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The Effect of Physical Activity Levels on Hydration Markers in Non-Athletic Free-Living Individuals

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by

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ABSTRACT

Higher levels of physical activity requires increased fluid intake due to increased water losses via sweating. **PURPOSE:** To determine the effect of physical activity on hydration status and water intake. **METHODS**: This study involved 8 visits to the Human Performance Laboratory over 22 days. Body weight and urine measurements were taken every visit. Physical activity was assessed by the International Physical Activity Questionnaire (IPAQ), water intake by the Water Frequency Questionnaire (WFQ), and hydration status by urine osmolality (UOsm), urine specific gravity (USG), and urine color (UC). From the IPAQ subjects were classified as low, moderate and high physical activity levels and the total amount of physical activity was expressed as MET-min·w^{-1}. All values represent means across 22 days of measurements except physical activity and water intake, which was a mean of two measurements. Participants were excluded if they exercised more than 4 hours a week or if they were on medications that effected fluid balance. One-way analysis of variance was computed to determine differences in hydration status between groups. Additionally, 2-tailed Pearson correlations were computed to determine relationships between all measurements while using physical activity as a continuous variable (i.e., MET-min·w^{-1}). **RESULTS:** 95 participants (45 males, 40±13.2 y, 1.76±0.07 m, 27.3±10.8 %BF 25.9±4.6 kg·m^2; 50 females, 41.1±14.8 y, 1.63±.06 m, 35.0±11.0 %BF, 26.6±6.2 kg·m^2) completed the study; n=39 for low activity (822±698 MET-min·w^{-1}), n=48 for moderate activity (1791±1195 MET-min·w^{-1}), n=8 for high activity (4,728±1150 MET-min·w^{-1}). Hydration status and water intake did not differ across all levels of physical activity; UOsm: 587±209, 596±223, 562±290; and TWI: 2.6±.9, 2.9±1.4, 3.0±1.5 L for low, moderate, and high physical activity levels, respectively ($P\leq0.05$). UOsm (589±290) was strongly correlated to Total Water Intake (2.83±1.22 L, P=0.000), and weight (76.4±17.3 kg, P=0.000) at a significance level of $P\leq0.01$

but not strongly correlated to MET·min·w^{-1} (1640±1453). **CONCLUSION:** No significant differences in hydration or water intake were observed across groups. However, the small numbers of participants in the high physical activity levels may have made this comparison difficult. A wider range of MET-min·w^{-1} between groups may need to be studied.

ACKNOWLEDGEMENTS

Special thanks are due to Dr. Kavouras, Dr. Gray, and Dr. Ganio for advising me through the degree and being a part of the committee. Also, a special thanks goes out to Dr. Johnson and the research team for mentoring me and making this all possible while giving me the opportunity to gain valuable knowledge and learning from great experiences.

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Introduction: Water is one of the most important nutrients in the human body (Sawka, Cheuvront & Carter III, 2005). It is the largest component of the human body accounting for about 50-70% of total body mass according to Maughan (2003), Sawka and colleagues (2005), and Armstrong (2005). It is crucial for metabolism, temperature regulation, and several other physiological functions (Armstrong, 2005). Research has found that fluid loses of only 1-2% has its negative effect on the body including alertness, work performance, headaches, concentration, and tiredness along with several other health issues (Maughan, 2003). However, it is often ignored in the general population's nutrition to replace water loss appropriately (Sawka et al., 2005). Due to it being ignored, recommendations are often not met. The recommended adequate intake of water is found to be about 3.7L a day for adult men and 2.7 L a day for adult females (*Dietary Reference Intake for Water*, 2005). Water intake includes drinking water, water in beverages, and water in food (Sawka et al. 2005). Fluids from drinking water and beverages provide about 81% of total water intake, and water from food provides about 19% of total water intake according to the *Dietary Reference Intake for Water* (2005). Urinary loss is the major route of water loss followed by sweat loss, respiratory loss, fecal loss, insensible loss, and metabolic production (Armstrong, 2005). Water loss or lack of appropriate total water intake can result in body water deficits. Body water deficits result when fluid intakes are reduced or fluid losses are increased (Sawka et al., 2005).Water intake will be assessed using the Water Frequency Questionnaire (WFQ). Westerterp (1999) found that self-reported dietary records show an underestimate of actual consumption of fluids and food by about 17% the first week, 27% the second week, and 35% the third week when compared to ^{2}H and ^{18}O. This could be due to the participant not accurately recalling what they have eaten when the intake is recorded (Westerterp, 1999). However, it is the next best tool to use to assess water intake. To measure hydration, urine specific gravity, urine osmolality, and urine color will be used to discover if each individual is euhydrated or hypohydrated. These methods have been used in most hydration research in the past to determine hydration status including Volpe and colleagues (2009), Kavouras and colleagues (2012), and Cleary and colleagues (2012). Aside from hydration, another major health concern that is often ignored is physical activity which can lead to a healthier longer life or the lack of can lead to obesity, hypertension, diabetes, arthritis, dyslipidemis, depression, osteoporosis, cancer, as well as several other health concerns (Marcos et al., 2014). Several studies have found no change in total water intake with age or gender (Bossignham, Carnell & Campbell, 2005; De Castro et al., 1992). However, there are changes seen with physical activity. If physical activity is vigorous, total water intake requirements may exceed 6 L a day (Sawka et al. 2005). To maintain and promote good health, healthy adults aged 18-65 years need moderate aerobic physical activity for a minimum of 30 minutes on five days a week, vigorous aerobic physical activity for a minimum of 20 minutes on three days a week, or a combination of the two (Haskell, Lee, Pate, Powell and Blair, 2007). Using the International Physical Activity Questionnaire (IPAQ) scoring protocol, this physical activity would place an individual at a minimum of 600 MET-minutes/week which is in the Moderate Intensity category of physical activity (IPAQ Scoring Protocol, 2005). Reports indicate that as age increases physical activity decreases and the fluid regulatory capacity decreases due to reduced renal concentration and diluting capacity partnered with less thirst drive (Armstrong, 2005). Therefore, we should see a decline in physical activity and lack of replacing fluids properly with an increase in age. Also, physical activity and environmental exposure will increase water losses and therefore increase daily fluid needs (*Dietary Reference Intake of* Water, 2005). We will assess this using the IPAQ Short Form. Research has shown that the self-report short form IPAQ is a valid instrument for physical activity assessment (Heesch, van Uffelen, Hill & Brown, 2010). The IPAQ has reasonable measurement properties for monitoring population levels of physical activity among 18 to 65 year old individuals in diverse settings (Craig et al., 2003). Water balance studies suggest that going from minimal activity to sedentary activity levels in temperate environments increased daily water requirements from about 2.5 to 3.2 L/day, respectively (Fusch et al., 1998; Leiper et al., 1996).Water turnover studies indicate that individuals with more vigorous levels of activity (> 60 minutes per day of activity) compared to individuals engaging in relatively sedentary activity (i.e., less than 60 minutes per day of activity) in temperate environments have increased daily total water requirements of approximately 3.0 to 4.5 L/day in men (Fusch et al., 1998; Leiper et al., 1996). Higher levels of physical activity further increase water requirements; for example, very active fire fighters had daily water requirements of about 7 L/day (Ruby et al., 2002). Hydration and physical activity are both very important concepts that when combined can promote well-being and health or be detrimental to the individual. The purpose of the present study was to study the effects of physical activity on hydration status and total water intake in healthy and active but non-athletic adults. We hypothesize that there will be a negative correlation between hydration markers and physical activity levels (as physical activity increases, hydration markers will show more signs of dehydration). More physically active individuals will sweat and excrete more water and not replace the lost water affectively.

Methods

Participants: This study included 102 individuals ages 18-65 years old from the local Northwest Arkansas area in an attempt to obtain a representative sample. Once subjects with incomplete questionnaires or other errors were excluded from the data 95 subjects (45 males, 40±13.2 y, 1.76±0.07 m, 27.3±10.8 %BF 25.9±4.6 kg·m^2; 50 females, 41.1±14.8 y, 1.63±0.06 m, 35.0±11.0 %BF, 26.6±6.2 kg·m^2) remained. Participation was based on inclusion criteria such as medical clearance, healthy and active (but not a competitive athlete), and signed informed consent prior to the initiation of any trial procedure. Exclusion criteria included inability to understand and write English, evidence of clinically relevant metabolic, cardiovascular, hematologic, hepatic, gastrointestinal, renal, pulmonary, endocrine or psychiatric history of disease (based on the medical history questionnaire), pregnancy or breast feeding, surgical operation on digestive tract (except possible appendectomy), regular drug treatment within 15 days prior to start of the study, currently exercising more than 4 hours per week, inability to participate in the entire study, changes in diet during the last month, or change in weight more than 2.5 kg (about 5 pounds) in the last month.

Research Design: A random stratified sampling of participants by age group and gender to represent the population of the United States was used for the study. All participants reported to the Human Performance Lab at the University of Arkansas. Each participant that completed the full study received $150.00.

Measures: This project involved 8 visits to the Human Performance Laboratory at the University of Arkansas over 3 weeks plus one day (i.e., 22 days). Prior to the start of the study participants met with a scientist for completion of the medical history and informed consent forms. Morning urine samples were collected to assess hydration status, weekly water intake was assessed using the Water Frequency Questionnaire (WFQ), and weekly physical activity was assessed using the International Physical Activity Questionnaire (IPAQ). Body weight was recorded every visit to the closest 100 g using a balance beam (Seca, model: 7701321004, Vogel & Hamburg,Germany), and standing height was recorded the first visit with a wall-mounted stadiometer (Seca, model: 770, Vogel & Hamburg, Germany). Body weight and height were recorded with the subjects wearing only their shorts and a t-shirt, with no shoes directly after they urinate. On the first day, a body composition measurement also took place via the use of dual x-ray absorptiometry (DXA) scan, (Lunar Prodigy, General Electric Healthcare, Waukesha, WI). Urine osmolality was measured in duplicate, by freezing point depression (3D3 Advanced Osmometer, Advanced Instruments Inc, Norwood, MA, USA). Urine Specific Gravity was measured with a hand held refractometer (ATAGO SUR-NE, Tokyo, Japan) in duplicate. Urine Color was measured standing in a well-lighted room (temperature 20-22°C) with samples placed in a clear glass tube against a white background. Urine Color was evaluated using the Urine Color Chart (UCC). The evaluations were carried out by a trained group of researchers at all times. These evaluation tools were also done by several past studies including Stearns et al. (2009), Cleary et al. (2012), Bardis, Kavouras, Arnaoutis, Panagiotakos, and Sidossis (2013), and Kavouras et al. (2012).

Procedures: This project involved eight visits to the Human Performance Laboratory at the University of Arkansas over three weeks plus one day (i.e., 22 days). Prior to the start of the study participants met with a scientist for completion of medical history and consent forms. Following the briefing, written guidelines were provided with all necessary questionnaires. After explaining the scope of the study and signing the consent form during the initial meeting of the first day, the individual completed the medical history questionnaire. Next, the Water Frequency Questionnaire (WFQ) and the International Physical Activity Questionnaire (IPAQ) were completed. We then collected a morning urine sample and measured height and weight followed by a measurement of body fat with dual x-ray absorptiometry (DXA). During the study body fat and height was measured once, eight morning urine samples were collected in small urine containers that were clearly labeled with the date, individual participant number and the sample identification code, (i.e., “#1 - Morning”, “#2 - Morning”, etc.). Body weight was measured eight times directly after the subject urinated, and the WFQ and IPAQ were assessed three times all assessing the week prior to the questionnaire. The participant’s visits and what data is collected in each visit (Table 1) serve to explain the study in more detail.

Table 1-Explains when the participants visit the lab each week over the entire study and what data is collected for each day (DXA, Urine, Weight, IPAQ, and WFQ).

	WEEK 1			WEEK 2	WEEK 3			WEEK 4
Day	Monday	Tuesday	Friday	Monday	Monday	Tuesday	Friday	Monday
Lab Visit	1	2	3	4	5	6	7	8
DXA	X							
Height	X							
Urine	X	X	X	X	X	X	X	X
Weight	X	X	X	X	X	X	X	X
IPAQ	X			X				X
WFQ	X			X				X

Water Frequency Intake Records: The Water Frequency Questionnaire (WFQ) is assessed on the first, fourth, and eighth visits to assess total water intake (L). The WFQ on the first visit is used for familiarization. The WFQ on the fourth visit is assessing week one, and on the eighth visit week three. Using this tool, we find the total weekly water consumed (L) and find the average daily water intake (L). We can then correlate these values to the individual's hydration status and physical activity level. The recommended adequate intake of water is found to be about 3.7L a day for adult men and 2.7 L a day for adult females (*Dietary Reference Intake for Water*, 2005). The WFQ assesses water from beverages (milk, juice, alcohol, soft drinks, coffee, etc.) and food (vegetables, fruits, dairy, starches, etc.).

Hydration Markers: Urine Specific Gravity (USG), Urine Osmolality (UOsm), and Urine Color are collected on all eight visits. The values are used to find the average hydration status for each individual and to correlate to the physical activity level and water intake of that individual. The first four urine samples are to find the average of week one and the last four samples are used to find the average value for week three. Hydration markers that are used for the study are:

- Urine Specific Gravity (USG) - measures hydration by a refractometer to analyze the amount of light that passed through a small drop of urine and assess urine specific gravity (Volpe et al., 2009). The cut off point for euhydration is based on Urine Specific Gravity less than 1.020 (Sawka et al., 2007). Values equal to or greater than 1.020 indicate hypohydration (Cleary et al., 2012).
- Urine Osmolality (UOsm) - measures the osmolality of urine by freezing point depression in an osmometer. A person is in the euhydrated state if less than 700 mOsm/kg of water or in the hypohydration state is equal to or greater than 700 mOsm/kg of water (Sawka et al., 2007).
- Urine Color Chart- measures the urine concentration. Most researchers use an 8-level chart. Values at 4 or lower indicate euhydration, and values greater than 4 indicate hypohydration (Cleary et al., 2012).

IPAQ: The IPAQ short form is scored by High, Moderate, or Low physical activity. These scores are used in correlation with hydration scores and total water intake. The IPAQ is given on the first visit for familiarization, the fourth visit to assess week 1, and the eighth visit to assess week 3. To analyze the IPAQ, walking is equivalent to 3.3 METs, moderate Intensity is equivalent to 4.0 METs, and vigorous intensity is equivalent to 8.0 METs (IPAQ Scoring Protocol, 2005). Categorizing of the IPAQ is as follows:

- High physical activity category- an individual must have vigorous-intensity activity on at least 3 days and at least 1500 MET-minute·w^{-1} or 7 or more days of any combination of walking, moderate or vigorous intensity activities and at least 3000 MET-minutes·w^{-1}.
- Moderate physical activity category an individual must have 3 or more days of vigorous activity of at least 20 minutes per day, 5 or more days of moderate activity and/or walking of at least 30 minutes per day, or 5 or more days of any combination of walking, moderate or vigorous intensity activity achieving a minimum of at least 600 MET-minutes·w^{-1}.
- Low physical activity category an individual must have no activity reported or some activity but not enough to meet the prior listed categories (IPAQ Scoring Protocol, 2005).

Statistical Analysis: Mean and SD values were computed for all variables to describe the data. A T-test to look at differences between gender across all variables, A One-way Anova ($P \leq 0.05$) was used to compare physical activity level with each variable, and also a post hoc test for multiple comparisons by using Bonferroni's method ($P \leq 0.05$) with physical activity level in week 1 as an independent variable, physical activity level in week 3 as an independent variable, and average physical activity across the study as an independent variable were also computed. A two-tailed Pearson's Correlation was calculated for all variable averages ($P \leq 0.05$). Linear

Regressions were also calculated for all variable averages to analyze patterns of change. The analysis was performed using SPSS.

Results:

Week 1: For week 1 (Table 2) there was no significant difference between males and females (45 males, 40±13.2 y, 1.76±0.07 m, 27.3±10.8 %BF 25.9±4.6 kg·m^2; 50 females, 41.1±14.8 y, 1.63±0.06 m, 35.0±11.0 %BF, 26.6±6.2 kg·m^2) in UOsm (639±203, 543±240), USG (1.016±0.006, 1.014±0.006), UC (3.0±0.79, 3.0±0.86), weight (81.6±16.1 kg, 71.9±17.2 kg), MET-minutes·w^{-1} (2053±2178 MET-min·w^{-1}, 1297±957 MET-min·w^{-1}), water from food (0.51±0.27 L, 0.54±0.26 L) water from beverages (2.8±1.5 L, 2.1±1.0 L), or Total Water Intake (3.2±1.7 L, 2.7±1.1 L), respectively.

Table 2- Week 1 differences in variables in males and females. TWI- Total Water Intake (L).

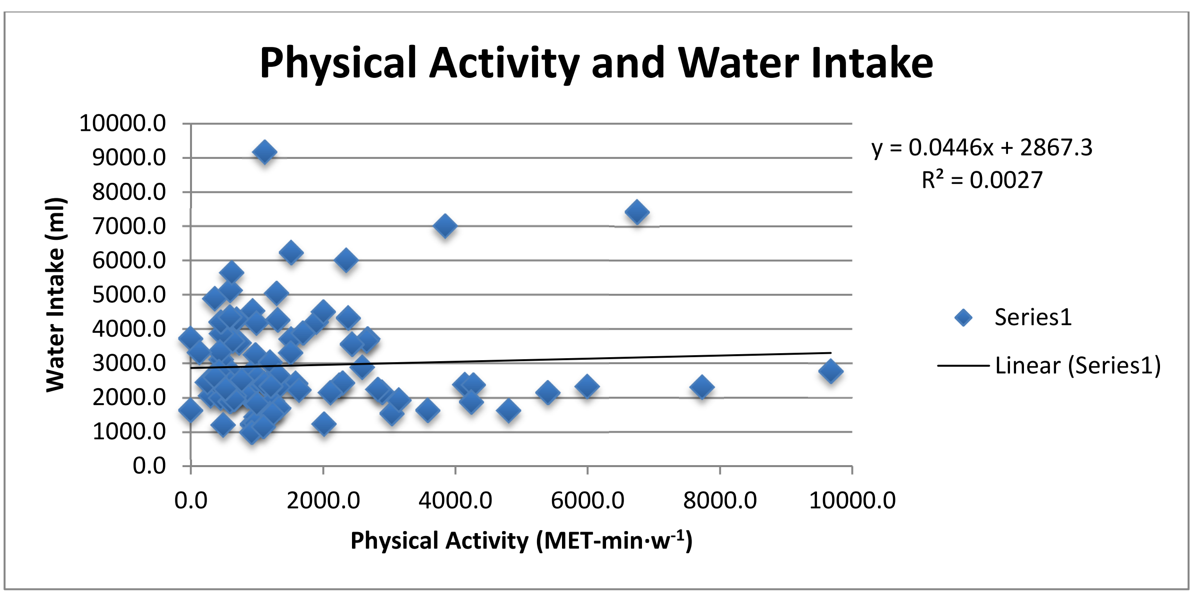
Variable	Male	Female
Subjects	45	50
Age (y)	40±13.2	41.1±14.8
Height (m)	1.76±0.07	1.63±0.06
Weight (kg)	81.6±16.1	71.9±17.2
%BF	27.3±10.8	35.0±11.0
BMI	25.9±4.6	26.6±6.2
UOsm (mmol/kg)	639±203	543±240
USG	1.016±0.006	1.014±0.006
UC (1-8)	3.0±0.79	3.0±0.86
TWI (L)	3.2±1.7	2.7±1.1
MET-min·w^{-1}	2053±2178	1297±957

Males and females were combined for the rest of the results as there were no significant differences between genders and we wanted to keep a large sample in each physical activity category. There was no significant difference (Table 3) between the low physical activity (n=23), moderate physical activity (n=59), and high physical activity (n=13) categories in UOsm (595±205, 586±235, 584±227), USG (1.014±0.005, 1.015±0.006, 1.015±0.007), UC (3.0±0.8, 2.9±0.8, 2.7±0.8), weight (75.9±19.3 kg, 75.1±16.6 kg, 83.9±15.9 kg), water from food (0.46±0.24 L,0.55±0.29 L, 0.50±0.16 L), water from beverages (2.21±0.83 L, 2.49±1.4 L, 2.6±1.9 L) or Total Water Intake (2.65±0.94 L, 3.03±1.49 L, 3.04±1.94 L), respectively. However, as most would assume, there was a significant difference ($P \leq 0.05$) in Met-min·w^{-1} as physical activity level increased from low (533±375 MET-min·w^{-1}) to moderate (1344±718 MET-min·w^{-1}, P=0.002), low to high (5052±2029 MET-min·w^{-1}, P=0.000), and from moderate to high (P=0.000).

Table 3- Change in variables between physical activity levels during Week 1. *-significant at the $P \leq 0.05$ Level.

Activity Level	Subjects	UOsm Mmol/kg	USG	UC (1-8)	Weight (kg)	TWI (L)	METs-min· w^{-1}
Low	23	595±205	1.014±0.005	3.0±0.8	75.9±19.3	2.65±0.94	533±375*
Moderate	59	586±235	1.015±0.006	2.9±0.8	75.1±16.6	3.03±1.49	1344±718*
High	13	584±227	1.015±0.007	2.7±0.8	83.9±15.9	3.04±1.94	5052±2029*

A)



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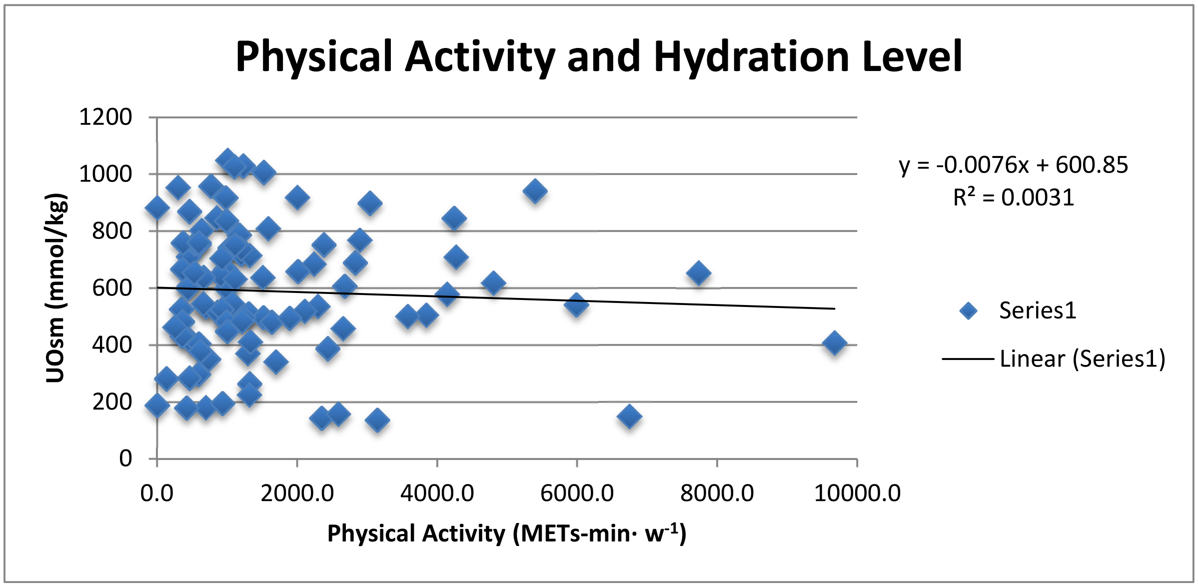

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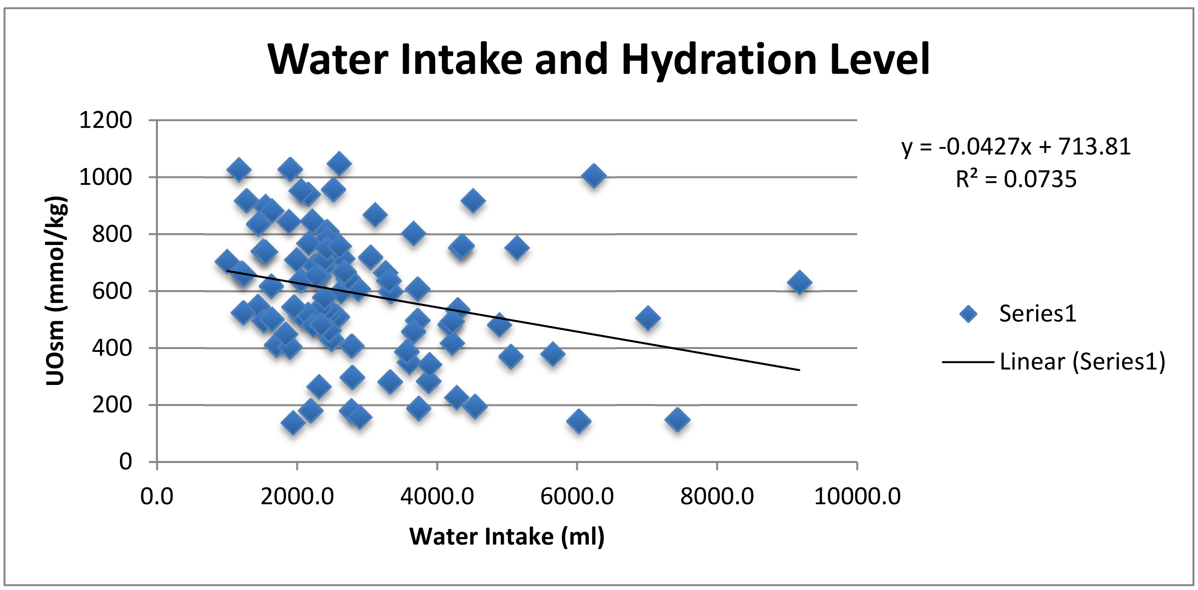


Figure 1- Correlation of A) Physical Activity (MET-min·w^{-1}) and Water Intake (ml), B) Physical activity (MET-min·w^{-1}) and UOsm (mmol/kg), C) Water Intake (ml) and UOsm (mmol/kg)

Although not significant, we do see a higher score of UOsm, USG, MET-min·w^{-1}, water from beverages and Total Water Intake in males than females. Also, we do see a slight decrease in UOsm and UC, and a slight increase in weight, water from beverages and Total Water Intake from low, moderate, to high physical activity levels.

In week 1 (Fig. 2), 31% (n=14) of males, 40% (n=20) of females, and 36% (n=34) total reached the daily water intake recommendations of 2.7 L for females and 3.7 L for males (*Dietary Reference Intake for Water*, 2005). For the recommended percentages of water from food and beverages we selected participants who were 1% below or above 19% for food to be in the range seen in past research. 39% (n=37) of participants did not reach the recommended percentages of water from food intake of about 19% (*Dietary Reference Intake for Water*, 2005), and only 17% (n=16) were in the 18-20% range of water from food, 66% (n=63) of the participants were in the euhydrated state, or less than 700 mmol/kg of water when using UOsm as the hydration marker (Sawka et al., 2007), and 76% (n=72) of the participants reached the daily physical activity recommendations of moderate physical activity of 3 or more days of vigorous activity of at least 20 minutes per day, 5 or more days of moderate activity and/or walking of at least 30 minutes per day, or 5 or more days of any combination of walking, moderate or vigorous intensity activity achieving a minimum of at least 600 MET-minutes$\cdot w^{-1}$ (IPAQ Scoring Protocol, 2005).

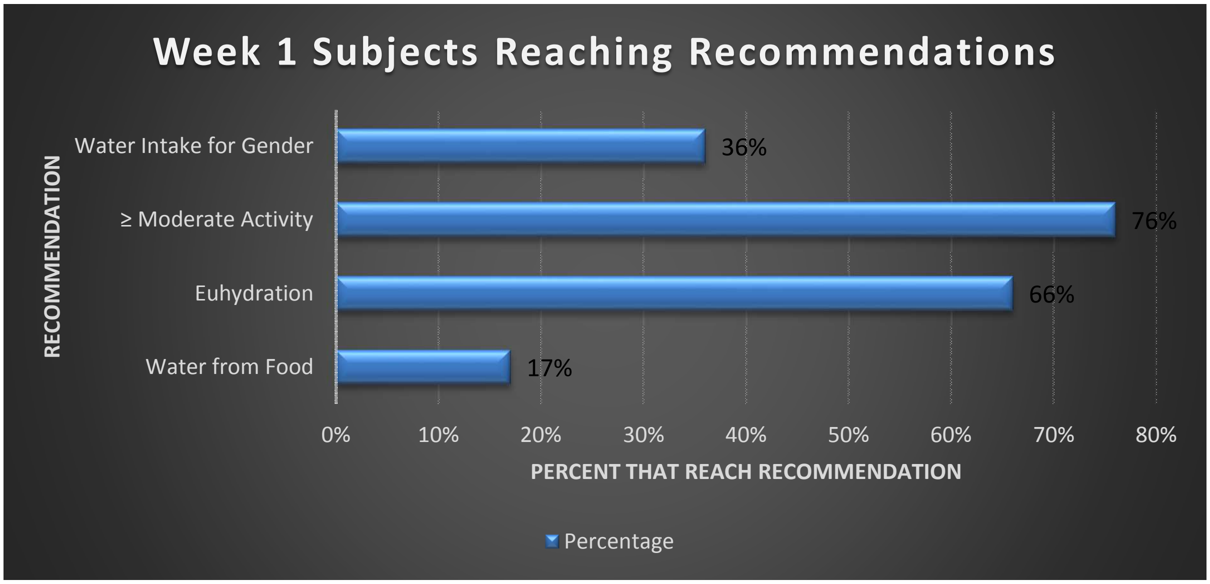


Figure 2- Subjects in Week 1 that reached the recommendations. Water from food- (n=16), euhydrated (n=63), ≥ Moderate Physical Activity (n=72), Total Water Intake in males (n=20), females (n=14).

Week 3: For week 3 (Table 4) there was no significant difference between males and females in UOsm (642±234, 543±225), USG (1.017±0.006, 1.014±0.006), UC (3.0±0.87, 3.0±0.76), weight (81.4±15.9 kg, 71.8±17.3 kg), MET-minutes·w^{-1} (1972±1818 MET-min·w^{-1}, 1314±1217 MET-min·w^{-1}), water from food (0.50±0.28 L, 0.49±0.21 L) water from beverages (2.3±1.2 L, 2.2±1.0 L), and Total Water Intake (2.8±1.3 L, 2.6±1.1 L), respectively.

Table 4- Week 3 differences in males and females. TWI-Water Intake (L).

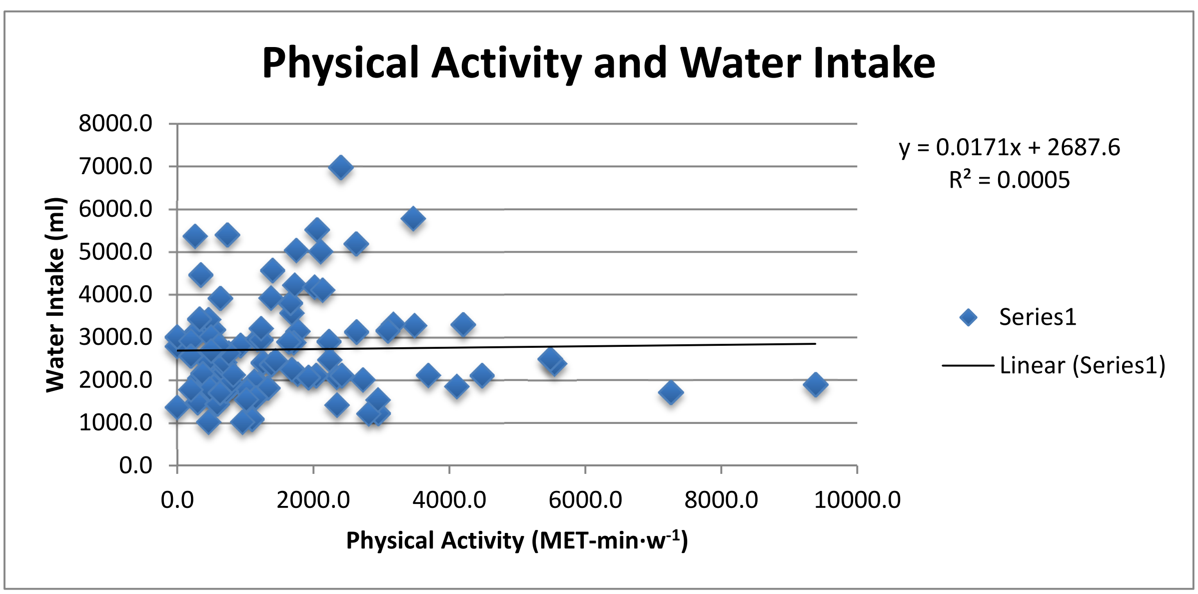
Gender	Subjects	Weight (kg)	UOsm	USG	UC (1-8)	TWI (L)	METs-min·w^{-1}
Male	45	81.4±15.9	642±234	1.017±0.006	3.0±0.87	2.8±1.3	1972±1818
Female	50	71.8±17.3	543±225	1.014±0.006	3.0±0.76	2.6±1.1	1314±1217

Again, there was no significant difference (Table 5) between the low physical activity (n=30), moderate physical activity (n=52), and high physical activity (n=13) categories in UOsm (573±225, 606±221, 565±310), USG (1.015±0.006, 1.016±0.006, 1.015±0.009), UC (2.9±0.9, 3.0±0.6, 2.8±1.2), weight (75.9±17.8 kg, 75.8±18.0 kg, 79.9±13.7 kg), water from food (0.45±0.25 L,0.51±0.23 L,0.53±0.29 L), water from beverages (1.97±0.83 L, 2.34±1.20 L, 2.49±1.14 L) and Total Water Intake (2.43±0.97 L, 2.83±1.23 L, 2.94±1.34 L), respectively. Also, there was a significant difference (P≤0.05) in MET·min·w^{-1} as physical activity level increased. However, from low (922±1589 MET-min·w^{-1}) to moderate (1475±772 MET-min·w^{-1}) there was no significance (p=0.179), but was a significant difference from low to high (3853±1917 MET-min·w^{-1}, P=0.000), and from moderate to high (P=0.000).

Table 5- Differences in variables between physical activity levels in Week 3. *-Significance at the *P*≤0.05 level.

Activity Level	Subjects	UOsm Mmol/kg	USG	UC (1-8)	Weight (kg)	TWI (L)	MET-min·w^{-1}
Low	30	573±225	1.015±0.006	2.9±0.9	75.9±17.8	2.43±0.97	922±1589*
Moderate	52	606±221	1.016±0.006	3.0±0.6	75.8±18.0	2.83±1.23	1475±772*
High	13	565±310	1.015±0.009	2.8±1.2	79.9±13.7	2.94±1.34	3853±1917*

A)



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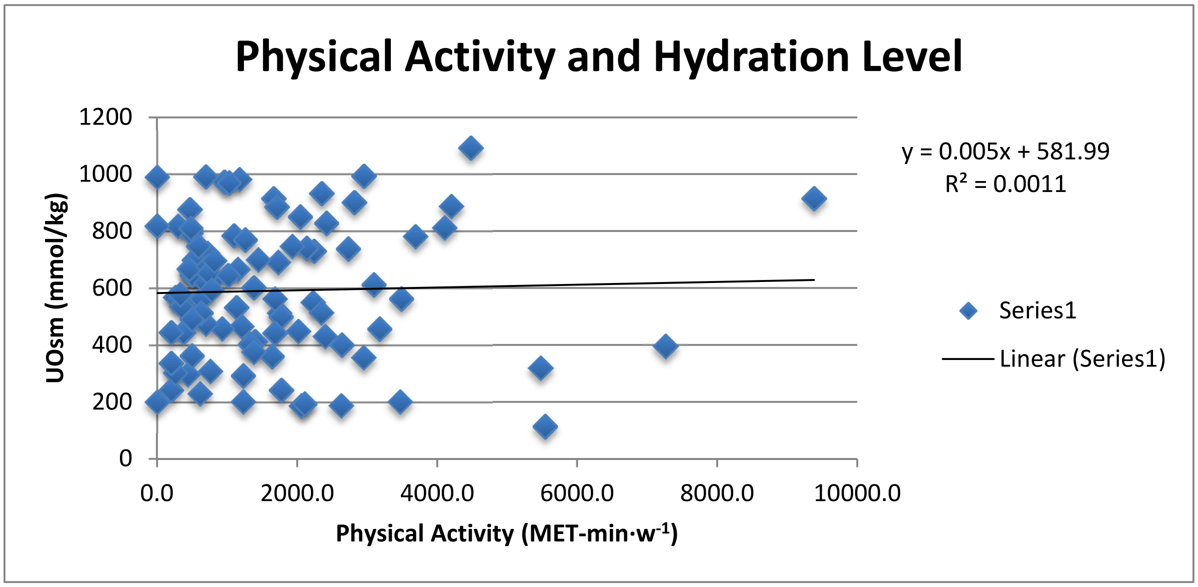

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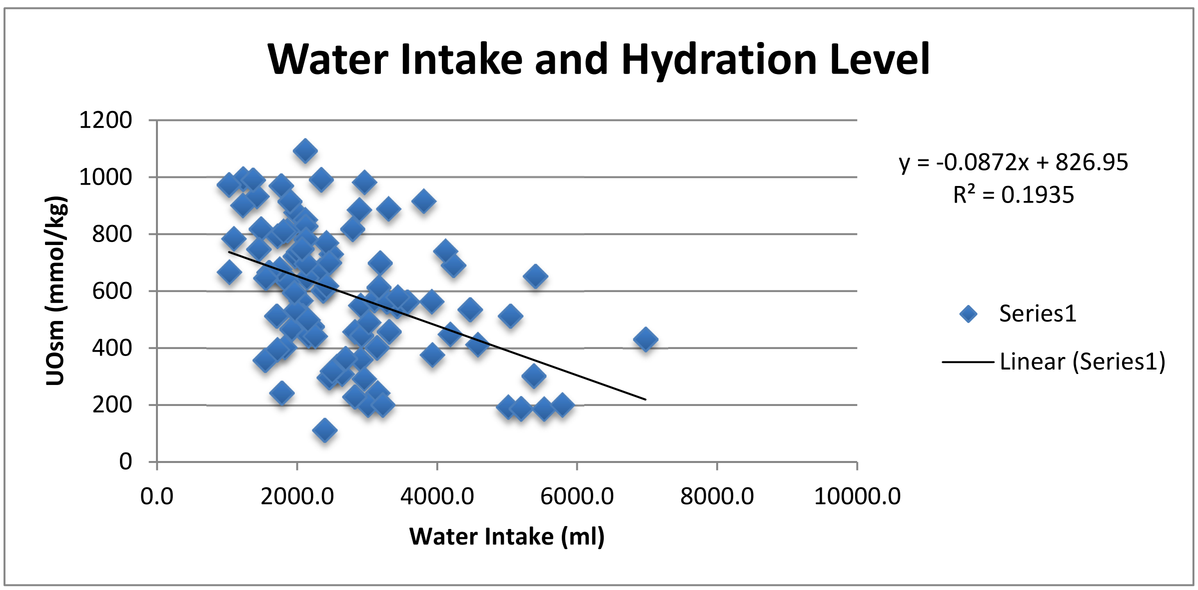


Figure 3- Correlation of A) Physical Activity (MET-min·w^{-1}) and Water Intake (ml), B) Physical activity (MET-min·w^{-1}) and UOsm (mmol/kg), C) Water Intake (ml) and UOsm (mmol/kg)

Although not significant, we do see higher levels of UOsm, USG, MET-min·w^{-1}, and Total Water Intake in males than females. Also, we see a slight increase in weight, water from food, water from beverages, and Total Water Intake as physical activity increases from low, moderate, to high physical activity levels.

In week 3 (Fig. 4), 18% (n=8) of males, and 38% (n=19) of females, and 28% (n=27) total reached the daily water intake recommendations, 47% (n=45) of participants did not reach the recommended percentages of water from food, and only 6% (n=6) were in the 18-20% range of water from food, 67% (n=64) of participants were euhydrated, and 68% (n=65) of the participants reached the daily physical activity recommendations.

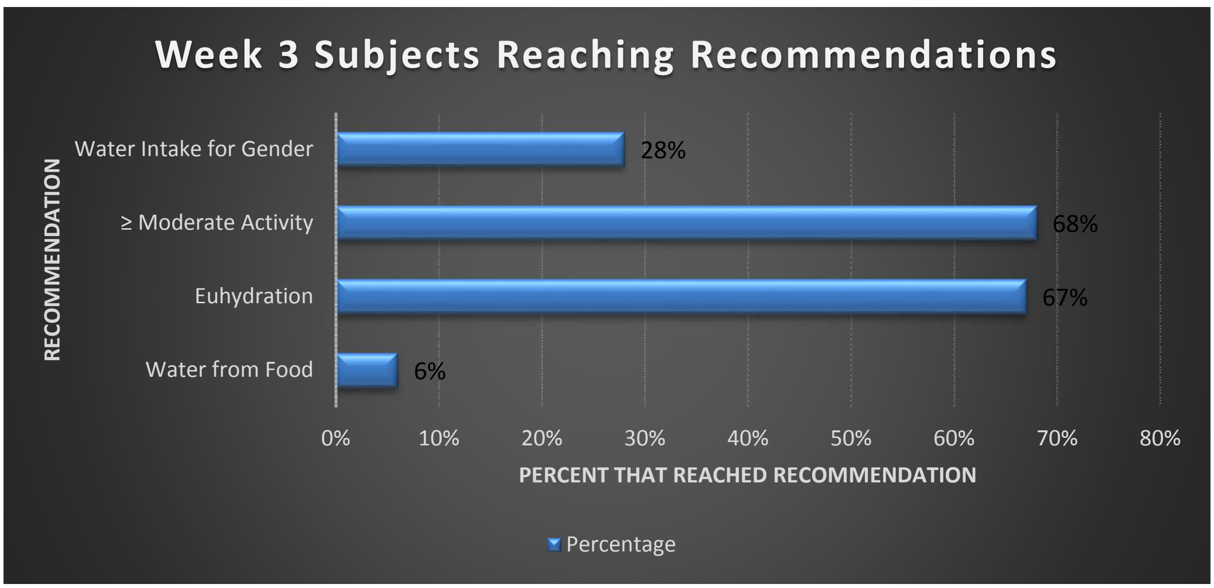


Figure 4- Subjects in Week 3 that reached the recommendations. Water from food (n=6), euhydrated (n=64), ≥ Moderate Physical Activity (n=65), Total Water Intake (n=27), females (n=19), Males (n=8).

Total Average: For the total average of both weeks (Table 6) there was no significant difference between males and females in UOsm (641±205, 543±226), USG (1.017±0.005, 1.014±0.006), UC (3.0±0.75, 3.0±0.72), weight (81.5±16.0 kg, 71.8±17.3 kg), MET-minutes·w^{-1} (2012±1821 MET-min·w^{-1}, 1306±911 MET-min·w^{-1}), water from food (0.51±0.22 L,0.51±0.21 L) water from beverages (2.6±1.3 L, 2.2±0.98 L), and Total Water Intake (3.0±1.4 L, 2.6±1.0 L), respectively.

Table 6- Total Average differences in males and females. TWI-Water Intake (L).

Gender	Subjects	Weight (kg)	UOsm Mmol/kg	USG	UC (1-8)	TWI (L)	METs-min·w^{-1}
Male	45	81.5±16.0	641±205	1.017±0.005	3.0±0.75	3.0±1.4	2012±1821
Female	50	71.8±17.3	543±226	1.014±0.006	3.0±0.72	2.6±1.0	1306±911

For the average scores across the entire study there was no significant difference (Table 7) between the low physical activity (n=39), moderate physical activity (n=48), and high physical activity (n=8) categories in UOsm (587±209, 596±223, 562±290), USG (1.015±0.006, 1.016±0.006, 1.015±0.008), UC (3.0±0.7, 2.9±0.7, 2.8±1.1), weight (75.5±17.8 kg, 76.3±17.2 kg, 81.6±16.4 kg), water from food (0.46±0.18 L,0.54±0.23 L, 0.54±0.25 L), water from beverages (2.20±0.82 L, 2.43±1.32 L, 2.57±1.31 L) and Total Water Intake (2.64±0.89 L, 2.95±1.38 L, 3.0±1.54 L), respectively. Again, there was a significant difference ($P \leq 0.05$) in MET-min·w^{-1} as physical activity level increased from low (822±698 MET-min·w^{-1}, P=0.000), moderate (1791±1195 MET-min·w^{-1}, P=0.000), to high (4728±1150 MET-min·w^{-1}, P=0.000) levels.

Table 7- Differences in variables between physical activity levels for the total average across the study. *-Significance at the p≤0.05 level.

Activity Level	Subjects	UOsm Mmol/kg	USG	UC (1-8)	Weight (kg)	TWI (L)	METs-min·w^{-1}
Low	39	587±209	1.015±0.006	3.0±0.7	75.5±17.8	2.64±0.89	822±698*
Moderate	48	596±223	1.016±0.006	2.9±0.7	76.3±17.2	2.95±1.38	1791±1195*
High	8	562±290	1.015±0.008	2.8±1.1	81.6±16.4	3.0±1.54	4728±1150*

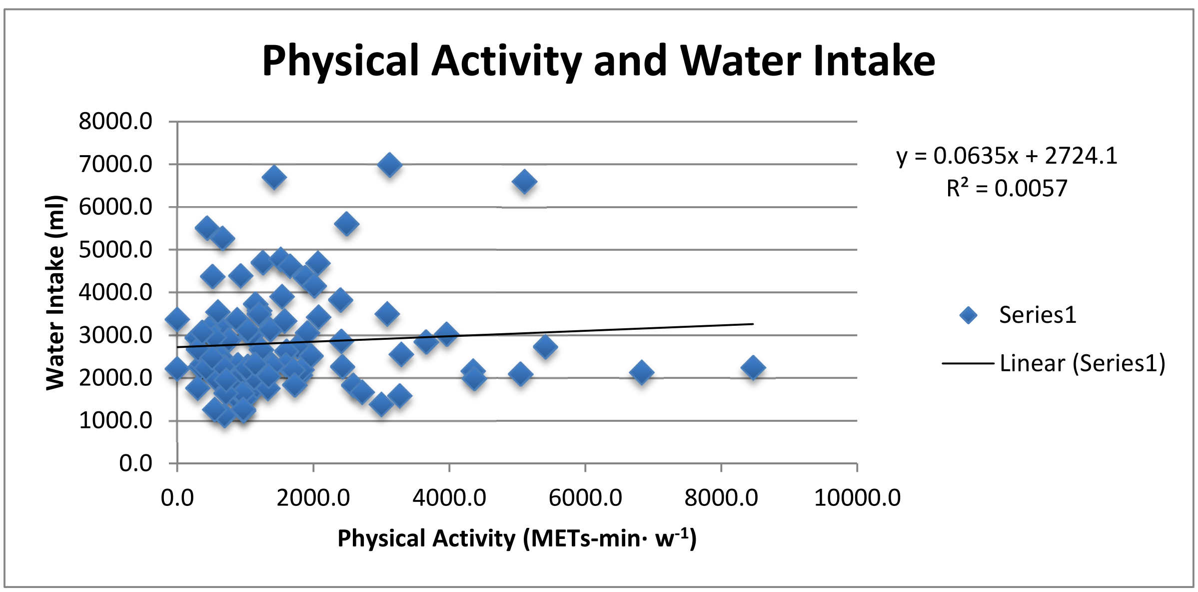
Although not significant, higher scores were found for UOsm, USG, MET-min· w^{-1}, water from beverages, and Total Water Intake in males than females. Also, although not significant, there was a slight decrease in UC, and a slight increase in weight, water from food, water from beverages, and Total Water Intake as physical activity increased from low, moderate, to high levels. For the average (Table 8), total average UOsm (589±290) is strongly correlated to total average water from food (0.51±0.22 L, P=0.022) at a significance level of P≤0.05 (2-tailed), total average water from beverages (2.34±1.13 L, P=0.000), total average water intake (2.83±1.22 L, P=0.000), total average USG (1.015±0.006, P=0.000), total average UC (2.9±.7, P=0.000), and total average weight (76.4±17.3 kg, P=0.000) at a significance level of P≤0.01 (2-tailed) but not strongly correlated to MET-min·w^{-1} (1640±1453). Total average USG (1.015±0.006) is strongly correlated to total average water from food (0.51±0.22 L, P=0.009), total average water from beverages (2.34±1.13 L, P=0.000), total average water intake (2.83±1.2 L, P=0.000), UOsm (589±290, P=0.000), UC (2.9±0.7, P=0.000), and total average weight (76.4±17.3, P=0.000) at a significance level of P≤0.01 (2-tailed) but not strongly correlated to MET-min·w^{-1}. Total average UC (2.9±0.7) is strongly correlated to total average water from food (0.51±0.22 L, P=0.002), total average water from beverages (2.34±1.13 L, P=0.000), total average water intake (2.83±1.2 L, P=0.000), total average UOsm (589±221, P=0.000), and total average USG (1.015±0.006, P=0.000) at a significance level of P≤0.01 (2-tailed) but not strongly correlated to total average weight, or total average MET·min·w^{-1}. Total average water from food (0.51±0.22 L) is strongly correlated to total average water from beverages (2.34±1.13 L. P=0.002), total average water intake (p=0.000), total average USG (1.015±0.006, P=0.009), total average UC (2.9±0.7, P=0.002) at a significance level of P≤0.01 (2-tailed) and UOsm (589±221, P=0.022) at a significance level of P≤0.05 (2-tailed) but not strongly correlated to total average weight or total average MET-min·w^{-1}. Total average water from beverages (2.34±1.13 L) is strongly correlated to total average water from food (0.51±0.22 L P=0.002), total average water intake (2.83±1.2 L, P=0.000), total average UOsm (589±221, P=0.000), total average USG

(1.015±0.006, P=0.000), and total average UC (2.9±0.7, P=0.000) at a significance level of $P\leq0.01$ (2-tailed) but not strongly correlated to total average weight or total average MET-min·w^{-1}. Total average water intake (2.83±1.2 L) is strongly correlated to total average water from food (0.51±0.22 L, P=0.000), total average water from beverages (2.34±1.13 L, P=0.000), total average UOsm (589±221, P=0.000), total average USG (1.015±0.006, P=0.000), and total average UC (2.9±0.7, P=0.000) at a significance level of $P\leq0.01$ (2-tailed) but not strongly correlated to total average weight or total average MET-min·w^{-1}. Total average weight (76.4±17.3) is strongly correlated to total average UOsm (589±221, P=0.000), and total average USG (1.015±0.006, P=0.000) at a significance level of $P\leq0.01$ (2-tailed) but not strongly correlated to total average weight, UC, MET-min·w^{-1}, total water intake, water from beverages, or water from food. Total average MET-min·w^{-1} is not strongly correlated with any of the variables in the study. For the regression, we used physical activity as a continuous variable instead of placing the participants in the three categories of low, moderate, and high physical activity. The only significant changes seen over time were as Total Water Intake increased, UOsm and USG tended to decrease. No changes were seen over time between physical activity and the other variables.

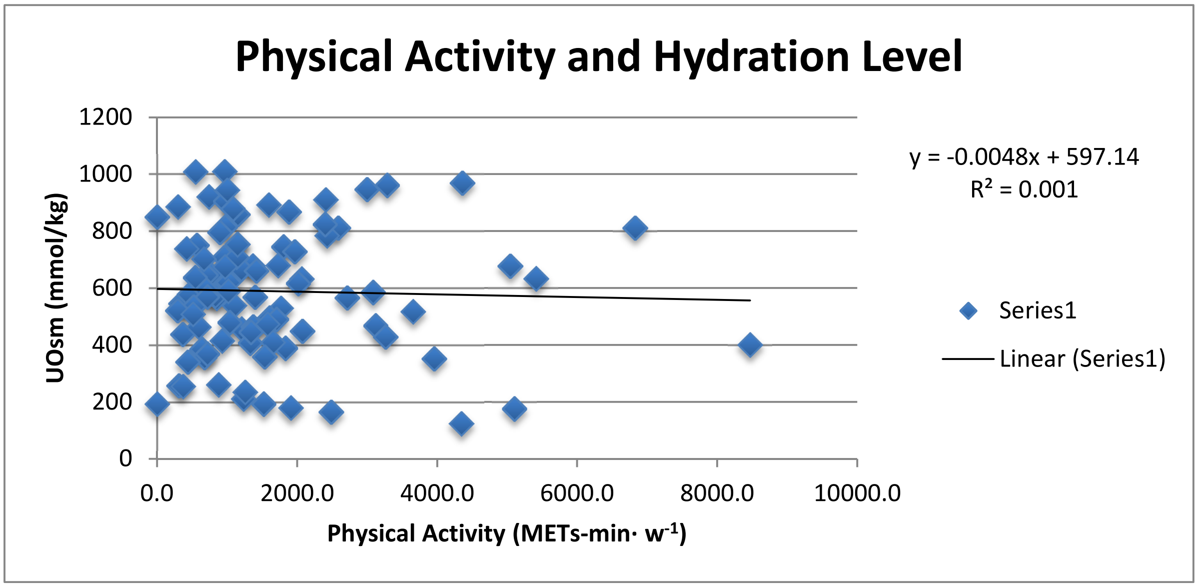
Table 8- Correlations (R- value) of the Total Average for each variable * significance of *P*≤0.05, ** significance of *P*≤0.01

Variable	UOsm	USG	UC	Weight	TWI	WFF	WFB	MET-min·w^{-1}
UOsm		0.977**	0.789**	0.405**	-0.375**	-0.234*	-0.359**	-0.032
USG	0.977**		0.861**	0.380**	-0.383**	-0.266**	-0.364**	-0.035
UC	0.789**	0.861**		0.145	-0.406**	-0.310**	-0.382**	-0.164
Weight	0.405**	0.380**	0.145		-0.763	-0.073	-0.892	0.156
TWI	-0.375**	-0.383**	-0.406**	-0.763		0.467**	0.983**	0.076
WFF	-0.234*	-0.266**	-0.310**	-0.073	0.467**		0.311**	0.085
WFB	-0.359**	-0.364**	-0.382**	-0.892	0.983**	0.311**		0.083
MET-min·w^{-1}	-0.032	-0.035	-0.164	0.156	0.076	0.085	0.083	

A)



B)



C)

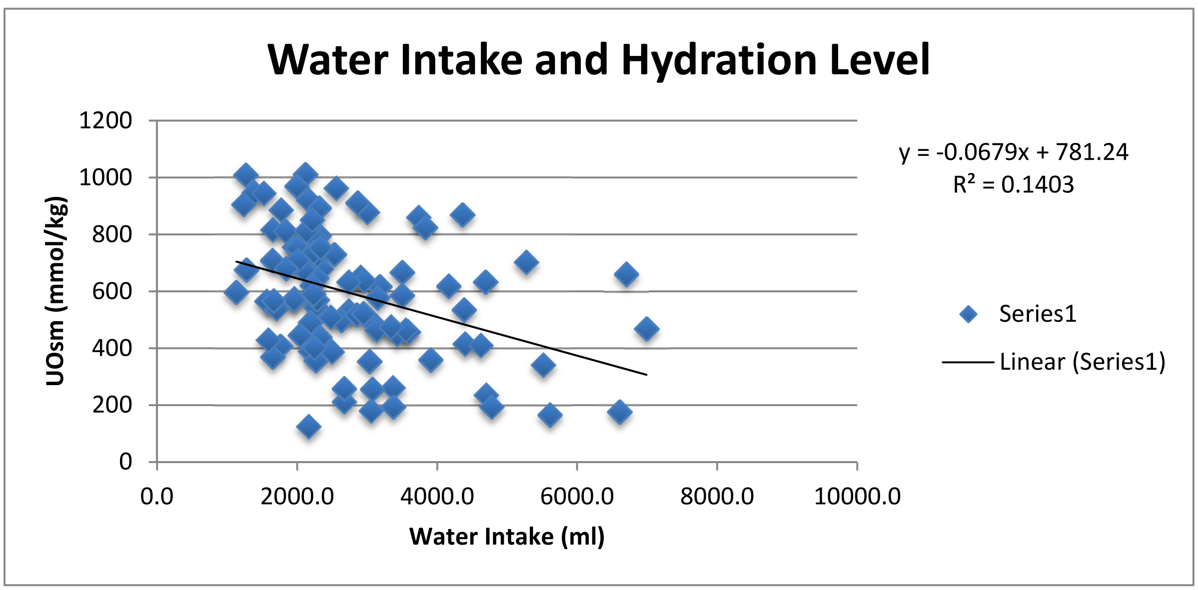


Figure 5- Correlations of A) Physical Activity (MET-min·w^{-1}) and Water Intake (ml), B) Physical activity (MET-min·w^{-1}) and UOsm (mmol/kg), C) Water Intake (ml) and UOsm (mmol/kg)

The average for the entire study (Fig. 6) consisted of 24% (n=11) of males, and 38% (n=19) of females, and 32% (n=30) total reached the daily water intake recommendations, 41%

(n=45) of participants did not reach the recommended percentages of water from food, and only 14% (n=13) were in the 18-20% range of water from food, 67% (n=64) of participants were euhydrated, and 59% (n=56) of the participants reached the daily physical activity recommendations (note: weeks 1 and 3 were averaged together however if one week a subject was low physical activity and the next week moderate physical activity, that subject would be placed in the low category because their average did not reach the moderate activity recommendations).

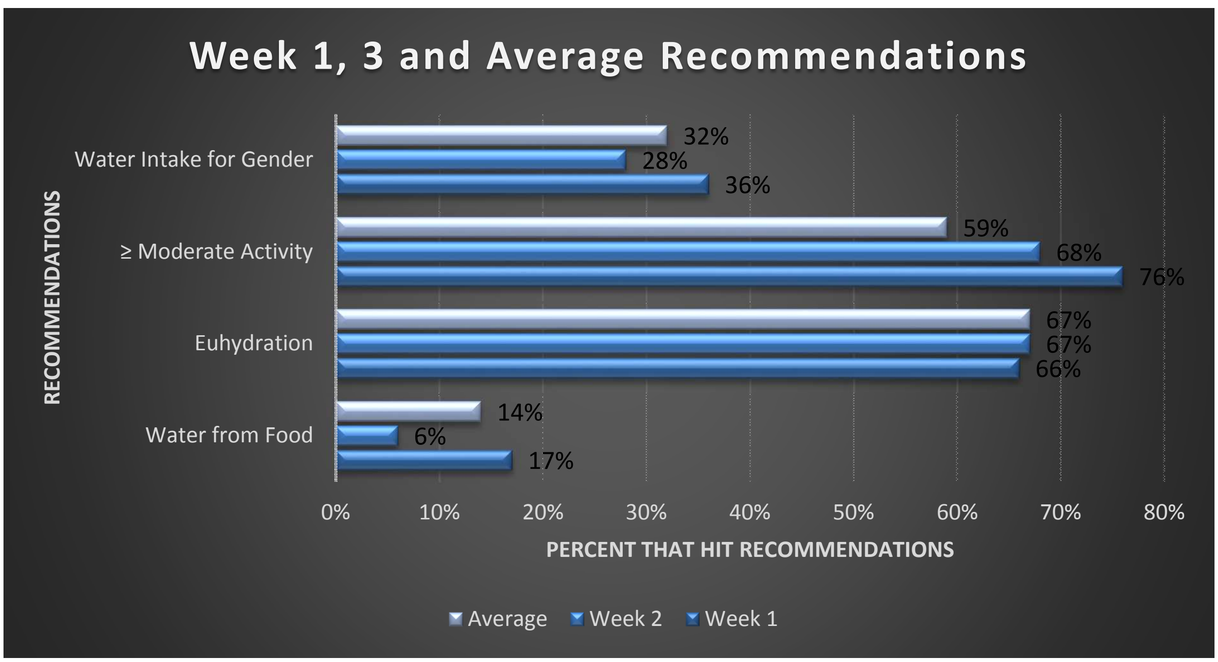


Figure 6- The average for the entire study (shown beside weeks 1 and 3 for comparison) consisted of water intake (n=11) of males, (n=19) of females, (n=30) total, water from food (n=13), euhydration (n=64), ≥ moderate activity (n=56).

Discussion The purpose of the present study was to study the effects of physical activity on hydration status and total water intake in healthy and active but non-athletic adults. We hypothesized that there would be a negative correlation between hydration markers and physical activity levels (as physical activity increases, hydration markers will show more signs of hypohydration). The results show that although there was a slight increase towards hypohydration in hydration markers as well as an increase in water intake as physical activity increased it was not significant. We believe this is due to the small range of physical activity level across the three categories low (822±698 MET-min·w^{-1}), moderate (1791±1195 MET-min·w^{-1}), and high (4728±1150 MET-min·w^{-1}). To put this into perspective, 822 MET-min·w^{-1} is equivalent to running a 6 minute pace for about 8 minutes and 30 seconds 6 days a week. 1791 MET-min·w^{-1} is equivalent to running a 6 minute pace for about 18 minutes and 30 seconds 6 days a week. 4728 MET-min·w^{-1} is equivalent to running a 6 minute pace for about 50 minutes 6 days a week. Although one may consume more fluids during or after a 50 minute run compared to an 8 minute and 30 seconds run it is not all that significant. However, if there was a wider range of physical activity across the three categories we may have seen stronger correlations between physical activity, water intake, and hydration status. Also, the lack of significance could also be due to the self-reporting of water intake and physical activity.

For week 1, 3, and the total average there was no significant difference between males and females in UOsm, USG, UC, Weight, MET-minutes·w^{-1}, water from food, water from beverages, and Total Water Intake. Males and females were combined for the rest of the results as there were no significant differences between genders and we wanted to keep a large sample in each physical activity category. There was no significant difference between the low physical activity, moderate physical activity, and high physical activity categories in UOsm, USG, UC, weight, water from food, water from beverages, and Total Water Intake in week 1, 3, or the total average. However, as most would assume, there was a significant difference in Met-min·w^{-1} as physical activity level increased from low, moderate to high. Although not significant, we do see a higher score of UOsm, USG, MET-min·w^{-1}, water from beverages and Total Water Intake in males than females. Also, we do see a slight decrease in UOsm, UC, and a slight increase in weight, water from beverages and Total Water Intake from low, moderate, to high physical activity levels. 31% (n=14), 18% (n=8), and 24% (n=11) of males; 40% (n=20), 38% (n=19), and 38% (n=19) of females; and 36% (n=34), 28% (n=27), and 32% (n=30) total reached the daily water intake recommendations in week 1, 3, and total average, respectively. 39% (n=37), 47% (n=45), and 41% (n=45) of participants did not reach the recommended percentages of water from food intake in week 1, 3, and total average, respectively. Only 17% (n=16), 6% (n=6), and 14% (n=13) were in the 18-20% range of water from food in week 1, 3, and total average, respectively.66% (n-63), 67% (n=64),67% (n=64) were euhydrated in week 1, 3, and total average, respectively. 76% (n=72), 68% (n=65), 59% (n=56) of the participants reached the daily physical activity recommendations in week 1, 3, and total average, respectively. Based on these results, there seemed to be a slight decrease in water intake and physical activity between week 1 and week 3. Also, UOsm is significantly correlated with USG, UC, weight, and Total Water Intake. Total Water Intake is significantly correlated with UOsm, USG, UC, water from food and water from beverages. Weight could be correlated to hydration status because of the increase (or lack of) of water in the body. Also, it could be due to muscle gains or food and beverage intake. The results show that the human body is very efficient at maintaining proper hydration across various physical activity levels on its own. However, more research needs to be done with higher levels of physical activity.

Limitations: Self-reported data was administered to the participants via the health history form, International Physical Activity Questionnaire (IPAQ), and water frequency questionnaire. The results of this study heavily rely on these results. Therefore, the results could be skewed if the questionnaires are not completed with accuracy and honesty.

Conclusion: Based on our results, there was no relationship between the three levels of physical activity and hydration and water intake status. Water intake did not significantly increase with the increase of physical activity, and hydration status did not significantly change either. This shows the human body can effectively control its own hydration levels to an extent. A larger range of MET-min·w^{-1} needs to be studied. As expected, there was a relationship with hydration status and water intake. There was an uneven dispersion of participants in the low, moderate, and high physical activity categories which could be skewing the data of each variable used. In the future, even dispersion of participants should be a focus. Further research in looking at physical activity, hydration, and water intake is needed with a larger sample size to even the physical activity categories and broader range of physical activity level.

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Appendix

UNIVERSITY OF ARKANSAS

Office of Research Compliance
Institutional Review Board

March 21, 2014

MEMORANDUM

TO:	Stavros Kavouras J.D. Adams Thomas Vidal Joseph Robillard Weldon Murray Ainsley Huffman	Evan Johnson Lynndee Summers Mikell Hammer Rebecca Mishler Ryan Peters Costas Bardis
FROM:	Ro Windwalker IRB Coordinator	
RE:	New Protocol Approval	
IRB Protocol #:	14-03-555	
Protocol Title:	*Assessing Dietary Water Intake: A Validation Study*	
Review Type:	☐ EXEMPT ☐ EXPEDITED ☒ FULL IRB	
Approved Project Period:	Start Date: 03/21/2014 Expiration Date: 03/16/2015	

Your protocol has been approved by the IRB. Protocols are approved for a maximum period of one year. If you wish to continue the project past the approved project period (see above), you must submit a request, using the form *Continuing Review for IRB Approved Projects*, prior to the expiration date. This form is available from the IRB Coordinator or on the Research Compliance website (http://vpred.uark.edu/210.php). As a courtesy, you will be sent a reminder two months in advance of that date. However, failure to receive a reminder does not negate your obligation to make the request in sufficient time for review and approval. Federal regulations prohibit retroactive approval of continuation. Failure to receive approval to continue the project prior to the expiration date will result in Termination of the protocol approval. The IRB Coordinator can give you guidance on submission times.

This protocol has been approved for 133 participants. If you wish to make *any* modifications in the approved protocol, including enrolling more than this number, you must seek approval *prior to* implementing those changes. All modifications should be requested in writing (email is acceptable) and must provide sufficient detail to assess the impact of the change.

If you have questions or need any assistance from the IRB, please contact me at 210 Administration Building, 5-2208, or irb@uark.edu.

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