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## The Effect of Cellular Dehydration on Glucose Regulation in Lean and Obese Females

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The Effect of Cellular Dehydration on Glucose Regulation in Lean and Obese Females

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

by

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Bachelor of Science in Biotechnology, 2012

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This thesis is approved for recommendation to the Graduate Council.

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## Abstract

A chronically elevated vasopressin level has been associated with an impaired glucose regulation system in humans. A recent study in patients with diabetes suggested that low water intake is associated with impaired glucose regulation. The aim of this study was to examine the effect of hypertonic saline infusion on glucose regulation in healthy females. Thirty healthy, non-diabetic females performed a 2-h infusion followed by a 4-h oral glucose tolerance test (OGTT). Every subject participated in two trials separated by one month, in a counterbalanced order. Two trials were identical in the procedure, differing in the concentration of saline infusion, with one trial being 3% NaCl (HYPER), another being 0.9% NaCl (ISO). The HYPER trial led to an increase in plasma osmolality (time point 0 min,  $305 \pm 0.91$  vs.  $287 \pm 0.61$  mmol/kg,  $P < 0.35$ ), plasma volume expansion ( $16.69 \pm 1$  vs.  $9.72 \pm 0.74$  %,  $P < 0.0001$ ) and plasma copeptin (time point -90 min  $7.16 \pm 1.07$  vs.  $3.37 \pm 0.3$  pmol/l,  $P < 0.0001$ ). Plasma glucose during the OGTT was greater in the HYPER trial. These data suggest that the acutely induced cellular dehydration impairs the glucose regulation in healthy females.

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## Introduction

Low water intake has been reported to be associated with chronic diseases (Thornton, 2009). Osmoreceptors that exist in the central nervous system, detect the increase in plasma osmolality, then signal to other parts of the brain to initiate the act of searching for drinking water by intriguing thirst. (Arsenijevic & Baertschi, 1985). Low level of drinking water activates these osmoreceptors in the brain and baroreceptors stimulate the hypothalamus to liberate arginine vasopressin (AVP) into the bloodstream to reabsorb the water in the kidney (Baylis, 1989). When osmolality is elevated, either plasma sodium or glucose levels might be elevated due to the decrease of total body water (Thomas et al., 2008).

Elevated AVP is correlated with plasma osmolality and it has been reported to be associated with the development of diabetes (Saleem et al., 2009). Furthermore, low insulin sensitivity and high risk of developing T2DM have been demonstrated having an association with high plasma copeptin level (Roussel et al., 2016). In a recent study, 565 subjects of a 3615 participants 9-year follow-up study became hyperglycemic and 202 developed diabetes, of whom were drinking less than 0.5 L water a day, compared with other participants drinking more than 0.5 L water a day, suggesting that water intake was inversely associated with hyperglycemia (Roussel et al., 2011). Carroll and her colleagues (2015) found in a study, where the relationship of plain water intake and type 2 diabetes risk was examined, that higher water intake has a positive association in reducing the risk of type 2 diabetes (Carroll, Davis, & Papadaki, 2015). Additionally, in a study by Stookey et al (2004), hypertonicity was independently associated with an increased risk of developing diabetes. Their study further suggested that hypertonicity magnified the effects of elevated glucose level so that

individuals with hypertonicity and high plasma glucose level were four times more likely to develop diabetes than those with elevated plasma glucose only (Stookey, Piper, & Cohen, 2013). It seems evident that associations of low water intake and metabolic syndromes were found in studies discussed above.

Roussel and his colleagues (2016) found high plasma copeptin was associated with reduced insulin sensitivity and an increased risk for IFG/T2DM in subjects investigated, where the participants in the higher quartile of plasma copeptin level had significantly lower insulin sensitivity (Roussel et al., 2016). In another study, AVP was determined to have an association with increased risk of diabetes in older men, which suggests a potential role of the AVP system in diabetes (Wannamethee et al., 2015). More studies show that not only there exists an association between low water intake or high AVP and hyperglycemia, but there are causality links between them. In a study that focused on determining the long-term influence of plasma vasopressin concentration in a rodent model prone to metabolic syndrome, researchers manipulated vasopressin levels in two opposite directions in lean and obese rats groups in a 4-week period. Lean rats with high AVP level had higher fasting glycemia compared to lean rats with normal AVP level. Conversely, utilizing an antagonist of AVP receptor 1A reduced glucose intolerance in obese rats compared to control group, with fasting insulin levels of  $634 \pm 180$  versus  $1526 \pm 163$  pmol/l,  $P < 0.001$  (Taveau et al., 2015). Another study in humans with type 2 diabetes showed that low water intake impaired blood glucose regulation probably due to cortisol responses (Johnson et al., 2017). AVP reduction through water supplementation appears as an attractive candidate for intervention to preventing diabetes and its cardiovascular complications (Mellander, 2016). Based on the existing

literature regarding the role of AVP in glucose regulation, it is positive that AVP system plays an important role in glucose regulation.

As suggested by previous studies that AVP is a small and unstable molecule, making it difficult to measure; copeptin is a precursor molecule of AVP that is produced in same mole amount with AVP in plasma, where it is very stable compared to AVP itself, thus it can be chosen as an alternative marker measured (Morgenthaler, Struck, Alonso, & Bergmann, 2006). Therefore, copeptin reflects the release of AVP and can serve as a reliable surrogate marker for circulating levels of AVP. One study analyzed copeptin baseline data from a population from Malmo Diet and Cancer Study cardiovascular cohort during 1991-1994 and conducted a follow-up experiment where 2064 subjects underwent an oral glucose tolerance test. They found that increased copeptin predicted incident abdominal obesity, diabetes mellitus, and microalbuminuria in diabetes individuals (Enhörning et al., 2013). Furthermore, Enhörning and her colleagues (2015) conducted another study including both diabetes and non-diabetes individuals; they found that copeptin could predict heart failure, coronary artery disease and death specifically in diabetes patients, but not in the non-diabetes individuals.

We hypothesized that hypertonic saline infusion would induce cellular dehydration, acutely raise copeptin levels and show an acute impairment of glucose regulation in healthy lean and obese females. Therefore, combining previous studies and current knowledge gap, the purpose of this study was to investigate the effect of elevated copeptin on glucose regulation in obese and lean females.



## **Methodology**

### **Subjects**

Thirty females with the absence of diabetes (HOMA-IR<6.5), not pregnant, aged between 32-50 years old, were recruited in this study. Fifteen of them were overweight or obese, with BMI 27.5-35 kg/m<sup>2</sup> and 36%-50.6% body fat, and 15 of them were lean, with BMI between 18.5-22.5kg/m<sup>2</sup> and 25.4%-37.6% body fat.

### **Subjects inclusion criteria**

Sedentary lifestyle, non-smoking within the past 6 months, no surgical operation on the digestive tract, no cardiovascular diseases, and no medications impairing water balance. All subjects recruited signed an informed consent prior to the initiation of any trial procedure.

### **Experiment design**

The effect of cellular dehydration on glucose regulation was studied using osmotic stimulation of AVP by hypertonic saline infusion followed by an oral glucose tolerance test (OGTT). All the subjects had two trials separated at least by a week. In one trial the subjects were infused with 3% NaCl (Baxter 3% Sodium Chloride Injection, USP), and in another trial, the subjects were infused with 0.9%NaCl (Baxter 0.9% Sodium Chloride Injection, USP). The hypertonic saline infusion was estimated to increase plasma osmolality from 285 to 300 mmol/kg, while isotonic saline infusion was infused to maintain osmolality at euhydrated thresholds of 285 to 290 mmol/kg. Both experimental trials were kept identical except for the difference in saline infusion concentration administered. Moreover, each

subject performed the hypertonic or isotonic trial in a random, counterbalanced order. All subjects performed both trials during their respective early follicular phase, approximately 1-6 days post menses onset to ensure low endogenous levels of estrogen and progesterone. Subjects consumed a normal diet rich in carbohydrates for 3 days leading up to and refrained from physical activity 24 h prior to each testing day. Dinner and 24h fluid intake pre-test day were standardized while alcohol and caffeine consumption were prohibited.

### **Protocol**

Subjects reported to the lab following 10 h of fasting. After providing a urine sample, the subject remained seated for 30 minutes. An intravenous catheter (BD Angiocath Antoguard 20GA 1.16 IN, 1.1\*30mm) was placed in an antecubital vein and intravenous infusion of either 3% NaCl or 0.9% NaCl commenced over a 120 minutes period at a rate of 0.1ml/min/kg of body weight, with blood samples being taken by technicians at baseline and then following an interval of 30 minutes. After the initial 2 h saline infusion period, the infusion line was removed from the catheter, which was flushed with an isotonic saline/heparin mixture to prevent the intravenous line from clotting, and subsequently a stop-cock (Medex 3-way stop-cock APV=0.5ml, Rx) was attached to allow for continuous blood sampling in 30 min intervals at no discomfort for the participant. Then, subjects started the OGTT that initiated with a 75 g glucose drink (Kosher Oral Glucose Tolerance Drink 75g) ingestion. The OGTT lasted for 240 minutes, during which blood samples were taken, every 30 minutes. Urine sample collection was standardized upon initial arrival to confirm hydration status for both trial days, at the end of the saline infusion and at the end of the

OGTT, while each participant was reminded to communicate urge for urination to the technician for additional urine sampling as needed. Blood pressure was recorded by a manual blood pressure cuff (Suntech Medical's Clinical-Grade Orbit-K Blood Pressure Cuffs) following each blood sampling. Whole blood samples were analyzed in triplicate to average hematocrit and hemoglobin measurements via microhematocrit centrifuge (Unico Micro Hematocrit Centrifuge, Model: C-MH30), reader (Damon/IEC Division Micro-Capillary Reader) and UV spectroscopy (Thermo Scientific Genesys 10S UV-VIS Spectrophotometer) respectively. Remaining whole blood was aliquoted into Lithium Heparin (Lithium heparin (LH) 75 USP Units Blood Collection Tubes), EDTA (EDTA (K2E) 10.8mg Plus Blood Collection Tubes) and Serum separator tubes (SST Plus Blood Collection Tubes) respectively and subsequently spun at 3000 rpm for 10 min. The supernatant was then removed from the spun sample and aliquoted and stored at -80°C for later hormonal analysis. 500µl of lithium heparin plasma was taken for assessment of plasma osmolality via freeze point depression (Advanced Instruments Model 3250 Osmometer). Urine samples were analyzed for specific gravity via refractometry (ATAGO Clinical Refractometer Master-SUR/NM) and urine osmolality via freeze point depression (Advanced Instruments Model 3250 Osmometer) respectively.

### **Statistical analysis**

Dependent variables measured were blood levels of insulin, glucose, and copeptin. Further, plasma osmolality, hematocrit, and hemoglobin values were analyzed for determination of plasma volume expansion and total plasma protein dilution in order to

confirm cellular de-/hydration of subjects. Statistical analysis was performed using JMP Pro 13.2.0. Between-condition differences for plasma osmolality, plasma sodium,  $\Delta$  plasma volume expansion, total plasma protein, glucose, insulin, and copeptin were assessed via two-way repeated measures analysis of variance with post-hoc contrast comparison.

The area under the curve for glucose and insulin was calculated to assess the actual total exposure following OGTT administration. Significant  $\alpha$  level was set at  $p < 0.05$ . Results described as means (SE).

## Results

A total of 30 females completed the study. Plasma sodium was significantly higher during the dehydrated condition starting from the time point -90 min. (euhydrated trial PNa:  $136.64 \pm 0.34$  mmol/L, dehydrated trial PNa:  $143.16 \pm 0.68$  mmol/L,  $P < 0.0001$ ,  $d = 2.23$ ); while at the baseline (-120min), no significant difference in plasma sodium level between conditions existed (euhydrated trial PNa:  $135.7 \pm 0.3$  mmol/L; dehydrated trial PNa:  $136.3 \pm 0.41$  mmol/L,  $P < 0.247$ ,  $d = 0.30$ ) (Figure 1.).

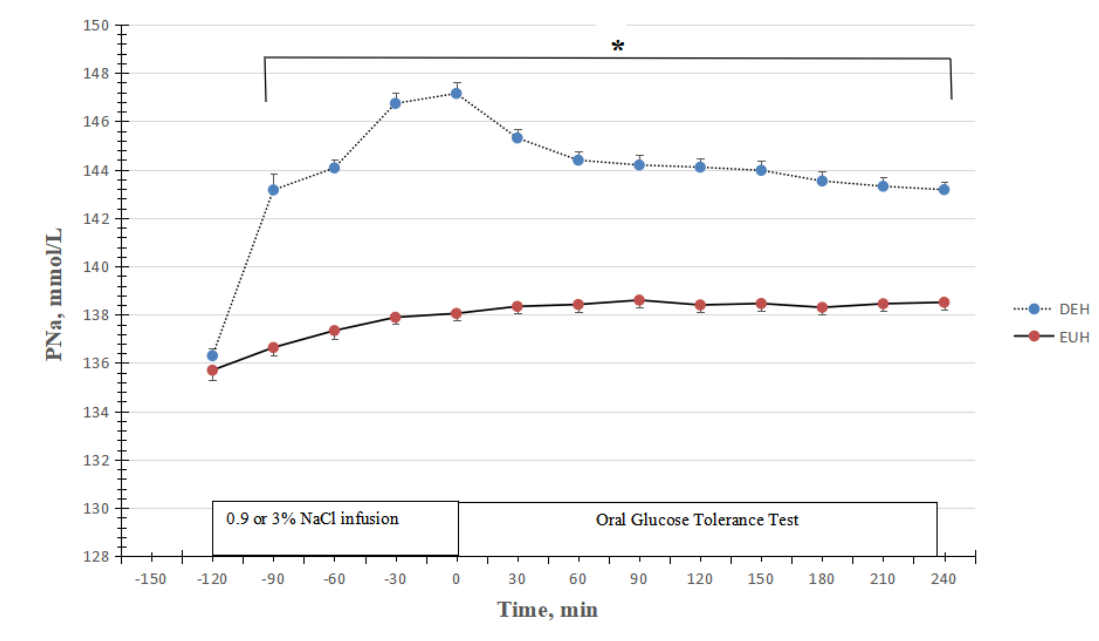


Figure 1. Plasma sodium concentration during the trial. \* denotes the statistically significant difference between trials for the same time point.

In concert, plasma osmolality of the dehydrated trial was significantly higher than that of the euhydrated trial from the time point -90 min (euhydrated trial POsmo:  $285 \pm 0.63$  mmol/kg; dehydrated trial POsmo:  $295.43 \pm 0.78$  mmol/kg,  $P < 0.0001$ ,  $d = 2.7$ ); at the baseline (-120 min), no difference existed between conditions (euhydrated trial POsmo:  $284.22 \pm 0.65$  mmol/kg; dehydrated trial POsmo:  $285.12 \pm 0.66$  mmol/kg,  $P < 0.3527$ ,  $\text{effect} = 0.25$ ) (Figure 2.).

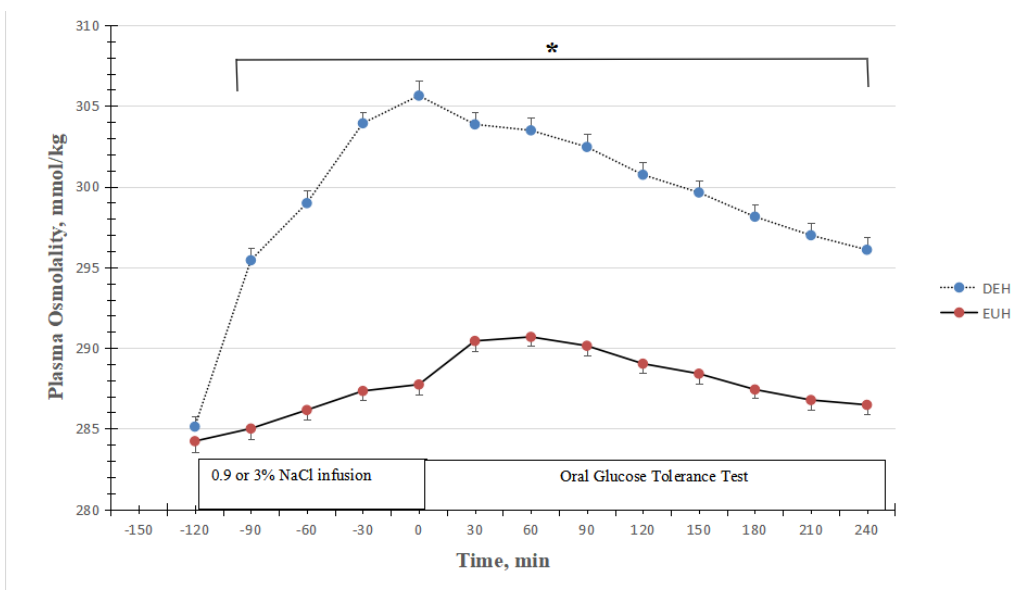


Figure 2. Plasma osmolality during the trial. \* denotes the statistically significant difference between trials for the same time point.

Furthermore, the difference of plasma copeptin between two conditions was significant. Plasma copeptin level during the dehydrated trial was significantly higher than during the euhydrated trial from the time point -90 min (euhydrated group PCopeptin:  $3.37 \pm 0.3$  pmol/L; dehydrated trial PCopeptin:  $7.16 \pm 1.07$  pmol/L,  $P < 0.0001$ , effect=0.88) to the end of the experiment; at baseline, no significant difference between two trials (euhydrated trial PCopeptin:  $3.64 \pm 0.36$  pmol/L; dehydrated trial PCopeptin:  $4.18 \pm 0.75$  pmol/L,  $P = 0.768$ , effect=0.16) (Figure 3.).

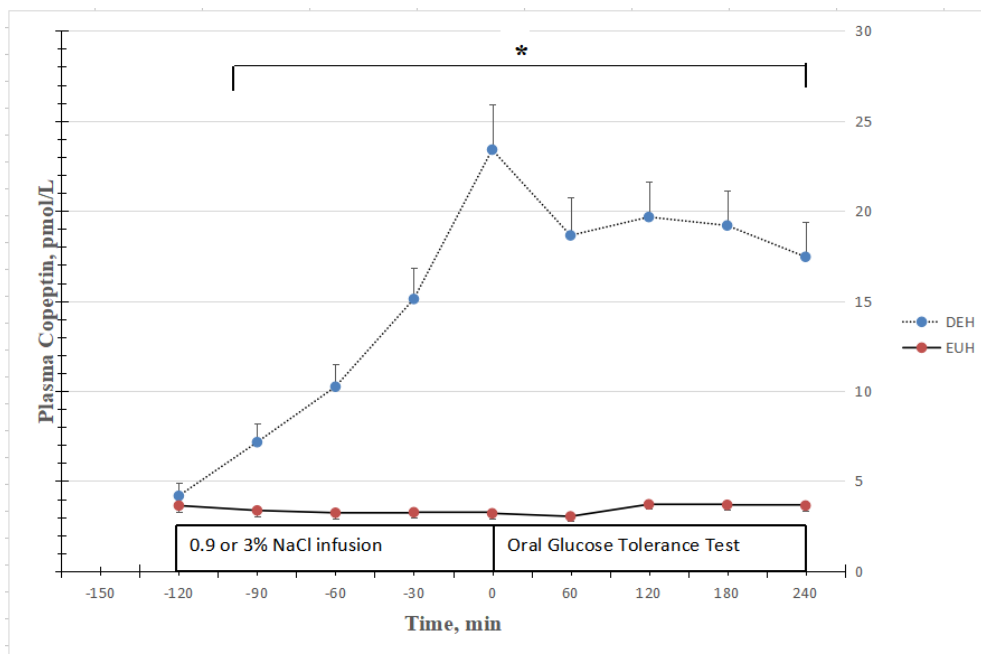


Figure 3. Plasma copeptin concentration during the trial. \* denotes the statistically significant difference between trials for the same time point.

The difference in change of plasma volume expansion was significant between trials starting from the beginning of the experiment to the end (Figure 4.).

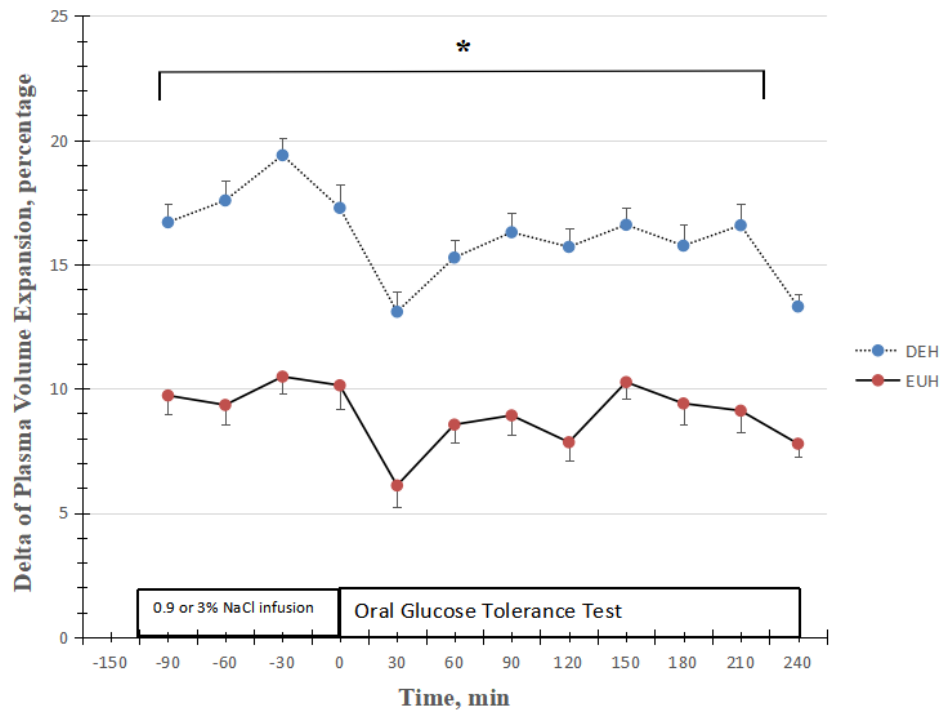


Figure 4. Percentage of the delta of plasma volume expansion during the trial. \* denotes the statistically significant difference between trials for the same time point.

The total plasma protein content of dehydrated trial was significantly lower than that of the euhydrated trial (mean difference between conditions is  $0.308\text{g/dL} \pm 0.016$ , 95% confidence interval (0.277-0.339),  $p=0.0001$ ) (Figure 5.).

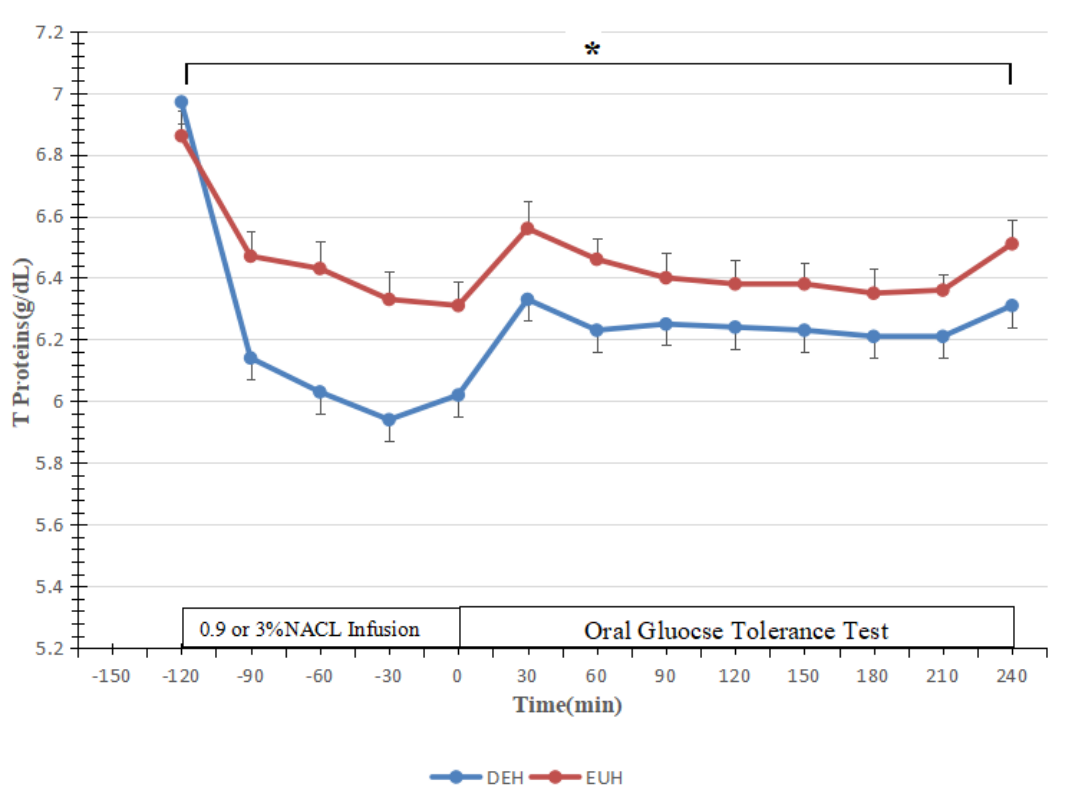


Figure 5. Total plasma protein concentration during the trial. \* denotes the statistically significant difference between trials for the same time point.

The difference of the serum insulin between two trials was not significant, except at the time point 30 min during the OGTT period, where the euhydrated trial had a significantly higher value than that of the dehydrated trial (euhydrated trial serum insulin:  $383.86 \pm 7.9$  pmol/L; dehydrated trial serum insulin:  $223.14 \pm 4.1$  pmol/L  $P < 0.0001$ , effect=4.66). Besides, at the time point 60 min, the comparison showed a trend of difference approaching significance (euhydrated trial serum insulin:  $451.02 \pm 8.34$  pmol/L; dehydrated trial serum insulin:  $376.33 \pm 5.21$  pmol/L,  $P < 0.0734$ , effect=1.96) (Figure 6).



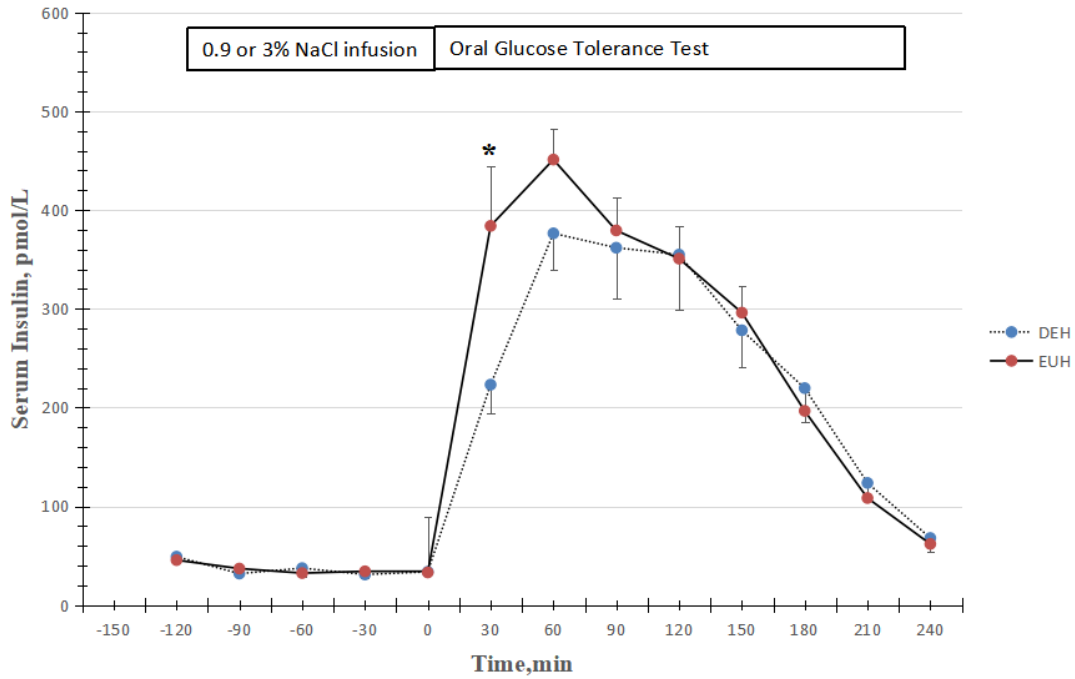


Figure 6. Serum insulin concentration during the trial. \* denotes the statistically significant difference between trials for the same time point.

There was no significant difference in fasting glucose between two trials from the beginning to the end of the experiment in terms of two-way ANOVA analysis (Figure 7).

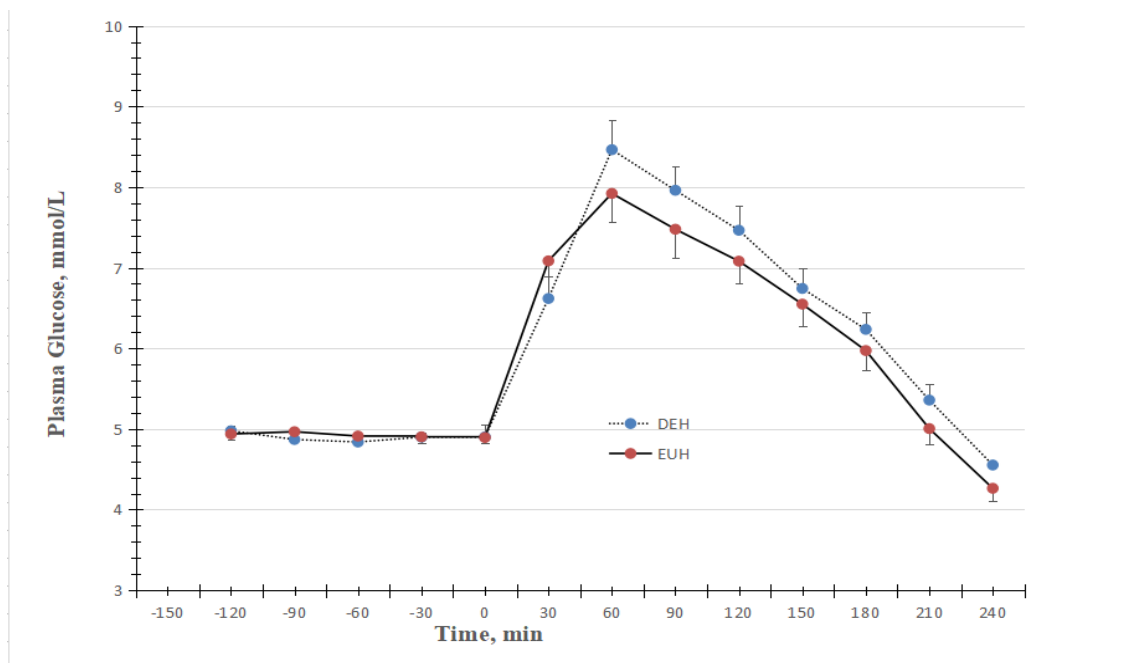
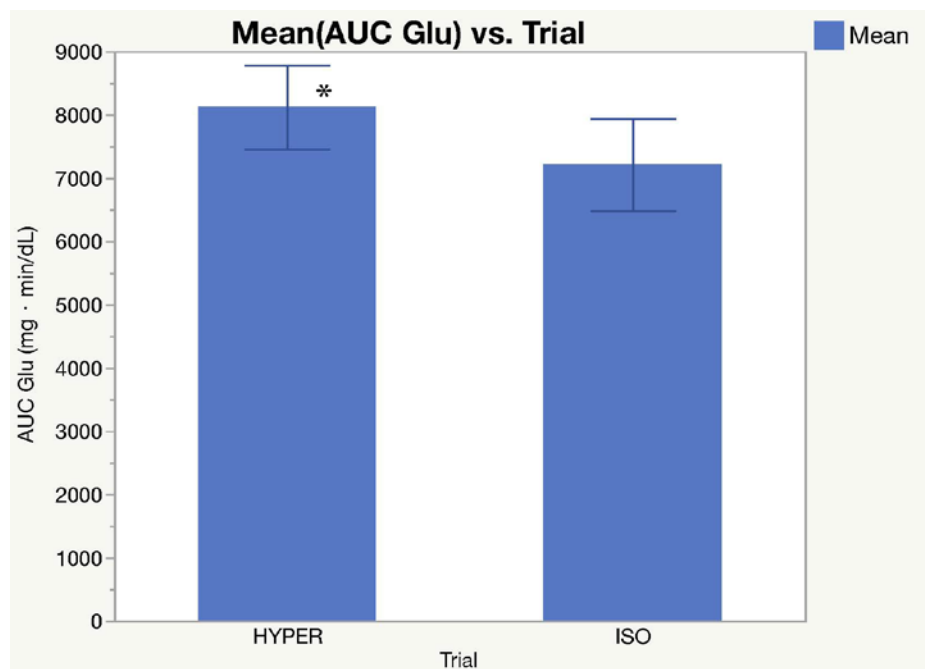


Figure 7. Plasma glucose concentration during the trial. \* denotes the statistically significant difference between trials for the same time point.

Nevertheless, further analysis of area under the curve found that the glucose levels were significantly higher in the dehydrated trial compared to the euhydrated trial (Figure-8).



## Discussion

This study tested the hypothesis that the saline infusion to the subjects would induce cellular dehydration, elevate the level of copeptin, acutely impair the ability of glucose regulation in healthy lean and obese females. Our results have shown that the 3% saline infusion successfully induced cellular dehydration, elevated the level of copeptin, and the glucose regulation was impaired in dehydrated trial.

**Cellular dehydration.** The data of plasma osmolality, plasma sodium, T protein, and delta plasma volume expansion have demonstrated that 3% saline infusion to the subjects indeed induced the cellular dehydration in the subjects of the dehydrated trial. Defining cellular dehydration in human subjects requires all dataset mentioned above and reasons are as follows. The osmolality threshold was defined earlier as extremes between 280-295

mOsm/l by Feig and McCurdy (1977). Our results show that dehydrated trials had osmolality higher than 295 mOsm/l from the time point -90 min till the end of the experiment(graph-2), hence based on the defined threshold of the osmolality the dehydrated trial showed a sign of an evident increase of osmolality compared to the normal daily range. Comparing dehydrated trial with euhydrated trial, data also show a significant difference between two groups starting from the time point -90 min. Therefore, the plasma osmolality of the dehydrated trial was significantly increased by the 3% saline infusion in this study. But as suggested by Feig and McCurdy (1977), the difference between hypertonicity and hyperosmolality cannot be distinguished by clinical methods that measure plasma osmolality, which we used to measure plasma osmolality in this study, so examining the plasma sodium to determine whether it is hypertonic under hyperosmolality is necessary. Our results show that the dehydrated trial had significantly higher plasma sodium starting from the time point -90 min till the end of the experiment. Moreover, the plasma sodium of dehydrated trial exceeded the normal range of 135-142 mmol/l, starting from the time point -90 min. Both of the plasma osmolality and plasma sodium followed a corresponding trend along the study. Combining the plasma osmolality and plasma sodium data, it might be safe to say the dehydrated trial successfully induced a hypertonic state in subjects. In addition to examining the plasma osmolality and plasma sodium, we also examined the change of plasma volume expansion. According to Feig and McCurdy (1977), there should be an extracellular volume expansion following the ingestion of solutes, such as sodium. Our results show that the dehydrated trial had a significantly higher change of plasma volume expansion during the experiment, starting from time point -90 min. This might suggest that there was more water excreted from the cell to

the plasma in the dehydrated trial. Furthermore, we measured the total plasma protein concentration to corroborate this volume expansion. Our results show that the total plasma protein concentration of dehydrated trial was significantly higher than that of the euhydrated trial, supporting that there might be more water excreted from the cell to plasma so that the total plasma protein concentration was diluted. Consequently, we could safely draw a conclusion that dehydrated trial in our study achieved the cellular dehydration starting from the time point -90 min to 0 min by infusion of 3% saline.

**AVP/Copeptin.** According to Thornton (2010), AVP regulates the water homeostasis within the human body, and the increase of the plasma osmolality will trigger the release of the AVP to the kidney to reabsorb water excreted from intracellular space. Since we induced the increase of plasma osmolality of subjects in dehydrated trial by infusion of 3% saline, we expected the rise of the AVP particularly in the dehydrated trial. Moreover, as previous studies (Enhoning et al., 2013; Morgenthaler, Struck, Alonso & Bergmann, 2006; Saleem et al., 2009) show that copeptin is a better surrogate to measure the AVP, therefore we measured plasma copeptin instead of AVP. Our results show that plasma copeptin of the dehydrated trial was significantly higher than that of the euhydrated trial, starting from the time point -90 min till the end of the experiment. Since AVP released as soon as plasma osmolality increased, the rise of first 2-hour 3% saline infusion corresponded with the well-defined AVP function, which is water retention. During the OGTT period, the dehydrated trial still had a significantly higher level of plasma copeptin than that of the euhydrated trial till the end of the experiment. This result might suggest that after a 2-hour of dehydration, the ingestion of glucose might still stimulate the increased release of the AVP in dehydrated trial compared to

the euhydrated trial, since the ingestion of glucose might have further aggravated the concentration of extracellular fluid, which induced the prolonged hypertonic state in fluid compartments, the AVP rise in dehydrated trial was induced to alleviate the cellular dehydration by directing the reabsorption of water in the kidney. Hence, it might safe to conclude from this study that the ingestion of glucose might aggravate the cellular dehydration following 2-hour dehydration period. This finding is relatively new compared to a previous study (Taveau et al., 2014) that only examined the long-term relationship between low water intake and high plasma copeptin level under hyperglycemia. Therefore, our results support that the 3% saline infusion is able to increase the AVP secretion.

**Serum insulin and fasting glucose.** Contrary to the previous studies (Enhörning et al., 2013; Vintila et al., 2016;), where significantly elevated levels of plasma osmolality, plasma copeptin are strong indicators for the impaired glucose regulation in then healthy human beings participated in the studies, our result of fasting plasma glucose show that there was no significant difference between dehydrate trial and euhydrated trial. The reason might have been that the subjects of our study are healthy females without diabetes symptoms, and it was only a 6-hour trial with low water intake compared to those years long studies with subjects having low water intake. Nonetheless, the level of fasting plasma glucose of dehydrated trial was higher than that of the euhydrated trial without significanc in terms of one-way ANOVA analysis. However, the analysis of area under the curve found that the glucose levels were significantly higher in dehydrated trial than that of the euhydrate trial.As for the level of serum insulin, there was a significant difference between two trials at the time point 30 min, with euhydrated trial having a higher serum insulin level. Moreover, at time

point 60 min, the difference between two trials nearly approached significant level. These results might suggest that the glucose regulation was impaired in the dehydrated trial.

### **Limitations of the Study**

The limitations of our study are that since it is human study, we cannot elaborate and find mechanism behind the reason as animal studies do, and females with body fat higher than 30% might have compounded the statistical analysis, as suggested by the study (Anastasiou et al., 2010) that insulin sensitivity is adversely associated with the increase of the body fat. Nevertheless, the specific topic and hypothesis our study investigated are relatively new in the field, because previous similar studies were only done in males and animals.

### **Conclusions and Future study**

We might conclude from this study that hypertonic saline infusion impairs glucose regulation in the healthy females.

Future study should investigate the reason why insulin was significantly higher at 30 min in the euhydrated trial under the same circumstance.

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## Appendix



Office of Research Compliance  
Institutional Review Board

September 28, 2016

### MEMORANDUM

TO: Stavros Kavouras Tabatha Teal Tracie Kirkland  
Elaine Lee Lisa Jansen J.D. Adams  
Hyun-Gyu Suh Yasuki Sekiguchi Audrey Smith  
Jordan Smith Zachary Lewis Zoe McKinney  
Adam Seal Jillian Fry Bryce Wall  
Alison Schoeder Kyle Cook Alexandria Aldridge  
Katherine Montgomery Cody Shopper Cameron Sprong  
Marshall Ward Cory Butts Jacob Clark  
Chunbo Young Dylan Scott

FROM: Ro Windwalker  
IRB Coordinator

RE: PROJECT MODIFICATION

IRB Protocol #: 14-12-360

Protocol Title: *The Effect of Vasopressin on Glucose Regulation*

Review Type:  EXEMPT  EXPEDITED  FULL IRB

Approved Project Period: Start Date: 09/27/2016 Expiration Date: 12/15/2016

Your request to modify the referenced protocol has been approved by the IRB. **This protocol is currently approved for 60 total participants.** If you wish to make any further 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.

Please note that this approval does not extend the Approved Project Period. Should you wish to extend your project beyond the current expiration date, you must submit a request for continuation using the UAF IRB form "Continuing Review for IRB Approved Projects." The request should be sent to the IRB Coordinator, 109 MLKG Building.

For protocols requiring FULL IRB review, please submit your request at least one month prior to the current expiration date. (High-risk protocols may require even more time for approval.) For protocols requiring an EXPEDITED or EXEMPT review, submit your request at least two weeks prior to the current expiration date. Failure to obtain approval for a continuation *on or prior to* the currently approved expiration date will result in termination of the protocol and you will be required to submit a new protocol to the IRB before continuing the project. Data collected past the protocol expiration date may need to be eliminated from the dataset should you wish to publish. Only data collected under a currently approved protocol can be certified by the IRB for any purpose.

If you have questions or need any assistance from the IRB, please contact me at 109 MLKG Building, 5-2208, or [irb@uark.edu](mailto:irb@uark.edu).