)			
Age, y	17.0 ± 3.72	12.0 ± 1.37	19.1 ± 0.64	**0.000***
Sex				
Female	26 (50.0)	8 (44.4)	18 (52.9)	0.560
Male	26 (50.0)	10 (55.6)	16 (47.1)	
Race				
White	30 (57.7)	2 (11.1)	28 (82.4)	**0.000***
African American	4 (7.7)	3 (16.7)	1 (2.9)	
Asian	2 (3.8)		2 (5.9)	
Native Hawaiian or other Pacific Islander	2 (3.8)		2 (5.9)	
Other	14 (26.9)	13 (72.2)	1 (2.9)	
Hispanic or Latino	38 (73.1)	13 (72.2)	25 (73.5)	0.919
IPAQ score (MET)	3639 ± 3714	1729 ± 1328	4540 ± 4194	**0.001***
BMI percentile	64.0 ± 24.8	58.0 ± 27.8	67.0 ± 24.7	0.278
Total water intake	1831± 841.4	1307 ± 543.8	2109 ± 843.9	**0.001***

USG (μG)	1.018 ± 0.005	1.020 ± 0.006	1.017 ± 0.005	0.072
BMD, g/cm^2	1.10 ± 0.17	0.90 ± 0.81	1.20 ± 0.10	**0.000***
BMC, g	2087 ± 636.7	1400 ± 300.6	2451 ± 431.4	**0.000***

**Significant difference between age groups at p<0.05 by independent sample t-test or Chi-square. Abbreviations: IPAQ, International Physical Activity Questionnaire, USG, urine specific gravity, BMD, bone mineral density, BMC, bone mineral content.*

Table 2 displays the linear regressions between BMD and BMC with hydration status assessed from USG in 24-h urine samples. After adjusting age, sex, and physical activity, no significant associations were found overall or by age groups.

Table 3 shows the linear regressions between BMD and BMC with total water intake from all sources assessed from 24-h food recalls. In the unadjusted model, there was a positive significant association between total water intake from all sources and BMD (B = 0.49, p<0.05) and BMC (B= 19.8, p<0.05) overall and for BMC (B = 9.23, p<0.05) in children (ages 10-13 years). However, after adjusting for sex and physical activity, the association was only significant for BMC (B = 9.48, p<0.05) in children (ages 10-13 years).

Table 2. Linear regressions between hydration status (assessed from USG in 24-h urine samples) and BMD and BMC (assessed from a DEXA scan) in children (10-13y) and adolescents (18-20y).

Bone parameter	Simple linear regression									Adjusted linear regression[1]								
	Overall			Children (10-13 years) (n = 18)			Adolescents (18-20 years) (n = 34)			Overall			Children (10-13 years) (n = 18)			Adolescents (18-20 years) (n = 34)		
	B	SE	P-value	B	SE	P-value	B	SE	P-value	B	SE	P-value	B	SE	P-value	B	SE	P-value
BMD	-6.86	4.11	0.101	-5.39	2.88	0.080	3.20	3.50	0.367	1.41	2.45	0.568	-5.93	3.15	0.080	3.40	3.55	0.345
BMC[2]	-23.2	15.45	0.139	-17.4	10.9	0.130	12.39	14.81	0.409	2.44	9.56	0.800	20.0	11.8	0.114	8.13	13.5	0.552

[1]Adjusted for age, sex, and physical activity overall and sex and physical activity only among age groups. [2]Variable was rescaled by dividing by 1000. *Statistically significant values ($p<0.05$) are in bold. Abbreviations: BMD, bone mineral density, BMC, bone mineral content.

Table 3. Linear regressions between total water intake (assessed from 24-h food recalls) and BMD and BMC (assessed from a DEXA scan) in children (10-13y) and adolescents (18-20y).

Bone parameter	Simple linear regression									Adjusted linear regression[1]								
	Overall			Children (10-13 years) (n = 18)			Adolescents (18-20 years) (n = 34)			Overall			Children (10-13 years) (n = 18)			Adolescents (18-20 years) (n = 34)		
	B	SE	P-value	B	SE	P-value	B	SE	P-value	B	SE	P-value	B	SE	P-value	B	SE	P-value
BMD	0.49	0.11	**0.000**	0.17	0.11	0.136	0.11	0.11	0.321	0.08	0.85	0.345	0.18	0.12	0.161	0.08	0.12	0.493
BMC[2]	19.8	3.79	**0.000**	9.23	3.68	**0.023**	7.28	4.65	0.127	4.42	3.29	0.186	9.48	4.08	**0.036**	2.98	4.53	0.516

[1]Adjusted for age, sex, and physical activity overall and sex and physical activity only among age groups. [2]Variable was rescaled by dividing by 100. *Statistically significant values ($p<0.05$) are in bold. Abbreviations: BMD, bone mineral density, BMC, bone mineral content.

Table 4 displays the logistic regression between hydration status or total water intake from all sources with BMD, and BMC. Hydration status was categorized as “euhydrated” and “dehydrated,” while total water intake from all sources, BMD, and BMC were categorized as “below the median” or “above the median”. Due to the small sample size, analyses were conducted for the overall sample only. No significant associations were found between BMD and BMC with hydration status in the unadjusted model or after adjusting for age, sex, and physical activity. However, in the unadjusted model for BMC, those with lower total water intake from all sources had 3.57 times higher odds of being below the median for BMC than those with higher total water intake from all sources (OR 3.57, 95% CI: 1.38, 11.19). After adjusting for age, sex, and physical activity, the association was similar in magnitude (OR 3.14 95% CI 0.97, 10.12, p=0.055).

Table 4. Logistic regression between hydration categories and total water intake categories and BMD and BMC categories in the sample.

Bone Categories[2]	Overall			
	OR (95% CI)	p-value	Adjusted OR[1] (95% CI)	p-value
BMD[2] and hydration status				
Euhydrated (USG < 1.020 μG)	1		1	
Dehydrated (USG ≥ 1.020 μG)	0.86 (0.29, 2.55)	0.781	0.60 (0.18, 2.02)	0.407
BMC[2] and hydration status				
Euhydrated (USG < 1.020 μG)	1		1	
Dehydrated (USG ≥ 1.020 μG)	0.46 (0.15, 1.39)	0.168	0.39 (0.11, 1.34)	0.132
BMD[2] and total water intake				
Above the median for total water intake	1		1	
Below the median for total water intake	2.56 (0.84, 7.83)	0.099	2.57 (0.80, 8.28)	0.113
BMC[2] and total water intake				
Above the median for total water intake	1		1	
Below the median for total water intake	3.57 (1.38, 11.19)	**0.029**	3.14 (0.97, 10.12)	0.055

[1]Logistic regressions were adjusted for age, sex, and physical activity.

[2]Reference category is above the median for all bone parameters.

*Statistically significant values (p<0.05) are in bold. Abbreviations: BMD, bone mineral density, BMC, bone mineral content.

Discussion

To our knowledge, this is the first study to explore an association between hydration status (measured from USG in a 24-h urine sample and assessed from three 24-h dietary recalls) with BMD and BMC (assessed using DEXA) in US children and adolescents. Total water intake from all sources was positively and significantly associated with BMC in the linear and logistic regressions. The linear regression model showed that a higher total water intake from all sources was associated with a greater BMC in the overall sample (B = 19.8, $p<0.05$) and in children (ages 10-13 years) (B = 9.23, $p<0.05$). The logistic regression models showed that those with a lower water intake had 3.57 times higher odds of having a low BMC (OR 3.57; 95 CI%: 1.38, 11.19).

There are no studies exploring the association between total fluid intake and bone health in children and adolescents. In older adults, those that were adequately hydrated (measured by TBW) had a lower risk of osteoporosis (OR = 0.76, 95% CI, 0.66-0.88, $p<0.0001$).[12] The evidence in children and adolescents is limited to evaluating bone parameters between drinkers of water with high levels of minerals or low level of minerals or by evaluating the intake of certain beverages. For example, a study conducted in China with 660 children ages 10-13 years found a significant positive association between consumption of high mineralized water and bone mineral content (BMC) ($p<0.01$).[23] However, BMC was estimated from bioelectrical impedance analysis, and they did not evaluate overall fluid consumption from the 3-day food records, but rather divided the group by low or regular levels of minerals in the water consumed. Another study conducted in Korea in 2,499 children and adolescents ages 12-25 years found a significant inverse association between a high consumption of cola sodas (assessed from

24-h dietary recall) and BMD (measured using DEXA) in males ($p<0.05$), however they failed to assess total fluid intake.[24] Another study conducted in 228 German children and adolescents ages 6-18 years found a significant inverse association between long-term consumption of soft drinks and non-caffeinated drinks (assessed from 3-day food records) with BMC (measured from XCT-2000) ($p<0.05$), however total fluid intake was not evaluated.[25]

Although the biological mechanism is not clear, total fluid intake is important for bones given its inverse relationship with BMD and strength from in vitro studies involving specimens of human femurs and cortical bone.[10,11] Since proper hydration is important for overall health,[26–28] it may also be important for bone mass accretion at the most important stage for bone development, from early puberty to late adolescences. Bone accretion depends on several factors, such as changes in the structure and composition of bone, in which water in the microscopic pores of bone and extracellular matrix may cause a decline in collagen as a result lead to dehydration.[9]

The strength of the present study is measuring hydration status using an objective marker from 24-h urine samples and from three 24-hour dietary recalls. In addition, this study also measured bone parameters using DEXA. Among the limitations is the small convenience sample size of children and adolescents. A larger sample may be needed to find significant associations between BMD and BMC and hydration status using USG. Also, USG was chosen as the objective marker for the present study instead of urine osmolality, urine color, FWR, and TBW due to its ability to adapt to pediatric populations and use without technical expertise, suitable to studies with a larger sample size and limited resources.[19] In addition, USG and urine osmolality have been used in conjunction due to similar specificity (91%) and sensitivity (89%) scores.[29] However, during acute exercise, both urine osmolality and USG measures could be overestimated, lowering their specificity.[21] Although the present study was not done among athletes, it did not record if participants had exercised while collecting the urine sample. In addition, other measures may impact the association with USG as protein, glucose, ketones, bilirubin, and urobilinogen may alter the results if present in the urine. [31] During proteinuria, each 10g/1 protein may increase USG by 0.003 when measured by refractometry. Similar is shown for every 10 g/1 of glucose may increase USG by approximately 0.002.[31] Therefore, other objective markers may need to be tested in this population to account for limitations.

In conclusion, our findings showed that total water intake from all sources was significantly associated with BMC in both the linear and logistic regression models, which may provide insight to the importance of children and adolescent's water intake to have a greater BMC during the most important stage for bone accretion. Future research should be conducted to explore other objective markers of hydration that may serve as a better predictor of bone mass along with a larger sample to explore the associations between hydration and bone mass in this population.

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CHAPTER V: SUMMARY AND CONCLUSIONS

The association between hydration status and total fluid intake

In a sample of 52 children (10-13 years) and adolescents (18-20 years), USG score (objective measure of hydration) was similar between age groups, and it was on average 1.018 ± 0.005 uG; with 36.5% categorized as dehydrated. Total water intake from all sources (foods and beverages) was significantly higher in adolescents (2109 ± 844 g/d) compared to children (1307 ± 544 g/d; $p<0.01$). A total of 86.5% failed to meet the DRIs for total water intake from all sources amongst both groups. Intake of beverages and foods with hydration status showed water intake, fruit juice, all beverages, total water intake from all sources, and vegetables were significantly and inversely correlated with USG in the overall sample. The logistic model showed intake of water (OR 1.001; 95% CI: 1.000, 1.002), fruit juice (1.011, 95% CI: 0.018), all beverages (OR 1.001, 95% CI: 1.000, 1.002), and total water intake from all sources (OR 1.001, 95% CI: 1.000, 1002) was significantly associated with higher odds of being euhydrated. Results showed total water intake from all sources and types of foods and beverages, are associated with hydration status.

The association between hydration status and body composition

In a sample of 52 children (10-13 years) and adolescents (18-20 years), body fat % was similar by age groups and it was on average 32.2 ± 7.31. FFM was significantly higher in adolescents (47.5 ± 8.16) compared to children (30.9 ± 6.92; $p<0.05$). Body fat % and FFM were not significantly associated with USG in the linear regression models. However, FFM was significantly associated with total water intake from all sources in the overall sample (B = 18.2, $p<0.05$) and in children 10-13 years old (B = 21.5, $p<0.04$). The logistic regression models showed those consuming total water intake from all sources below the median had significantly lower OR of being above the median for FFM (OR 0.23, 95% CI 0.05, 0.96); in other words, having a low total water intake was associated with having less FFM. Results may provide insight to the importance of children and adolescent's water intake and body composition.

The association between hydration status and bone

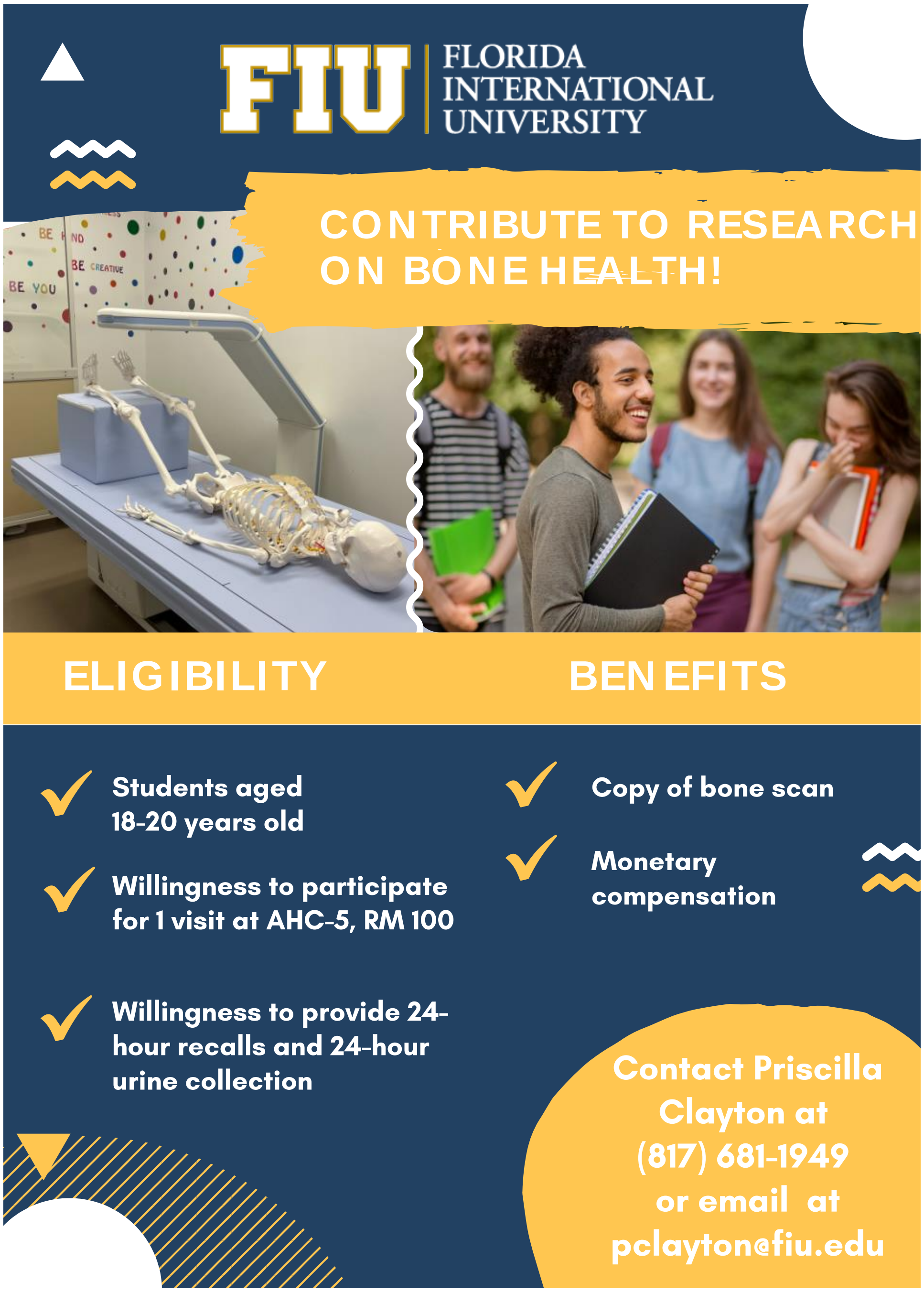
In a sample of 52 children (10-13 years) and adolescents (18-20 years), BMD, BMD-z score, and BMC were significantly higher in adolescents compared to children, as expected ($p<0.05$). No significant associations were found overall or by age groups between BMD and BMC with hydration status assessed from USG in a 24-h sample in both linear and logistic regressions. A positive significant association was found between total water intake from all sources and BMD (B = 0.49, $p<0.05$) and BMC (B = 19.8, $p<0.05$) in the overall group in the unadjusted model. Adjusting for sex and physical activity, BMC remained significant although only in children (B = 9.48, $p<0.05$). Logistic regression showed there was a significantly higher OR of being below the median for BMC among those consuming total water intake from all sources below the median (OR 3.57, 95% CI: 1.38, 11.19) compared to being above the median. Results showed that a higher total water intake from all sources is associated with greater BMC during the most pivotal stage of bone accretion.

CHAPTER VI: STRENGTH AND LIMITATIONS

The study presented evidence on the potential association between body composition and bone with hydration status measured by different methods in US children and adolescents as no studies to our knowledge have explored this association. The study was strengthened due to its ability to collect 24-hour urine samples to assess hydration status and the use of three 24-hour dietary recalls analyzed using NDSR to assess food and beverage intake. In addition, this study used the gold standard for measuring body composition using the DEXA scan, which several of the studies reported failed to use. Another strength is that all anthropometric data was measured and collected by trained study personnel, and this eliminated the use of self-reporting.

Among the limitations is the small convenience sample size of children and adolescent from south Florida, which is predominately Hispanic, and may not be generalizable to others from differing geographical, cultural, racial, and ethnic backgrounds. This can result in an inability to explore racial differences in dietary patterns, body composition, and hydration status. Furthermore, a small sample size may cause minimal to no statistical significance or trend that could be potentially significant in a larger sample. The present study was also a cross-sectional study and collecting longitudinal data could provide a better understanding of the associations being analyzed. Lastly, future studies should evaluate other objective markers of hydration when evaluating the associations with body composition and bone mass in US children and adolescents to determine whether the type of objective marker used is important when exploring this association. Despite these limitations, the present study's findings can provide an understanding of the role of total water intake from all sources in changes in children and adolescent's body composition and bone development.

APPENDIX 1 – Recruitment flyer


FIU FLORIDA INTERNATIONAL UNIVERSITY
CONTRIBUTE TO RESEARCH ON BONE HEALTH!
ELIGIBILITY
Students aged 18-20 years old
Willingness to participate for 1 visit at AHC-5, RM 100
Willingness to provide 24-hour recalls and 24-hour urine collection
BENEFITS
Copy of bone scan
Monetary compensation
Contact Priscilla Clayton at (817) 681-1949 or email at pclayton@fiu.edu

APPENDIX 2 – IRB Approved consent forms

FIU IRB Approval:	01/26/2022
FIU IRB Expiration:	01/26/2023
FIU IRB Number:	IRB-22-0045

FLORIDA INTERNATIONAL UNIVERSITY

ADULT CONSENT TO PARTICIPATE IN A RESEARCH STUDY
Hydration and the association of body composition and bone mass in college students

SUMMARY INFORMATION

Things you should know about this study:

- **Purpose:** The purpose of the study is to associate hydration, body fat percentage, fat-free mass, and bone mass in college students.
- **Procedures**: If you choose to participate, you will be asked to undergo a bone scan, body measurements, provide a 24-h urine sample, and complete questionnaires.
- **Duration:** This will take about 2 hours.
- **Risks**: The main risk from this research is very small radiation with the bone scan.
- **Benefits:** The main benefit to you from this research is receiving a copy of the bone scan with your body fat percentage, fat-free mass, and bone mass.
- **Alternatives:** There are no known alternatives available to you other than not taking part in this study.
- **Participation:** Taking part in this research project is voluntary.

Please carefully read the entire document before agreeing to participate.

PURPOSE OF THE STUDY
The purpose of this study is to associate hydration, body fat percentage, fat-free mass, and bone mass in college students.

NUMBER OF STUDY PARTICIPANTS
If you decide to be in this study, you will be one of 50 people in this research study.

DURATION OF THE STUDY
Your participation will involve a visit to our lab in AHC5-100 to complete questionnaires, undergo a bone scan, undergo weight and height measurements, and provide the first urine sample from the 24-h urine collection (about 1 hour). At home, you will continue with the urine sample collection (about 30 minutes) and 2 additional 24-h diet recalls (about 15 minutes each).

PROCEDURES
If you agree to be in the study, we will ask you to do the following things:

1. Initial questionnaire: you will provide your phone number, email, and address. You will also complete questions about your age, gender, race/ethnicity, and about your general health.
2. 24-h food recalls: you will complete questions about the foods and beverages consumed in the last 24-hours. You will repeat this on 2 different days at home.
3. Weight and height: we will measure your weight and height while wearing light clothes (shorts and t-shirt) and no shoes in a private room.
4. Bone scan: you will undergo a bone scan, which is a test to measure your body fat percentage, fat-free mass, and bone mass using an instrument called "DXA" (Hologic). This is a test similar to X-rays but improved and with much less radiation (about 1.5 mrem, which is less than what one is

exposed to when traveling on an airplane). It will not cause you any damage or injury. For this test, you will lie on a bed and the scanner will scan the body for approximately 7 minutes. If you are female, we will ask if you if you are pregnant. If pregnant, you will not undergo the bone scan and will not be able to continue in the study

5. Urine collection: you will collect urine for 24-hours. You will start the urine collection as part of this visit and then continue at home. We will explain in detail how to do this collection and we will give you written instructions. Your contact information will be used to remind you to return your collection once completed. In addition, we will provide collectors and bio-safety bags for the sample. You will bring these to the lab the next day. We will follow-up for 4 weeks to remind you to complete this task.

RISKS AND/OR DISCOMFORTS

The study has the following possible risks to you:

1. Risk of the bone scan: for this test, you will be exposed to small radiation that will go through immediately (1.5 mrem). X-rays usually do not have side effects, and the amount of radiation is extremely small, which is much less than the dose of an arm x-ray (less than what one is exposed to when traveling on an airplane). The radiation does not remain in the body when the test is completed. Ask the Principal Investigator if you have questions about this risk.
2. Discomfort when measuring weight and height: for this, you will wear no shoes and light clothes (shorts and t-shirt). This may cause a little discomfort.
3. Discomfort when collecting urine: you will have to urinate into a urine collector for 24-hours at home. This could be a little uncomfortable.
4. Risks of disclosure of personal information.

BENEFITS

The study has the following possible benefits to you: receive a copy of the bone scan, which provides your body fat percentage, fat-free mass, and bone mass. The results of the study will help us understand how hydration influences body fat percentage, fat-free mass, and bone mass.

ALTERNATIVES

There are no known alternatives available to you other than not taking part in this study. Any significant new findings developed during the course of the research which may relate to your willingness to continue participation will be provided to you.

CONFIDENTIALITY

The records of this study will be kept private and will be protected to the fullest extent provided by law. In any sort of report, we might publish, we will not include any information that will make it possible to identify you. Research records will be stored securely, and only the researcher team will have access to the records. However, your records may be inspected by authorized University or other agents who will also keep the information confidential.

USE OF YOUR INFORMATION

- Identifiers about you might be removed from the identifiable private information and identifiable biospecimens (urine) and that, after such removal, the information and biospecimens could be used for future research studies or distributed to another investigator for future research studies without additional informed consent from you.

- Your biospecimen (urine) may be used for commercial profit and you will not share in this commercial profit.

COMPENSATION & COSTS
You will receive a payment of $10 (online gift card) for completing the visit and $10 (online gift card) for completing the additional 24-h dietary recalls and the 24-h urine collections. You will also receive small study tokens after you complete all study procedures, such as snacks. There are no costs to you for participating in this study.

MEDICAL TREATMENT
Routinely, FIU, its agents, or its employees do not compensate for or provide free care for human subjects in the event that any injury results from participation in a research project. If you become ill or injured as a direct result of participating in this study, contact your regular medical provider. If you have insurance, your insurance company may or may not pay for these costs. If you do not have insurance, or if your insurance company refuses to pay, you will be billed. Funds to compensate for pain, expenses, lost wages and other damages caused by injury are not routinely available.

RIGHT TO DECLINE OR WITHDRAW
Your participation in this study is voluntary. You are free to participate in the study or withdraw your consent at any time during the study. You will not lose any benefits if you decide not to participate or if you quit the study early. The investigator reserves the right to remove you without your consent at such time that he/she feels it is in the best interest.

RESEARCHER CONTACT INFORMATION
If you have any questions about the purpose, procedures, or any other issues relating to this research study you may contact Cristina Palacios at (305) 348-3235 or cristina.palacios@fiu.edu.

IRB CONTACT INFORMATION
If you would like to talk with someone about your rights of being a subject in this research study or about ethical issues with this research study, you may contact the FIU Office of Research Integrity by phone at 305-348-2494 or by email at ori@fiu.edu.

PARTICIPANT AGREEMENT
I have read the information in this consent form and agree to participate in this study. I have had a chance to ask any questions I have about this study, and they have been answered for me. I understand that I will be given a copy of this form for my records.

______________________________ ________________
Signature of Participant Date

____
Printed Name of Participant

______________________________ ________________
Signature of Person Obtaining Consent Date

APPENDIX 3 – Researcher administered questionnaire

Recorder ID: ______ Participant ID: ______ Date: _ _/_ _/_ _ _ _

Health form

I. General information

1. What is your date of birth? (month/day/year): _ _/_ _/_ _ _ _
2. How old are you today? _______
3. Are you Hispanic? Yes____ No____
4. What is your race?

___White	___American Indian or Alaska Native
___Black or African American	___Native Hawaiian or Other Pacific Islander
___Asian	___Other Race, specify

II. Health history

1. Have you ever had a fracture? No____ Yes ____
   If Yes, how many times? ________ Date of last fracture: _ _/_ _/_ _ _ _

2. Do you use medications? No____ Yes ____
   If Yes, which ones? ____
   For what?____

3. Are you lactose intolerant? No ____ Yes _____ I do not know ____
   If yes: Who diagnose it? _________________________
   How many years ago? _________________________
   What changes have you done in your diet? ______________________________

4. Have you ever smoked? No ____ Yes _____
   If yes: How frequently do you smoke? _____ times per day
   _____ times per week
   _____ times per month
   When you smoke, how many cigarettes do you smoke? __________

5. Have you ever drunk alcohol (beer, wine, rum, breezers, gin, etc.) more than a sip?
   No ____ Yes _____
   If yes: How frequently do you drink? _____ times per day
   _____ times per week
   _____ times per month
   When you drink, how much do you drink? _________

III. Sun exposure

1. How many hours do you usually spend outdoors? _______________

2. When outdoors, do you usually have? (mark all that applies):
   _____ (1) Arms covered
   _____ (2) Legs covered

_____ (3) Head and face covered
_____ (4) Feet covered

3. How easily do you burn when exposed to the sun? ____ very quickly
____ quickly
____ not that quickly
____ not at all

4. Do you use sunscreen? No____ Yes ____
If yes: How much SPF? _____________
How frequently? ____________ days per week

IV. Sleep

In the last month, indicate the frequency for each question:

Question	Always	Almost always	Some-times	Almost never	Never
1. How often do you fall asleep or sleep during class hours?					
2. How often do you fall asleep or get sleepy while doing homework?					
3. Are you attentive or alert most of the day?					
4. How often do you feel tired and moody during the day?					
5. How often do you find it hard to get out of bed in the morning?					
6. How often do you fall asleep again after you woke up in the morning?					
7. How often do you need someone to wake you up in the morning?					
8. How often do you feel you need to sleep longer?					

V. Stress

In the last month, indicate the frequency for each question:

Question	Always	Almost always	Sometimes	Almost never	Never
1. Have you felt unable to control important situations in your life?					
2. Have you felt nervous and stressed?					
3. Have you ever felt confident in your way to manage your personal problems?					
4. Have you felt that things happen the way you want them to be?					
5. Have you had difficulties that you cannot solve?					

2

Recorder ID: _______ Participant ID: ______ Date:_ _/_ _/_ _ _ _

Physical Activity Questionnaire

<u>Vigorous activities</u>

This refer to activities that take hard physical effort and make you breathe much harder than normal. Think *only* about those that you did for at least 10 minutes at a time.

1. During the **last 7 days**, on how many days did you do **vigorous** physical activities such as like heavy lifting, digging, aerobics, fast bicycling, playing sports or games outdoors such as Football, Basketball, Running, Tag etc.

   ___**days per week** ___**None** → *Skip to question 3*

2. How much time did you usually spend doing vigorous physical activities on one of those days?

   ___**hours per day** ___**minutes per day**

<u>Moderate activities</u>

This refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those that you did for at least 10 minutes at a time

3. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads like book bags/grocery bags, bicycling at a regular pace, riding skateboards or scooters, playing with friends in a park, playing interactive video games like virtual reality 'VR' or Dancing, etc. Do not include walking.

   ___**days per week** ___**None** → *Skip to question 5*

4. How much time did you usually spend doing **moderate** physical activities on one of those days?

   ___**hours per day** ___**minutes per day**

SHORT LAST 7 DAYS SELF-ADMINISTERED version of the IPAQ. Revised August 2002.

Walking

This includes walking to school from home and from home to school, walking at the mall, walking around your neighborhood, etc.

5. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time?

   ___**days per week** ___**None** → *Skip to question 3*

6. How much time did you usually spend **walking** on one of those days?

   ___**hours per day** ___**minutes per day**

Sitting

This includes time spent at home or at school, while doing course work and during leisure time. This may include time spent sitting at a desk to study, read or do homework, visiting friends, sitting, or lying down to watch television, time spent playing videogames and using social media.

7. During the **last 7 days**, how much time did you spend **sitting** on a **week day**?

   ___**hours per day** ___**minutes per day**

Sleep

8. During **The last 7 days**, how many hours did you **sleep** at night?

   ___**hours per night**

SHORT LAST 7 DAYS SELF-ADMINISTERED version of the IPAQ. Revised August 2002.

Anthropometric Recording FORM

Participant ID: _ _ _
Recorder ID: ____
Date: ___/___/___

Measurement	Units	*Measurement #1	*Measurement #2	*Measurement #3
Weight [measured to the nearest tenth of a kilogram (0.1 kg)]	kg	_ _ . _	_ _ . _	Only if difference between #1 and #2 is >0.5 kg _ _ . _
Height [measured to the nearest tenth of a cm (0.1 cm)]	cm	_ _ _ . _	_ _ _ . _	Only if difference between #1 and #2 is >0.5 cm _ _ . _

Note if the participant is wearing …	Light clothing	Normal clothing	Heavy clothing	Jeans

COMMENTS:

____
____

APPENDIX 4 – IRB Forms

FLORIDA INTERNATIONAL UNIVERSITY

Office of Research Integrity
Research Compliance, MARC 414

MEMORANDUM

To:	Dr. Cristina Palacios
CC:	Priscilla Clayton
From:	Maria Melendez-Vargas, MIBA, IRB Coordinator
Date:	October 7, 2021
Protocol Title:	"Hydration and The Association of Body Composition and Bone Mass in Children (Secondary analysis of the MetA-Bone Trial).""

The Florida International University Office of Research Integrity has reviewed your research study for the use of human subjects and deemed it Exempt via the **Exempt Review** process.

IRB Protocol Exemption #:	IRB-21-0429	**IRB Exemption Date:**	10/07/21
TOPAZ Reference #:	110773		

As a requirement of IRB Exemption you are required to:

1) Submit an IRB Exempt Amendment Form for all proposed additions or changes in the procedures involving human subjects. All additions and changes must be reviewed and approved prior to implementation.
2) Promptly submit an IRB Exempt Event Report Form for every serious or unusual or unanticipated adverse event, problems with the rights or welfare of the human subjects, and/or deviations from the approved protocol.
3) Submit an IRB Exempt Project Completion Report Form when the study is finished or discontinued.

Special Conditions: N/A

For further information, you may visit the IRB website at http://research.fiu.edu/irb.

MMV/em

Office of Research Integrity
Research Compliance, MARC 414

Priscilla Clayton

February 14, 2022

"Hydration and the association with body composition in college students"

The Institutional Review Board of Florida International University has your study for the use of human subjects via the process. Your study was found to be in compliance with this institution's Federal Wide Assurance (00000060).

01/26/22

IRB Expiration Date: 01/26/23

As a requirement of IRB Approval you are required to:

Submit an IRB Amendment Form for all proposed

Receive annual review and re-approval of your study prior to your IRB expiration date. Submit the IRB Renewal Form at least 30 days in advance of the study's expiration date. or discontinued.

HIPAA Privacy Rule:

Special Conditions:

For further information, you may visit the IRB website at http://research.fiu.edu/irb.

VITA

PRISCILLA CLAYTON

Born, Calw, Germany

2012-2016	B.S., Dietetics and Nutrition Abilene Christian University, Abilene, Texas
2016-2018	M.S., Dietetics and Nutrition Florida International University, Miami, Florida
2017-2018	McNair Scholar Masters Fellow Florida International University, Miami, Florida
2017-2018	Masters student and Graduate Assistant Florida International University, Miami, Florida
2018-2022	Ph.D student and Research Assistant Florida International University, Miami Florida
2018-2022	McNair Scholar Doctoral Fellow Florida International University, Miami, Florida
2020	Recipient of the Robert Stempel College Oral Presentation Award Florida International University, Miami, Florida
2022	Recipient of the Robert Stempel College Publication Award Florida International University, Miami, Florida
2022	Recipient of the Real Triumph Graduate Award Florida International University, Miami, Florida

PUBLICATIONS AND PRESENTATIONS

Clayton P, Connelly J, Ellington M, Rojas V, Trak-Fellermeier MA, and Palacios C. Facilitators and barriers of children's participation in nutrition, physical activity, and obesity interventions: A systematic review. *Obes Rev*. 2021 Sep 1:e13335.

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