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## Hypohydration and Mood State in Free-Living Males and Females

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Kinesiology

by

Thomas Edward Vidal Henderson State University Bachelor of Science in Biology, 2012

> May 2015 University of Arkansas

This thesis is approved for recommendation to the Graduate Council
Dr. Stavros Kavouras Thesis Director
Dr. Matthew Ganio Committee Member
Dr. Brendon Mcdermott

Committee Member

#### **Abstract**

Previous research has shown that acute dehydration can result in changes in mood. These changes have been reported in less than a 1% loss in total body water. However, the effect of hypohydration (i.e., reflected through high urine concentration) on mood in free-living conditions has not been studied. **PURPOSE:** The present study was designed to determine if hydration status is associated with mood within the general population under free-living conditions. **METHODS:** A group of 103 apparently healthy subjects (49 male, 54 female, 41±14 y, 1.7±0.1 m, 76.1±16.9 kg) completed three visits separated by a week. Mood was assessed by the Profile of Mood States (POMS) questionnaire during each visit. Participants were familiarized to the POMS questionnaire on their first visit. Hydration was assessed via urine osmolality (uOsm), urine specific gravity (USG), and urine color (UC) done on both spot and twenty four hour (24-h) urine samples taken during the 2nd and 3rd visits. Urine indices and POMS data from the 2nd and 3rd visit were averaged to attain measurements for analyses. **RESULTS:** Overall USG displayed significance in predicting changes in Vigor/Acuity (P = 0.031). UOsm (P = 0.006) and USG (P = 0.012), as well as 24-h uOsm (P < 0.001) and USG (P < 0.001) 0.001) showed significance in predicting Vigor/Acuity in females. 24-h uOsm (P = 0.012) and USG (P = 0.004) were a significant predictor of a female's feelings of friendliness. No significant relationships were found for the male subjects. **CONCLUSION:** These data suggested that hydration status affects mood specifically in free-living females but not in males.

## Acknowledgements

First and foremost I would like to thank God for all that he has done for me and given me over the last couple years. I am certain that without his help and guidance I wouldn't be where I am today. I also want to thank my parents and siblings for their unwavering support in me throughout this entire process. Without their reassurance and guidance I believe I would have given up a long time ago. I would also like to thank Dr. Kavouras for the opportunity to participate in his research and all the guidance he has provided. I would like to thank Dr. Evan Johnson for the knowledge, wisdom and assistance he has provided without it this process would have taken much longer. Finally I want to thank everyone in my lab group and in the Human Performance lab that assisted with data collection, without their help I'm not sure we ever would have finished. Thank you to the College of Health and Education Professions for the opportunity of obtaining a master's degree.

## **Table of Contents**

INTRODUCTION	1
PURPOSE	4
SUBJECTS	5
EXPERIMENTAL DESIGN	5
PROCEDURES	5
MOOD ASSESSMENT	6
URINE ANALYSIS	7
STATISTICAL ANALYSIS	7
RESULTS	7
DISCUSSION	12
ASSUMPTIONS	19
CONCLUSION	19
REFERENCES	21
APPENDIX	2.4

List of Tabl	les & F	ligures	Page #
Table 1	-	Baseline Descriptive Variables	8
Table 2	-	Average mood scores and t-test P values for males and fema	les for visit 5,
	8, av	verage of both visits, and differences between visits.	9
Table 3	-	Linear regression p values of spot urine indices in relationsh	nip with mood.
			11
Table 4	-	Linear regression p values of 24-h urine indices in relationsl	hip with mood.
			12
Table 5	-	Correlation coefficients of linear regression analysis of urine	e indices and
	Vigo	or in females.	15
Figure 1	-	Overall Spot uOsm vs Vigor	13
Figure 2	-	Female and male Vigor scores vs spot USG.	14
Figure 3	-	Female Spot & 24-h uOsm vs Vigor	16
Figure 4	-	Female Spot & 24-h USG vs Vigor	17
Figure 5	-	Female 24-h USG vs Friendliness	18
Figure 6	-	Female 24-h uOsm vs Friendliness	19

## Introduction

Hydration can be defined as total body water volume. It is only one aspect that contributes to an individual's total body fluid volume. Other key contributors are the distribution and composition of the fluid. The fluid that is a key contributor to fluid balance is water. Water has been documented in its importance in various physiological processes which include: the solution that metabolism occurs within; its functions as a reactant and a product; a shock absorber (e.g. for the brain); the method of transport for essential substrates such as gases, heat, hormones, etc. Water also serves as the thermal safeguard of the body (Cotter, Thornton, Lee, & Laursen, 2014). The impact of acute or chronic reductions of total body water is known to have severe detrimental effects on the body, with the extreme circumstance being death. The importance of hydration and its possible relationship with performance, disease prevention and mood has been supported in various studies (Begum, 2010; Cheuvront, 2010; Pross, 2014).

Multiple studies have produced similar results when comparing hydration status to performance in endurance exercise. Lopez et al. (2011) demonstrated in their research with 14 individuals completing 2 trials of trail running in the heat, with one trial being in a euhydrated state and the other being in a hypohydrated state, that the hypohydrated trial times were significantly slower than the euhydrated trials. Research has also been done on cyclists where trained male cyclist completed an uphill 5 km course in the heat in either a euhydrated or a hypohydrated state. The hypohydrated individuals performed worse with slower times, than their euhydrated counterparts (Bardis, Kavouras, Arnaoutis, Panagiotakos, & Sidossis, 2013). The results of these studies support the idea that an individual's hydration status is highly correlated to his/her performance.

The research on whether or not the general population experiences the adverse effects of dehydration is inconclusive. However, there are specific populations that may be at a higher risk than others. These include but are not limited to younger populations such as children, and elderly populations.

Previous research has presented results that suggest children who participate in physical activity also experience voluntary hypohydration. In a study conducted by Y Bar-David, Landau, Z Bar-David, and Pilpel (2009) over 400 children's individual hydration status, determined by urine osmolality, showed that 67.5% of the children expressed an osmolality above 800 mOsmol kg(-1) H(2)O. This suggests that children may be dehydrated and at a higher risk for heat stroke when participating in physical activity in the heat. Unfortunately children are not the only population at risk of voluntary hypohydration; elderly individuals may also be at risk.

Epidemiological studies have shown an association, although not necessarily a causal one, between a low habitual fluid intake and some chronic diseases, including urolithiasis, constipation, asthma, cardiovascular disease, diabetic hyperglycemia, and some cancers. There is evidence to support the idea that acute hypohydration may be a precipitating factor in a number of acute medical conditions in elderly persons (Maughan, 2012). Also research has been conducted in which hydration status was related to cognitive skill performance, such as processing speed and memory skills. In this study dehydration was associated with a negative effect on processing speed and memory. (Suhr, Hall, Patterson, & Niinisto 2004). Based on this information it can be inferred that elderly populations are at a higher health risk when hypohydrated. Hydration status is essential for many physiological processes that contribute to overall health the least of which is mood.

While there is an abundance of evidence on the negative impact of hypohydration on specific populations as well as performance during endurance related competition, there is also substantial evidence of the impact of hypohydration on mood. Some research has observed the effects of hypohydration on mood in which an inverse relationship was calculated as the level of hypohydration increased (Lieberman, 2005; Masento, 2014; Pross, 2013). This relationship has been supported through research done on specific populations including college athletes and military personal.

Ganio et al., (2011) demonstrated, in healthy college males, that both exercise and diuretic-induced hypohydration result in decreased self-reported scales of cognition and mood. A similar study was also done with females in which mild dehydration was induced using moderate exercise; a subsequent decrease in mood resulted (Armstrong et al., 2012). The implications of these results are important because in theory an individual's mood may affect his/her motivation to continue physical or daily activity. This makes the management of mood important for consideration when applied to populations that benefit from adherence to consistent physical activity. Military personal usually don't have problems with adherence to physical activity but they can also benefit from a positive mood state.

Lieberman et al., (2005) demonstrated that when elite military personnel are exposed to prolonged stressful combat simulations, severe decrements in mood are observed. Even though mood can be influenced by multiple external and internal factors, individuals in the study did experience a loss of 6% total body water. It therefore should not be overlooked when Pross et al., (2014) demonstrated that low fluid-drinkers experience less of a change in self-reported mood scores when fluid intake is increased. Less of a change in a soldier's mood during combat could improve the odds of that individual making a critical decision that could save his/her life as well

as the lives of others. While there is clear evidence of a possible relationship between hypohydration and mood under extreme conditions, there also exists some evidence to suggest that free-living individuals may experience a change in mood with a subsequent change in hydration status.

A dramatic change in mood state could possibly affect how that individual works, communicates, and acts with others in his/her everyday life. A negative mood state could result in negative consequences that may affect the stability of his/her relationships with others. An individual's mood state can also affect how a student performs in the classroom due to his/her ability to focus on the task at hand (Edmonds & Jeffes, 2009). Unfortunately very little research has been done on how a free-living individual's hydration status might affect his/her mood.

While the general population may not have to make life or death decisions or run an extreme endurance race in everyday life, the previous implications should not be underestimated. The majority of research that has been done has induced hypohydration through a > 2% loss in total body water (Armstrong, 2012; Lieberman, 2004; Masento, 2014). However, research has been conducted that supports the idea that a change in total body water that is < 2% results in a negative change in mood (Ganio, 2011). It is unclear whether or not individuals in everyday life suffer from chronic or acute dehydration as a result of a loss in total body water that is < 2% but it seems more likely that the general population experiences a loss in total body water that is < 2%. Whether or not this change affects his/her mood is unclear.

## **Purpose**

The present study was designed to determine if hydration status is associated with mood within the general population under free-living conditions.

## **Subjects**

The current study was conducted under the predetermined guidelines of the Declaration of Helsinki and the modified protocol was approved by the University of Arkansas Institutional Review Board for human studies on May 1, 2014. Written informed consent was received by all 103 participants (49 male, 54 female, 41±14 y, 1.7±0.1 m, 76.1±16.9 kg) before participation. Any participant that expressed evidence of any chronic diseases based on the medical history questionnaire, was pregnant, breastfeeding, was a woman who had unprotected sex in the last 60 days, had recently had a surgical operation on the digestive tract, started a regular drug treatment 15 days prior to the start of the study, was currently exercising >4 h per week, changes in diet during the last month, change in weight >2.5 kg (~5 lbs.) in the last month, or was unable to participate in the entire study were excluded from participation.

## **Experimental Design**

In this cross-sectional study stratified random sampling was utilized to account for the convenient sample taken from the Northwest Arkansas area. This study consisted of 103 participants completing three visits with a total of one week in between visits. Prior to the first visit participants filled out a medical history form and a consent form to ensure that each participant meet the inclusion criteria and Institutional Review Board requirements.

## **Procedures**

To become more familiar with the protocol every participant completed one visit in which they provided a urine spot sample, body weight, height, completed a Profile of Mood States (POMS) questionnaire and completed a DEXA scan. The POMS data collected at this time point were not used in the final statistical analysis. Also this was the only time height and a DEXA

scan data were collected for the entire study. The next two visits were identical in terms of procedures and only differed in time of data collection. Approximately 6 days after the first visit the participant met with a technician at which time they were provided with a 24-h urine container and instructed to only urinate in the container for the next twenty four hours. The time of instruction for the 24-h container was scheduled as close to 24-h before the next visit as was conveniently possible for the participant. Twenty four hours after the container was provided the participant returned to the lab at which time they provided the 24-h container with the urine sample. During this visit the participant also provided a spot urine sample, body weight, and completed a POMS questionnaire. The procedure for 24-h urine collection and visit 2 were repeated for visit 3. At the end of every visit the technician scheduled a visit with the participant. Times of each visit were kept as consistent as possible. Following visit three the participant was provided with monetary compensation for completing the study.

#### **Mood Assessment**

The Profile of Mood states 2 Adult short is a self-administered scale that assesses various mood states. The POMS is adaptable to capturing transient and fluctuating feelings, or relatively enduring affect states in adults aged 18+ years.

Results from the POMS 2-A Short contribute to a comprehensive assessment by providing indications of potential mood disturbance. The questionnaire consist of 35 words that are answered on a 5 point-scale in response to the question: "How are you feeling right now?" The answers are grouped and factored into 7 different sub-scales; six of these sub-scales are used to calculate an individual's overall Total Mood Disturbance score. The POMS 2A-short was administered at all 3 visits.

## **Urine Analysis**

Spot urine samples were analyzed for urine osmolality using freezing point depression. Urine was also tested for urine specific gravity (USG) and urine color. This was completed for every urine sample within 1 hour of receiving the sample. The same procedures were repeated for the 24-h urine that was collected in addition to the mass of the urine being recorded.

## **Statistical Analysis**

Linear Regression was performed when determining whether or not a relationship exist between hydration status and mood state. This was repeated for all mood sub-scales and TMD against all urine indices. The statistical level of significance was fixed at P < 0.05 (2 tailed). Descriptive variables, such as height and weight, were taken from the DXA scan that was administered on the first visit. Multiple independent-samples t-test were conducted to compare mood in males and females for visits 5, 8, the average of both visits, and differences between visits.

## **Results**

One hundred and three participants (49 male, 54 female, 41±14 y, 1.7±0.1 m, 76.1±16.9 kg) completed the study. Complete participant demographics separated by gender are provided in Table 1. Since multiple measures of mood and hydration status were taken at different times in order to obtain a representative sample of the subject's mood and hydration status, the results of all assessments were pooled and averaged for final analysis. Each mood sub-scale and TMD was compared to each urine indices for both the spot urine and 24-h urine samples. The data was then split by gender and repeated.

Table 1. Demographics for all participants and separated by gender.

Sex		N	Minimum	Maximum	Mean	Std. Deviation
Both	Age		18.80	64.9	40.71	13.92
	Height		1.36	1.9	1.70	0.10
	Weight	102	47.22	118.0	76.11	16.91
	BMI		14.60	44.8	26.44	5.52
	PercFat		4.30	54.4	31.55	11.60
Female	Age		20.3	64.9	41.53	14.47
	Height		1.35	1.77	1.63	0.074
	Weight	53	47.99	118.02	71.33	16.48
	BMI		18.4	44.8	26.9	6.25
	PercFat		18.8	54.4	37.7	9.57
Male	Age		18.8	63.9	39.83	13.38
	Height		1.61	1.91	1.77	0.067
	Weight	49	47.22	117.48	81.28	15.95
	BMI		14.6	37.2	25.9	4.59
	PercFat		4.3	52.0	24.9	9.78

All demographics were taken from the DEXA scan administered on the first visit. Age (years); Height (meters); Weight (kilograms);

Multiple independent t-test was conducted to compare mood in males and females. These test compared gender against mood scores for visits 5 and 8, as well as the average of the two visits and the difference between the two visits. There was no significant difference in the scores for males and females for any of the previous conditions. (Table 2). This suggest that males and females did not differ in mood scores on visits 2 and 3.

Table 2. Average mood scores and t-test P values for males and females for visit 5, 8, average of both

visits, and differences between visits.

Subscales	Male	Female	P value
Visit 5			
Anger/Hostility	$0.96 \pm 1.7$	$0.55 \pm 1.6$	0.215
Confusion/Bewilderment	$1.65 \pm 2.3$	$1.85 \pm 3.7$	0.748
Depression/Dejection	$1.02 \pm 1.8$	$0.43 \pm 1.2$	0.054
Friendliness	$12.88 \pm 3.5$	$13.89 \pm 3.7$	0.162
Fatigue/Inertia	$3.20 \pm 3.3$	$3.94 \pm 3.9$	0.305
Tension/Anxiety	$1.98 \pm 2.3$	$1.66 \pm 2.8$	0.534
Vigor/Acuity	$10.3 \pm 4.4$	$10.0 \pm 4.8$	0.769
Total Mood Disturbance	$11.6 \pm 10.1$	$12.3 \pm 12.1$	0.750
Visit 8			
Anger/Hostility	$0.78 \pm 1.7$	$0.92 \pm 1.5$	0.640
Confusion/Bewilderment	$2.18 \pm 2.9$	$1.83 \pm 2.6$	0.518
Depression/Dejection	$1.02 \pm 1.8$	$0.43 \pm 1.2$	0.185
Friendliness	$12.61 \pm 4.3$	$12.91 \pm 4.0$	0.722
Fatigue/Inertia	$3.24 \pm 3.5$	$3.70 \pm 3.8$	0.531
Tension/Anxiety	$1.80 \pm 2.3$	$1.94 \pm 2.7$	0.771
Vigor/Acuity	$10.29 \pm 4.9$	$8.75 \pm 5.0$	0.122
Total Mood Disturbance	$11.51 \pm 11.1$	$13.11 \pm 11.0$	0.467
Average of visits			
Anger/Hostility	$0.87 \pm 1.5$	$0.74 \pm 1.4$	0.652
Confusion/Bewilderment	$1.92 \pm 2.4$	$1.84 \pm 2.5$	0.872
Depression/Dejection	$1.03 \pm 1.8$	$0.50 \pm 1.0$	0.074
Friendliness	$12.74 \pm 3.7$	$13.40 \pm 3.4$	0.357
Fatigue/Inertia	$3.22 \pm 3.1$	$3.82 \pm 3.3$	0.352
Tension/Anxiety	$1.89 \pm 2.1$	$1.80 \pm 2.4$	0.849
Vigor/Acuity	$10.3 \pm 4.4$	$9.39 \pm 4.3$	0.298
Total Mood Disturbance	$11.55 \pm 9.9$	$12.71 \pm 10.2$	0.564
Difference between visits			
Anger/Hostility	$-0.18 \pm 1.5$	$0.38 \pm 1.4$	0.051
Confusion/Bewilderment	$0.53 \pm 2.2$	$-0.02 \pm 3.8$	0.379
Depression/Dejection	$0.02 \pm 1.7$	$0.13 \pm 1.3$	0.706
Friendliness	$-0.27 \pm 2.6$	$-0.98 \pm 3.6$	0.261
Fatigue/Inertia	$0.04 \pm 2.7$	$-0.25 \pm 3.8$	0.663
Tension/Anxiety	$-0.18 \pm 1.9$	$0.28 \pm 2.8$	0.329
Vigor/Acuity	$0.00 \pm 3.0$	$-1.26 \pm 4.7$	0.108
Total Mood Disturbance	$0.08 \pm 7.6$	-0.81 ±	0.637

Values are means  $\pm$  SD, n = 103 (male n = 49, female n = 53) comparisons. P values result from paired t tests comparing males to females for all subscales for visit 5, visit 8, average of the visits, and difference between the two visits.

A simple linear regression was calculated to predict mood based on hydration status.

Hydration status was determined by spot and 24-h urine indices. Linear regression analysis was conducted for all urine indices (uOsm, USG, UC) for both spot and 24-h urine against all subscales and TMD. Gender was also controlled for and analysis were repeated. The resulting regression p values are documented in Tables 3 and 4.

When gender was not accounted for spot USG displayed significance in predicting changes in Vigor/Acuity ( $F_{[1,99]} = 4.773$ ,  $\beta = -138$ , P = 0.031) with an  $R^2 = 0.046$ . Overall spot uOsm and UC were not significant predictors of any sub-scale or TMD (Table 3). Likewise none of the 24-h urine indices were significant predictors of any sub-scale or TMD (Table 4). When the data was split by gender spot uOsm ( $F_{[1,51]} = 8.178$ ,  $\beta = -.372$ , P = 0.006) with an  $R^2 = 0.138$  and USG ( $F_{[1,51]} = 6.87$ ,  $\beta = -211$ , P = 0.012) with an  $R^2 = 0.119$ , as well as 24-h uOsm ( $F_{[1,51]} = 15.968$ ,  $\beta = -0.012$ , P < .001) with an  $R^2 = 0.238$  and USG ( $F_{[1,51]} = 16.312$ ,  $\beta = -466$ , P < .001) with an  $R^2 = 0.242$  showed significance in predicting changes in Vigor/Acuity in females. Spot urine indices as related to all other sub-scales and TMD showed no significance (Tables 3 & 4). In males none of the spot urine indices significantly predicted changes in mood (Tables 3 & 4). 24-h uOsm ( $F_{[1,51]} = 6.807$ ,  $\beta = -15.957$ , P = 0.012) with an  $R^2 = 0.118$  and USG ( $F_{[1,51]} = 9.26$ ,  $\beta = -295$ , P = 0.004) with an  $R^2 = 0.154$ , were both significant predictors of a female's feelings of friendliness. Neither UC from spot or 24-h urine were significant predictors of mood state in either males or females (Tables 3 & 4).

Table 3. Linear regression p values of spot urine indices in relationship with mood.

<b>N</b> / 1		USG	Urine Color
Mood	uOsm	I I N C T	Lirine Color

All subjects			
Anger/Hostility	0.494	0.451	0.402
Confusion/Bewilderment	0.440	0.417	0.553
Depression/Dejection	0.475	0.423	0.209
Friendliness	0.757	0.768	0.934
Fatigue/Inertia	0.860	0.718	0.982
Tension/Anxiety	0.883	0.763	0.627
Vigor/Acuity	0.057	0.031*	0.222
Total Mood Disturbance	0.825	0.758	0.909
Females			
Anger/Hostility	0.276	0.204	0.379
Confusion/Bewilderment	0.584	0.464	0.648
Depression/Dejection	0.293	0.234	0.496
Friendliness	0.316	0.404	0.855
Fatigue/Inertia	0.447	0.661	0.636
Tension/Anxiety	0.986	0.854	0.878
Vigor/Acuity	0.006*	0.012*	0.225
Total Mood Disturbance	0.528	0.705	0.793
Males			
Anger/Hostility	0.974	0.902	0.752
Confusion/Bewilderment	0.577	0.676	0.706
Depression/Dejection	0.510	0.559	0.220
Friendliness	0.419	0.495	0.700
Fatigue/Inertia	0.728	0.788	0.581
Tension/Anxiety	0.822	0.762	0.545
Vigor/Acuity	0.785	0.481	0.596
Total Mood Disturbance	0.840	0.864	0.650
4.11 1 . D 1 C	1.	1 . 0	1

All values represent P values from linear regression analysis. Significance was designated at p < 0.05. \* designates significance

Table 4. Linear regression p values of 24-h urine indices in relationship with mood.

Mood	uOsm	USG	Urine Color
All subjects			
Anger/Hostility	0.891	0.707	0.846
Confusion/Bewilderment	0.657	0.766	0.545
Depression/Dejection	0.952	0.815	0.888
Friendliness	0.261	0.162	0.375
Fatigue/Inertia	0.520	0.524	0.847
Tension/Anxiety	0.704	0.644	0.988
Vigor/Acuity	0.052	0.051	0.391
Total Mood Disturbance	0.527	0.487	0.998
Females			
Anger/Hostility	0.919	0.993	0.473
Confusion/Bewilderment	0.573	0.581	0.693
Depression/Dejection	0.850	0.930	0.652
Friendliness	0.012*	0.004*	0.360
Fatigue/Inertia	0.213	0.345	0.471
Tension/Anxiety	0.254	0.363	0.965
Vigor/Acuity	< 0.001*	< 0.001*	0.154
Total Mood Disturbance	0.134	0.226	0.787
Males			
Anger/Hostility	0.932	0.764	0.802
Confusion/Bewilderment	0.227	0.318	0.589
Depression/Dejection	0.505	0.650	0.676
Friendliness	0.424	0.464	0.948
Fatigue/Inertia	0.818	0.616	0.842
Tension/Anxiety	0.546	0.783	0.995
Vigor/Acuity	0.832	0.826	0.868
Total Mood Disturbance	0.886	0.845	0.935

Significance was designated at p < 0.05. \* designates significance

## **Discussion**

The aim of this research was to determine if a possible relationship exist between an individual's mood and hydration status under free-living conditions. Based on our results a relationship may exist between hydration status and feelings of Vigor and friendliness in free-living females. Specifically 24-h uOsm, 24-h USG, spot uOsm and spot USG may be moderate predictors of Vigor/Acuity in females (Figures 2 & 3). This finding is similar to that found in previous research suggesting that hydration status has an effect on Vigor (Ganio et al. 2011). Also 24-h uOsm and USG may be moderate predictors of a female friendliness (Figures 4 & 5).

Demonstrated by Tables 2 and 3 UC showed no significant correlation in either spot or 24-h urine. This scale may not be sensitive enough to detect changes in mood due to its limited range. Also during data collection the urine was analyzed by multiple technicians and could have led to greater variance in the reported urine colors. Figure 1 demonstrates that overall spot USG is significantly correlated with Vigor. This can be explained when gender is controlled for showing that females are significantly correlated while males are not (Table 3 & Figure 6).

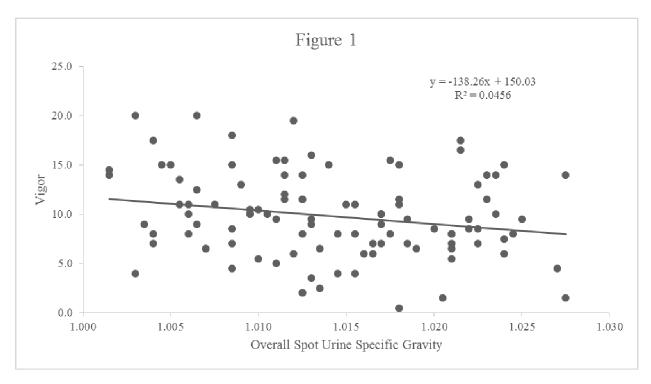


Figure 1. Plots of Vigor mood scores over Overall Spot USG.

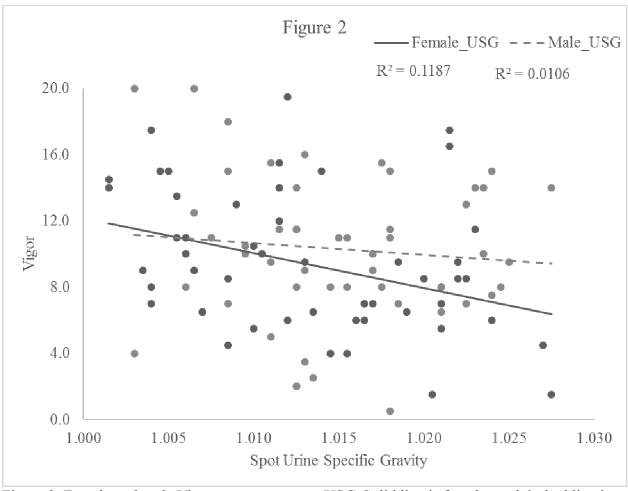


Figure 2. Female and male Vigor scores over spot USG. Solid line is females and dashed line is males.

To our knowledge very few studies have utilized 24-h urine collection as a measure of hydration status in relation to mood. This may be due to feasibility or subject inconvenience since the subjects had to carry around a urine container for twenty four hours. This was compensated for by also supplying a bag with the urine container to make it more discreet. Our results suggest 24-h urine may be a better predictor of Vigor in free-living females (Table 5). Given our limited data it cannot be determined whether or not uOsm or USG is a better predictor of changes in mood in females. Future research should determine if this relationship is dependent

on time of day, whether or not uOsm or USG is a better predictor of changes in mood, and determine the mechanism by which this relationship occurs.

Table 5. Correlation coefficients of linear regression analysis of urine indices and Vigor in females.

	uOsm	USG
24-h	0.488	0.492
Spot	0.372	0.345

All R values are significant.

Of particular interest is the finding that 24-h uOsm and USG are moderate predictors of a female's feelings of friendliness (Figures 4 & 5). To our knowledge this is the first time this has been documented in the research, especially one that did not experimentally induce dehydration. Consequently spot urine indices were not significantly correlated to friendliness in females (Table 3). The fact that 24-h urine is not used very often in mood research may explain why this is not a common finding. These results suggest that feelings of friendliness in females can be adversely effected by changes in hydration status in everyday life. Future research should try and determine if this relationship can be supported in dehydration protocols.

It should be noted that the POMS questionnaire ask the question "How are you feeling right now?" Some may argue that spot urine is a better indication of a person's immediate hydration status when compared to 24-h urine. However, an individual's hydration status is determined by fluctuations in total body water. These fluctuations can be acute and if continuous in occurrence result in a chronic change in total body water. In this respect 24-h urine may serve as a better representation of an individual's hydration status because it encompasses a larger number of fluctuations then spot urine. Future research should determine if changing the POMS question to one that is more representative of a twenty four hour period, such as "How have you been feeling in the last twenty four hours?" yields similar results when compared to 24-h urine.

Our research implies that under free-living conditions females are more susceptible to adverse changes in mood with fluctuations in hydration status then men. These gender differences have been supported in previous research in which greater adverse changes were documented in females when compared to males in dehydration induced protocols (Armstrong et al. 2012, Ganio et al. 2011). The exact mechanism by which this occurs is still unknown. However, our exclusion criteria did not control for the female menstrual cycle and this could have affected our female mood scores. Future research should try to determine if these results are replicated when controlling for the female menstrual cycle.

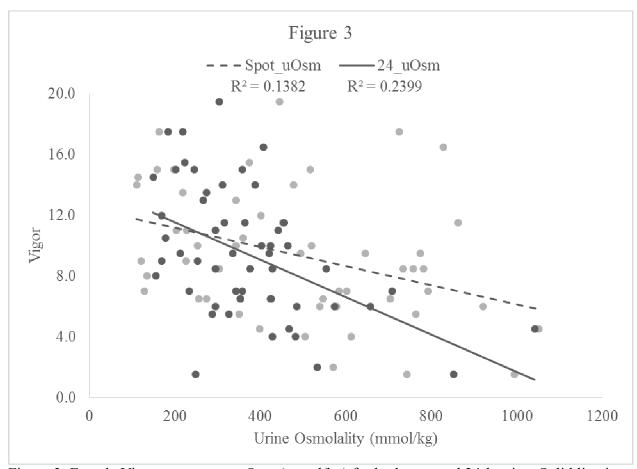


Figure 3. Female Vigor scores over uOsm (mmol/kg) for both spot and 24-h urine. Solid line is 24-h and dashed is spot.

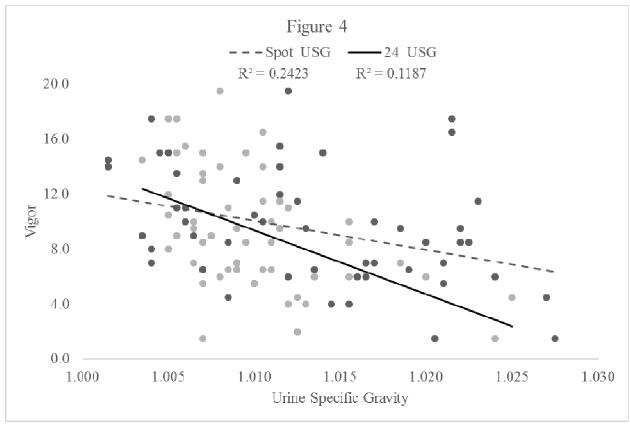


Figure 4. Female Vigor scores over USG for both 24-h and spot urine. Solid line is 24-h urine and the dashed line is spot urine.

The finding that spot uOsm and USG can be used as significant predictors of Vigor and Acuity in females is important due to its feasibility. The convenience of collecting a spot urine sample from a participant is not as invasive as having the participant collect a 24-h sample. This allows future research to be confident that although 24-h uOsm and USG may be a better predictor of Vigor/Acuity in females, spot urine is also reliable (Figures 2 &3).

The area of hydration and mood is a relatively new area of research however these findings do contribute to the overall body of knowledge and provide researchers evidence to support uOsm and USG as both a valid and reliable measures in predicting a female's feelings of Vigor and friendliness. The exact mechanism by which hydration status affects cognitive function is still unknown.

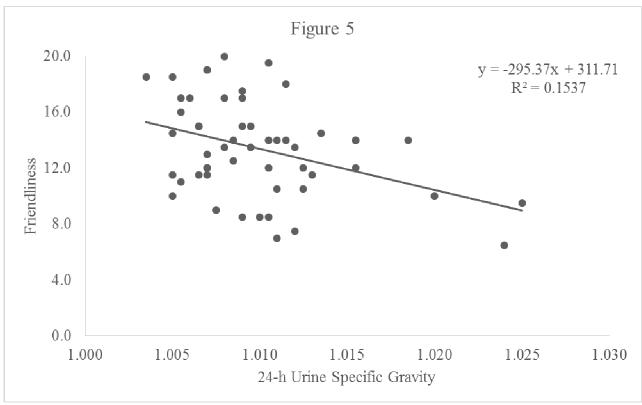


Figure 5. Female Friendliness scores over 24-h USG.

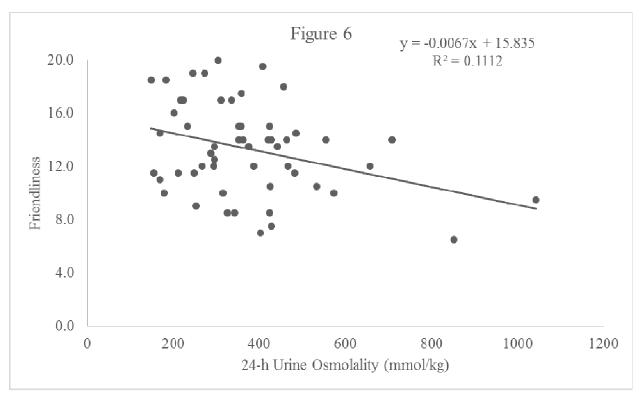


Figure 6. Female Friendliness scores over 24-h uOsm (mmol/kg).

## **Assumptions**

The assumptions of this study are that the participants have not lied about their medications and are not ingesting any kind of diuretic or mood altering medication. We also assume that the participant's will not be exercising more than four days a week. The idea that none of the participants will experience practice effects was assumed due to the administration of the POMS on the first visit.

## Conclusion

In conclusion our results suggest that spot and 24-h uOsm and USG are moderate predictors of Vigor/Acuity in females (Figures 2 & 3). Twenty four hour uOsm and USG were moderate predictors of feelings of friendliness in females (Figures 4 & 5). While the relationship of hydration and Vigor is supported by previous research, the finding of friendliness and

hydration in females to our knowledge has not been documented before. Future researcher should try to replicate these findings and design a more controlled environment to monitor hydration status with mood. Such as assessing mood through an online questionnaire that the participant can take at home, this may be more representative of the individual's mood state. These findings propose that females who experience changes in hydration status in their everyday lives also have adverse changes in mood, specifically feelings of Vigor and Friendliness.

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## **Appendix**



Office of Research Compliance Institutional Review Board

#### March 21, 2014

#### MEMORANDUM Stavros Kavouras Evan Johnson TO: J.D. Adams Lynndee Summers Thomas Vidal Mikell Hammer Joseph Robillard Rebecca Mishler Weldon Murray Ryan Peters Ainsley Huffman 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.

> 210 Administration Building • 1 University of Arkansas • Fayetteville, AR 72701 Voice (479) 575-2208 • Fax (479) 575-3846 • Email irb@uark.edu

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