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The Effect of Dehydration on Metabolism and Energy Substrate Utilization

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The Effect of Dehydration on Metabolism and Energy Substrate Utilization

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Abstract

The purpose of this study was to measure hormonal and metabolic markers in humans to see if simulating dehydration would emulate measures that are seen in prediabetic humans. 60 volunteers were divided into equal categories by sex and body mass index of normal or overweight. Participants completed two oral glucose tolerance tests (OGTT) after being intravenously infused with a isotonic or hypertonic saline solution for 120 minutes in a counter balanced order. All trials began with a euhydrated subject with a urine specific gravity (USG) <1.020. Participants remained sedentary and were infused with the same amount of volume of solution in each trial, blood samples were taken every 30 minutes and respiratory exchange ratio was measured every 60 minutes. The hypertonic (HYPER) trial 120 minutes after OGTT was administered had significantly higher carbohydrate oxidation then that of the isotonic trial. Blood markers such as cortisol and aldosterone lowered significantly from baseline, but glucose was significantly elevated 60 and 90 minutes post infusion, reaching blood glucose levels of 155mg/dL. Plasma osmolality rose from baseline and remained elevated throughout the duration of the trial. Copeptin rose significantly during the HYPER trial 30 minutes into infusion and remained elevated the entire length of the trial falling slightly after infusion stopped. This may indicate that the elevated copeptin is associated with elevated glucose levels, and carbohydrates as the primary fuel source

Table of Contents

Introduction

A report from the US Centers for Disease Control and Prevention 2014 statistics report claims that in the United States 29.1 million people have been diagnosed with Diabetes Mellitus and another 8.1 million people are currently undiagnosed with the disease. That's almost 1 out of 11 Americans with the disease and 1 out of 3 Americans not knowing they are prediabetic. This disease accounts for $254 billion dollars between direct (medical costs) and indirect costs (lost wages). The New England Journal of Medicine examined individuals' fasting glucose to determine if those in a diabetic state (126 mg/dL) have a higher risk to develop heart disease, stroke, kidney failure, and premature death. The study associated diabetes with the development of cancers, infectious disease, intentional self-harm, and degenerative disorders, but a novel finding of their study was that on average 50-year old males diagnosed with diabetes died, approximately six years before that of their aged matched non-diabetic males. Is it possible that fluid intake plays a bigger role in the development of diabetes then we think?

Fetissov and Thornton conducted a study with type II diabetic participants in which a low water intake negatively impairs glucose regulation. Fluid homeostasis and blood pressure are largely due to two systems, arginine vasopressin (AVP) and renin-angiotensin-aldosterone (RAAS). The mechanisms of AVP and RAAS play significant roles in the regulation of glucose. AVP has a short half-life, so its surrogate marker, copeptin is often used (Boertien, W.E., Riphagenm I.J., Drion, I. 2013). Copeptin was elevated in non-diabetic and diabetic individuals with low water intake (Bankir L, Bardoux P, Ahloulay M 2001)(Enhorning et al. 2010)(Johnson EC et al. 2016). Boertein et al. also suggest that copeptin has an inverse relationship with kidney function. The mechanism for this is not yet known, but looking at diabetic rodent models there is an inverse relationship between copeptin and kidney function, it can be suggested that AVP leads

to hyperfiltration and then to albuminuria and glomerulosclerosis (Bankir et al 2001). Besides kidney function AVP plays an important role in glucose levels. As seen in Spruce et al. human subjects infused with AVP had an acute rise in blood glucose levels. This is most likely occurring at receptors V1aR and V1bR. V1aR has been connected with liver glycogenolysis and V1bR is associated with glucagon and insulin secretion (Keppens S, de Wulf H 1979)(Abu-Basha EA, Yibchok-Anun S, Hsu WH. 2002). AVP can also stimulate the V1bR to release pituitary adrenocorticotropic hormone (ACTH) (Holmes CL, Landry DW, Granton JT 2003). An increase in ACTH also causes an increase in cortisol, which can lead to cortisol-mediated gluconeogenesis (Perraudin et al. 1993)(Rizza RA, Mandarino LJ, Gerich JE 1982). As said previously, the RAAS can be triggered by disturbances in body water and blood pressure (usually deals with a decrease in blood volume) thus causing an increase in aldosterone and renin (Underwood PC, Adler GK 2013). Aldosterone has been linked to insulin resistance from mechanisms that are involved with inhibiting effects on insulin signaling and insulin-glucose uptake via the glut-4 translocation in adipocytes, skeletal muscle, and vascular and smooth muscle cells (Luther JM, Luo P, Kreger MT et al. 2011)(Wada T et al. 2009)(Selvaraj J, Sathish S, Mayilvanan C, Balasubramanian K 2012). Renin also plays a role in insulin resistance due to the RAAS cascade it is hard to determine whether elevated renin causes a change in sensitivity or renin's effects on downstream components, such as, aldosterone has a slowing effect on the removal of glucose from the blood (Bochud M, Nussberger J, Bovet P et al. 2006)(Wada et al. 2009).

Long-term low water intake has been linked with the development of type II diabetes. Roussel et al. conducted a 9-year follow-up study in which water intake had an inverse relationship with hyperglycemia. The group observed that people who drank more than 1 L/day

of water had a 27% less chance of developing diabetes than people who drank less than 0.5 L/day. When you look at dehydration and substrate utilization you will find that carbohydrates tend to be the primary fuel source. Hargreaves et al. conducted a study on males that exercised until 3% BM loss in a temperate environment (20C–22C). At 60 and 120 min of exercise a higher RER was measured during the fluid-restricted trial and also reported a 16% increase in glycogen content. Another study with similar findings is this one conducted by Gonzalez-Alonso et al. with seven male cyclists exercising in a hot environment (35C). They cycled until volitional exhaustion while developing progressive dehydration to approximately 3.9% BM loss. They reported increased carbohydrate oxidation, muscle glycogen use at 45% greater than the Euhydration trials. The aim of this study is to associate dehydration with pre-diabetic markers and the effects this has on metabolism and substrate utilization. I hypothesize that there will be elevated hormonal markers and carbohydrates will be the main energy substrate used in a hypohydrated state.

Methods

Participants

Sixty total participants were needed to volunteer for this study. Males and females were divided into equal and separate groups. The 60 participants were divided up into two groups dependent upon their body mass index (BMI). This study looked at BMI's of normal weight (18.5-25) and overweight individuals (27.55-35). Prior to the start of the study, the participant met with a research team member and filled out a health history and consent form. After consent was given, anthropomorphic (height, weight, waist & hip circumference and body composition via dual-energy x-ray absorptiometry) measurements were recorded. Also, a small sample of blood was taken via finger stick for a glycosylated hemoglobin test (HbA1c). A value less than 6.5% was

needed to continue in the study, once confirmed the participant was then familiarized with a few perceptual scales pertaining to perception of thirst and fatigue

Experimental Design

To assess the effects of hydration status on glucose regulation in people, participants completed two identical 6 h trials. Participants were blinded to the infusion of either a isotonic saline solution (ISO) or hypertonic saline solution (HYPER). This allowed the subject to drink freely up until the start of the trial and then in the lab we kept them euhydrated with the isotonic saline solution, or we simulated a dehydrated state with the hypertonic saline solution. The infusion lasted 2 h and immediately after the infusion stops, the subject was given an oral glucose tolerance test (OGTT). Metabolic and endocrine markers were assessed at baseline -150 (i.e. immediately before) and at -120, -90, -60, -30, 0, 30, 60, 90, 120, 150, 180, 210, 240 minutes. Respiratory exchange ratio (RER) was assessed at baseline and every hour during the trial (-120, -60, 0, 60, 120, 180, 240 min).

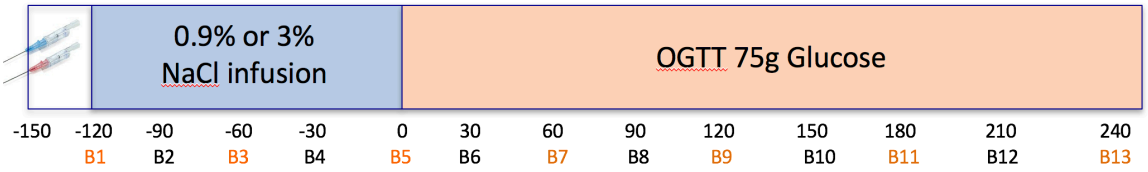

Figure 1.
Experimental Protocol

Experimental Procedures

Following an approved screening procedure and participant's consent, height and weight was recorded. Next, a dual x-ray absorptiometry scan (DXA; Lunar Prodigy, General Electric Company, Fairfield, CT, USA) was performed to assess body composition. Glycosylated

hemoglobin (HbA_{1c}) was assessed via a single finger stick (Alere AfinionTM HbA_{1c}, Waltham, MA, USA). The euhydrated and hypohydrated experimental visit was separated by a minimum of 48 hours for males. Female trials were separated by a minimum of one month and on the same day of their menstrual cycle. Participants are required to record a food log 24 hours prior to the trial, participants were instructed to choose foods high in carbohydrates. The last meal was 12 hours before the start time of the trial. During the fast participants were instructed to drink non-caloric beverages ad libitium. Subjects were also asked to refrain from exercise, alcohol, and caffeine 24 hours prior to the trial day. All of the above procedures were replicated for the second trial.

Experimental Trials

Upon arrival, a urine sample was collected for immediate hydration status assessment via urine specific gravity (USG) measurement (refractometer ATAGO, MASTER-SUR/Nα, Inc., Bellevue, WA) and urine osmolality by freezing point depression (Model 3250, Advanced Instruments, Norwood, MA). All trials began with a euhydrated subject with a USG <1.020. Upon confirmation of hydration status, participants were seated and a catheter was placed into an antecubital vein. Following 20 min seated rest, a 15- mL fasted blood sample was drawn, blood pressure, and respiratory exchange ratio (RER) were measured. The infusion was started and blood samples were taken, once every 30 min until the end of the 6-hour protocol. RER was taken once an hour (TrueOne 2400 Parvo Medics Metabolic Measurement System) where five-minute respiratory gas measures were taken. Volume of oxygen consumed (VO_2), and volume of carbon dioxide (VCO_2) produced was used to determine the respiratory exchange ratio (Peronnet and Massicotte 1991) and whole body carbohydrate (CHO) and fat oxidation with the use of the nonprotein RER table and the following equations: CHO oxidation (g) = 4.585 (VCO_2) - 3.226

(VO_2), and fat oxidation (g) = 1.695 (VO_2) - 1.701 (VCO_2) (Saltin 1980; Ferrannini 1988). Upon completion of the trial participants were free to leave the lab, participants were given water and offered a meal and instructed to resume normal acts of daily living.

Biochemical Analysis

Blood samples were transferred into appropriate plasma or serum Vacutainer tubes immediately. Untreated whole blood was analyses in triplicate for hematocrit via micro-centrifugation and for hemoglobin via colorimetric analysis (Drabkin's Reagent, RICCA Chemical Laboratories, Arlington, TX). Vacutainer tubes were centrifuged at 1,500 g for 15 min at 4 °C. From blood plasma, copeptin, aldosterone (ALDO), cortisol (CORT), were measured via enzyme-linked immunosorbent assay (Alpco, Salem, NH) and read on a microplate spectrophotometer (PowerWave HT, Biotek Winooski, VT) at 450 nm. Intra assay CVs, ALDO, and CORT were 6.2, 7.2, 6.5, and 5.8%, respectively.

Statistical Analysis

A statistical analysis was completed using the Statistical Package for the Social Sciences (SPSS, V22, IBM New York, NY, U.S.A.). Fasting measurements, and all measures of insulin resistance / sensitivity were compared between conditions using paired- sample t-tests. Hormone measurements over the course of each 4 h OGTT were compared using repeated measure analysis of variance. This allowed for analysis of main effects of time, condition, and the interaction between the two factors. When main effects were shown to be significant, individual time points were compared between conditions, while using a Bonferroni correction for multiple comparisons. Significance was established a priori at $\alpha = 0.05$.

Results

The subject characteristics of the 60 participants are presented in Table 1.

Table 1
Subject characteristics
Data are mean ± SD.

	Female (N=30)		Male (N=30)	
	Normal	**Obese**	**Normal**	**Obese**
Age (years)	41.7±9.0	41.0±7.7	35.4±6.0	37.8±7.9
HbA1c (%)	5.4±0.3	5.1±0.2	5.1±0.2	5.3±0.3
Height (m)	1.6±0.1	1.7±0.1	1.8±0.1	1.7±0.1
BMI	22.9±2.2	30.5±2.9	24.5±1.4	29.6±2.9
Body Fat(%)	31.5±6.1	43.3±7.3	21.4±5.8	29.7±5.4
Total Fat (g)	18659.1± 5011.1	36237.1± 9536.4	16522.9± 5019.2	27548.5± 7533.6
Trunk Fat(g)	9225.1± 3155.4	18405.1± 5281.4	9269.6± 3033.9	16270.2± 4959.7

Blood Measurements

Copeptin began to rise immediately during the HYPER trials and reached approximately a five-fold increase (20pmol/L) by the end of infusion and remained elevated throughout the remainder of the trial. During the ISO trial, copeptin remained at or near baseline (4pmol/L) during the

entire trial (Figure 2). During the HYPER trials glucose was elevated over 126mg/dL at time points 60-90 min (Figure 3). Cortisol levels in both trials nearly halved from baseline measures (Figure 4). Aldosterone decreased in both trials from baseline measures, but after the infusion during the isotonic trial, measures rose slightly through the remainder of the trial (Figure 5). Plasma osmolality increased immediately from baseline in the HYPER trial and remained elevated throughout the entire trial (Figure 6).

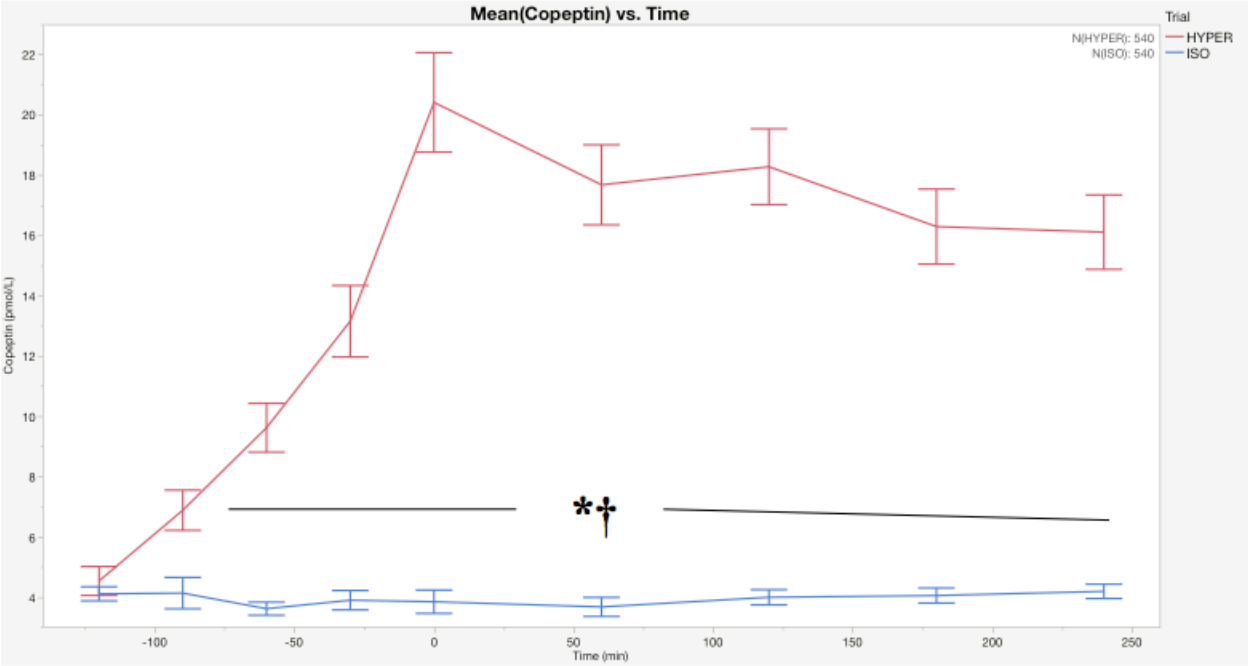

Figure 2. Mean Copeptin vs. Time between Hypertonic (HYPER) and Isotonic (ISO) trials. *, denotes differences between trials for the same time-point. †, denotes differences in comparison to baseline value for the same trial.

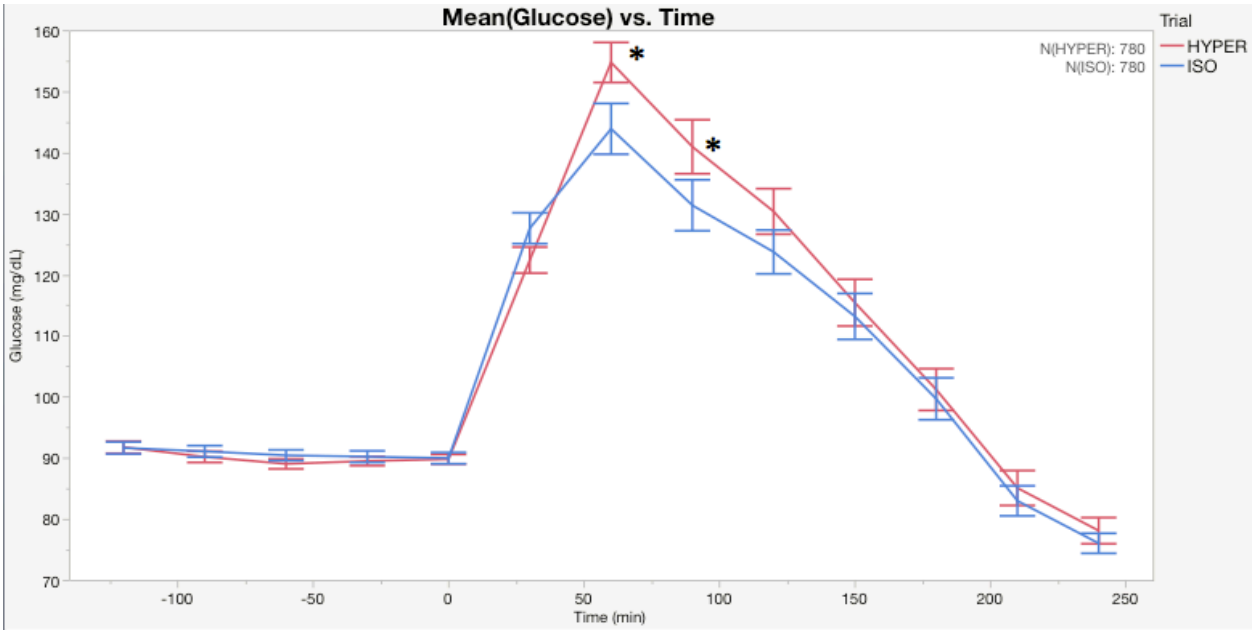

Figure 3. Mean Glucose vs. Time between Hypertonic (HYPER) and Isotonic (ISO) trials. *, denotes differences between trials for the same time-point.

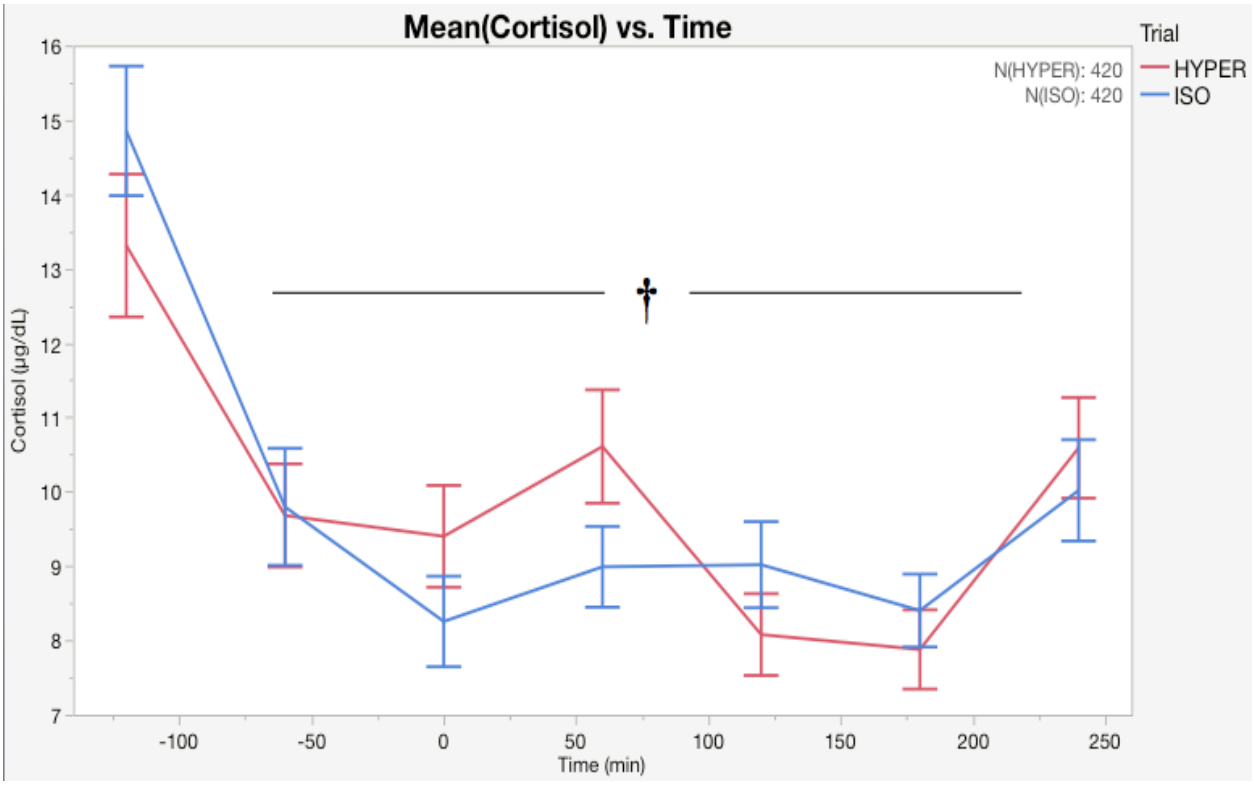

Figure 4. Mean Cortisol vs. Time between Hypertonic (HYPER) and Isotonic (ISO) trials. †, denotes differences in comparison to baseline value for the same trial.

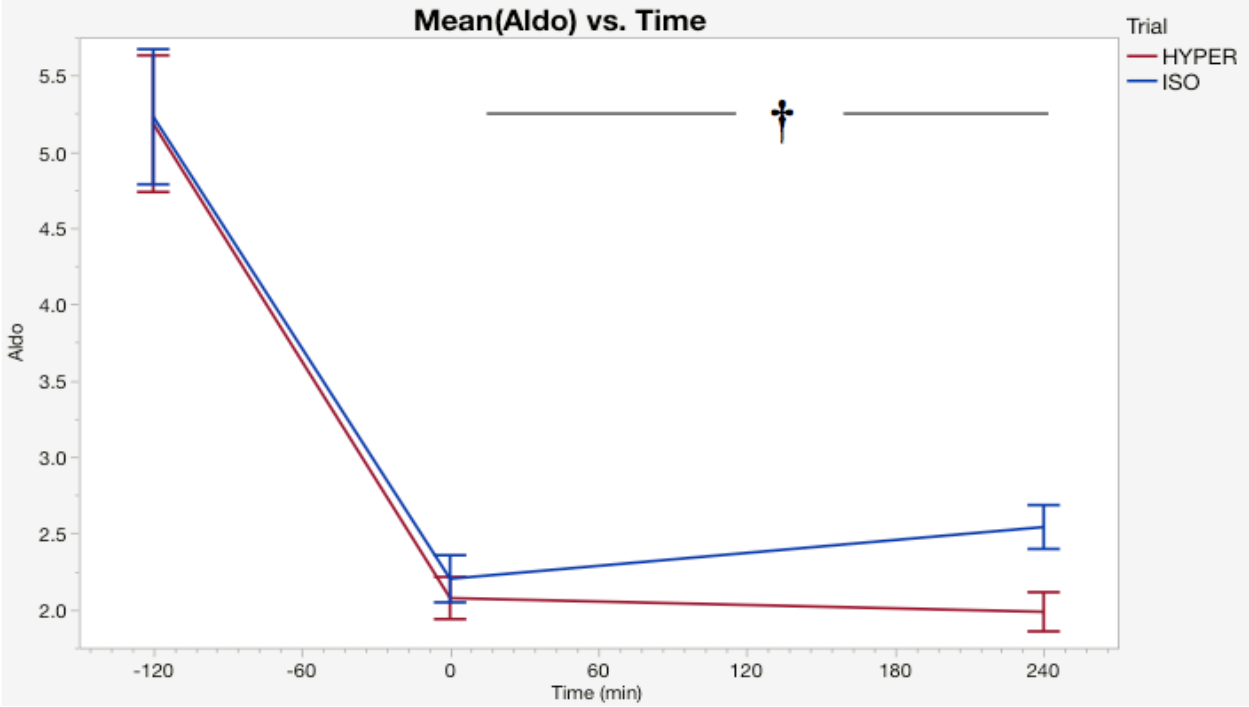

Figure 5. Mean Aldosterone vs. Time between Hypertonic (HYPER) and Isotonic (ISO) trials. †, denotes differences in comparison to baseline value for the same trial.

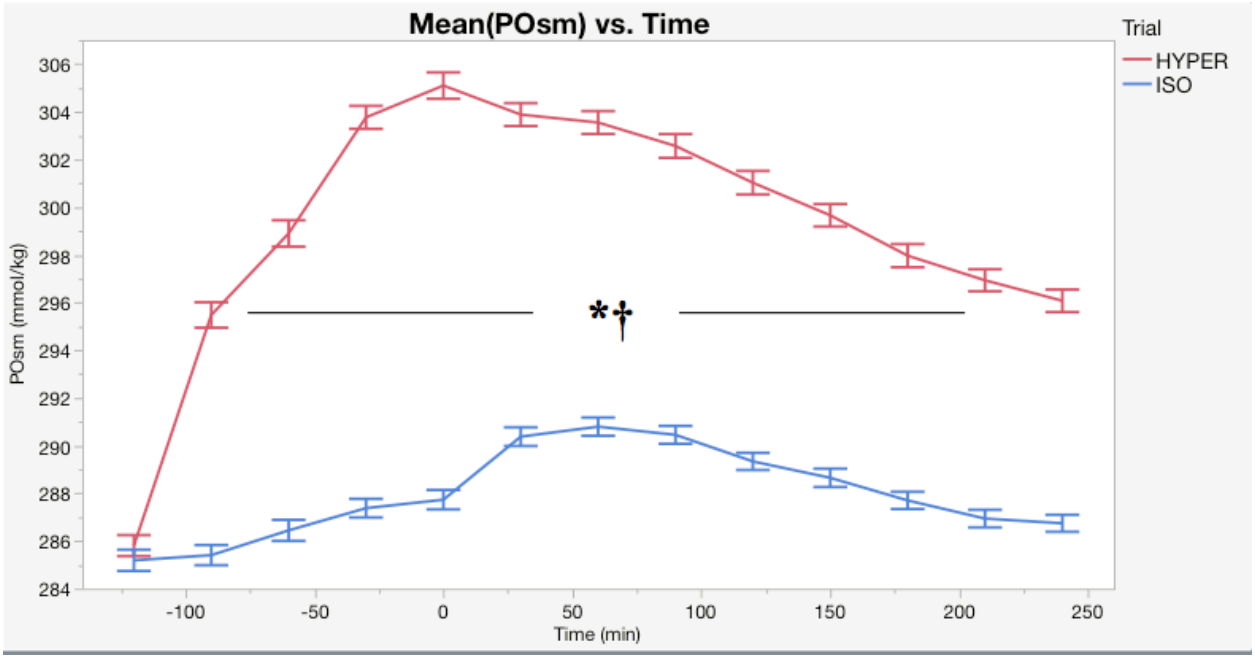

Figure 6. Mean Plasma Osmolality vs. Time between Hypertonic (HYPER) and Isotonic (ISO) trials. *, denotes differences between trials for the same time-point. †, denotes differences in comparison to baseline value for the same trial

Substrate Utilization

The RER increased in both the HYPER and ISO trials from 60-180 minutes post infusion as a response to the 75g of glucose ingestion (Figure 7). Post infusion during the HYPER trial at 120 min there was a statistically significant rise in RER (Figure 7). When RER was examined with Body Mass Index (BMI) the data suggests that RER is elevated post infusion 60-180 min in the normal BMI category and 120-180min in the obese category (Figure 8). Carbohydrate oxidation was elevated 60-180 min post infusion (Figure 9).

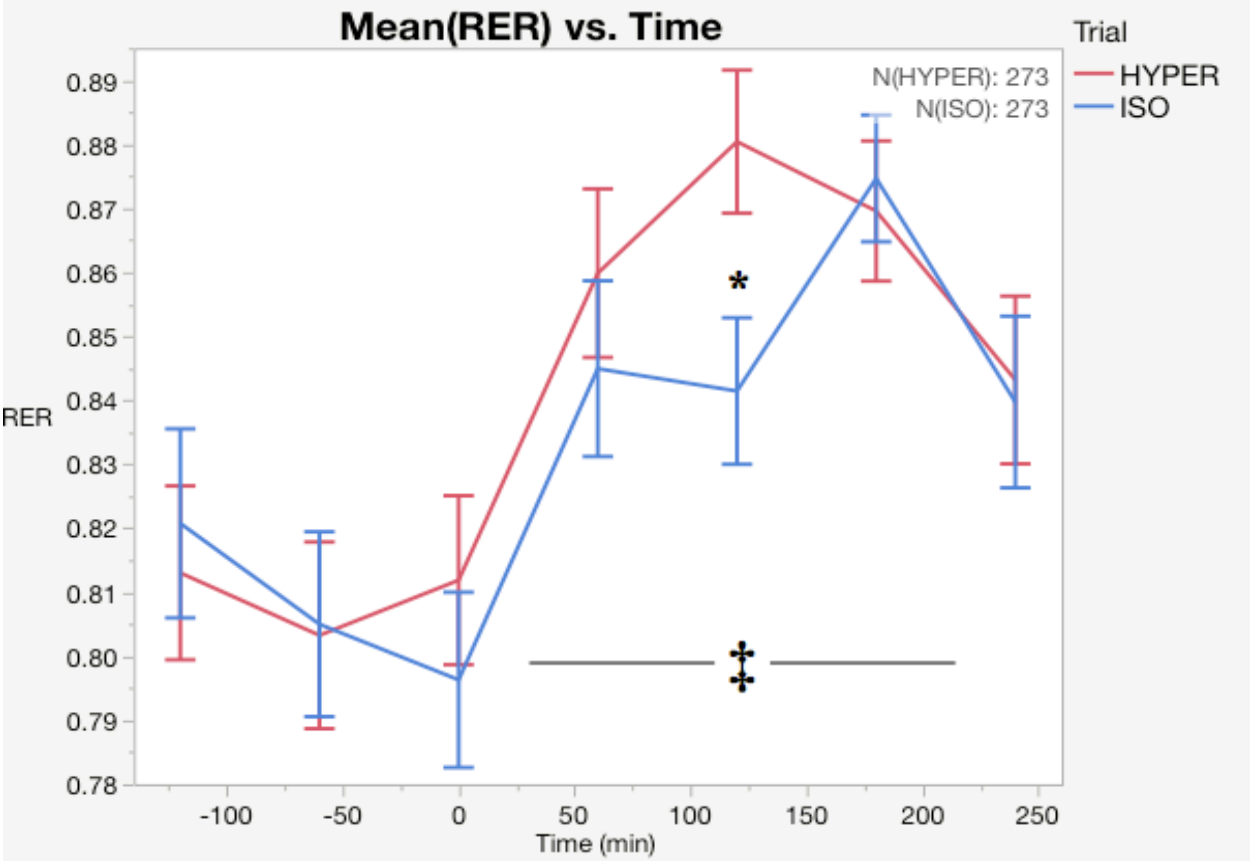

Figure 7. Mean RER vs. Time during Hypertonic(HYPER) and isotonic (ISO) trials. *, denotes differences between trials for the same time-point. ‡, denotes differences in comparison to post-infusion (0 minutes) value for the same trial.

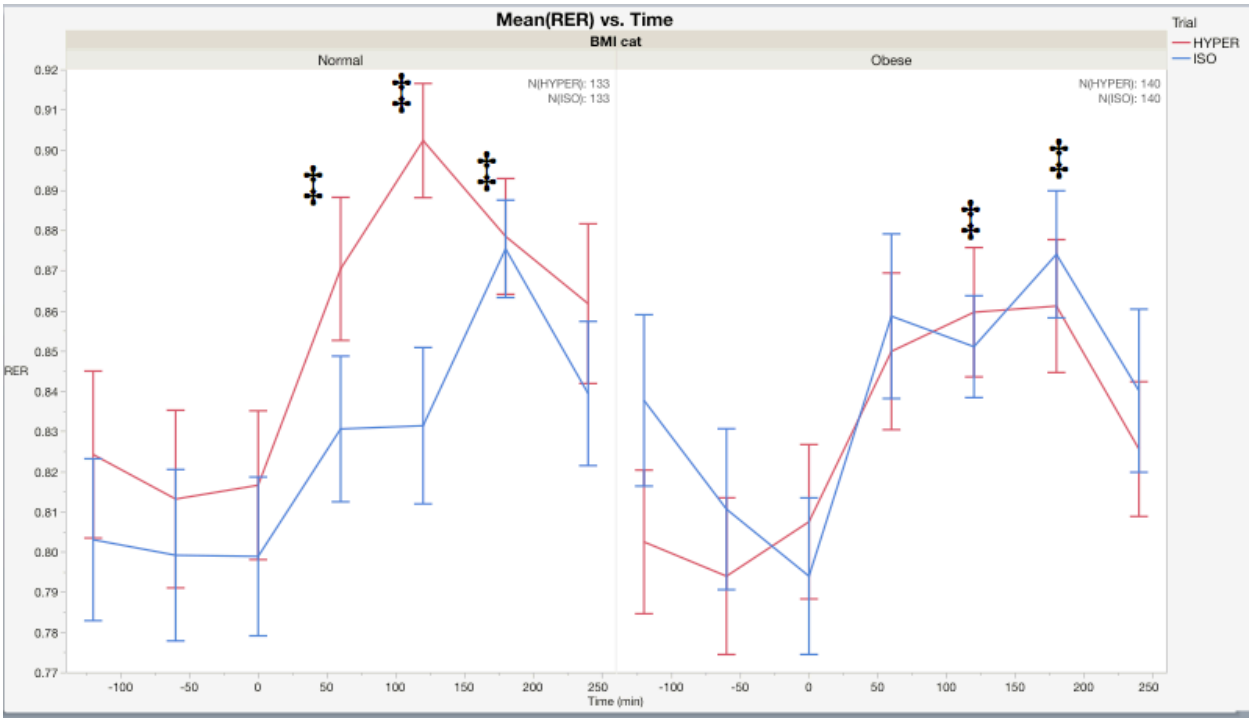

Figure 8. Mean RER vs. Time between BMI categories. ‡, denotes differences in comparison to post-infusion (0 minutes) value for the same trial.

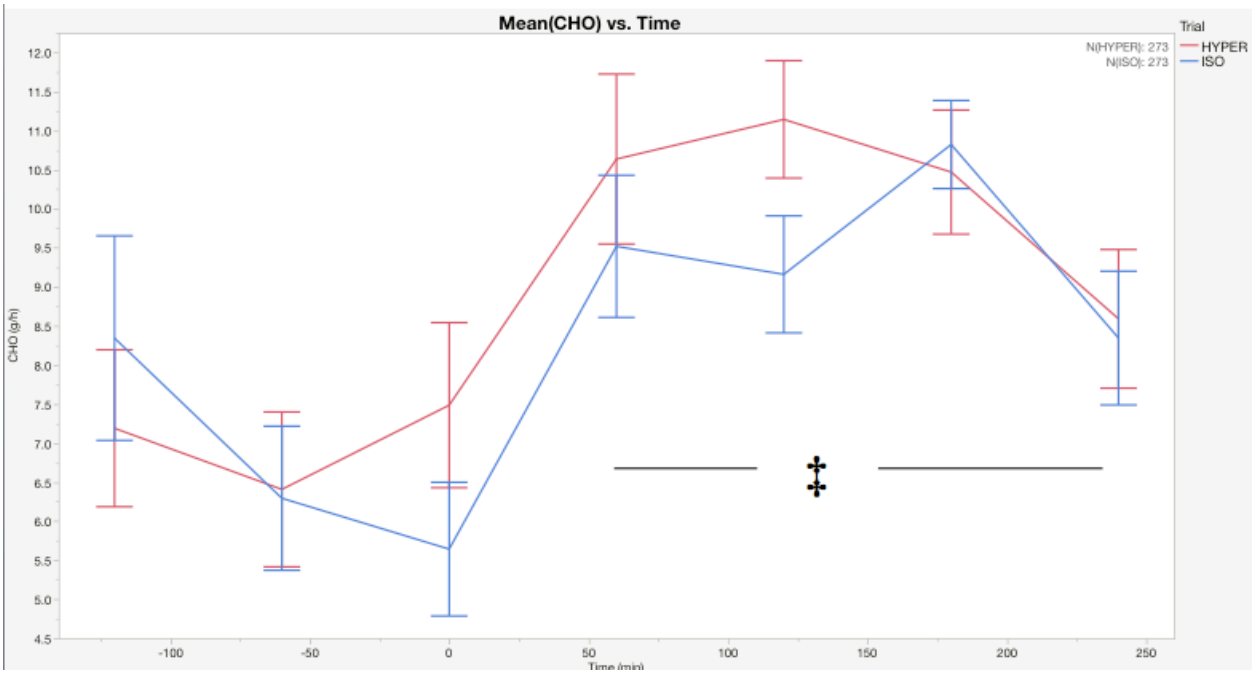

Figure 9. Mean CHO vs. Time between hypertonic (HYPER) and isotonic (ISO) trials. Figure 6. Mean RER vs. Time between BMI categories. ‡, denotes differences in comparison to post-infusion (0 minutes) value for the same trial.

Discussion

The purpose of this study was to measure hormonal and metabolic markers in humans to see if simulating dehydration would emulate measures that are seen in prediabetic humans. As hypothesized, after the 75g of glucose were administered, there was an elevation in carbohydrate metabolism in both trials. But, the hypertonic (HYPER) trial 120 minutes after OGTT was administered had significantly higher carbohydrate oxidation then that of the isotonic trial. Blood markers such as cortisol and aldosterone lowered significantly from baseline, but glucose was significantly elevated 60 and 90 minutes post infusion, reaching blood glucose levels of 155mg/dL. Plasma osmolality rose from baseline and remained elevated throughout the duration of the trial. Increased plasma osmolality plays a significant role in stimulation AVP and copeptin. Copeptin rose significantly during the HYPER trial, the upward trend began 30 minutes into infusion and remained elevated the entire length of the trial falling slightly after infusion stopped.

Fetissov and Thorton presented in their data that inadequate water consumption could negatively impact glucose regulation in humans with type II diabetes. This dysregulation comes from two systems in charge of regulation of body water and blood pressure, arginine vasopressin (AVP) and the renin-angiotensin-aldosterone system (RAAS). From our data we can see that the RAAS system, specifically the aldosterone system, was not a major factor as much as copeptin was since our data had approximately a 5-fold increase during the HYPER trial. Our participants started the trials in a euhydrated state, thus bypassing the RAAS system, which is activated mostly by changes in body water disturbances. When the RAAS system detects a change in body water or more typical; a decrease in blood volume it signals for aldosterone to up regulate as well as plasma renin activity. But since there was no change in blood volume or body water due to the

control of hydration status and infusion rates, this system did not seem to play a major role in glucose regulation. This is important because aldosterone has been shown to interrupt insulin signaling and removal of glucose from the bloodstream (Wada T, Ohshima S, Fujisawa E, Koya D, Tsuneki H, Sasaoka T 2009). But, in our study aldosterone decreased as infusion started, leaving copeptin as the main culprit for the rise in carbohydrate oxidation and glucose left in the plasma.

Other research suggests that when plasma osmolality is elevated a rise in AVP or its surrogate marker, copeptin can cause stimulation of V1a receptors in the liver which effects hepatic glycogen degradation and gluconeogenesis (Kirk CJ, Rodrigues LM, Hems DA 1979; Whitton PD, Rodrigues LM, Hems DA 1978). This can also be seen in a study from The European Journal of Clinical Nutrition where the group found an increase hepatic gluconeogenesis and higher plasma glucose concentrations when osmolality and AVP were elevated during a hyperosmolality state (Keller U, Szinnai G, Bilz S, et al. (2003). Our data would also suggest that increasing copeptin plays a role in an increase in plasma glucose, which is consistent with previous research. Our study is the first to simulate dehydration by intravenous infusion in humans and to measure gas exchange. During the HYPER trial while the body is in a simulated dehydrated state, glucose was readily available in the plasma, which could be a possible explanation for an increased carbohydrate oxidation from our data. Logan-Sprenger et al. conducted a study on female cyclists exercising in a temperate environment in a euhydrated and dehydrated state. The study recorded elevated carbohydrate metabolism within the dehydrated trials and hypothesized three possibilities for the increase in carbohydrate metabolism. 1) an augmented sympathoadrenal response leading to greater glycogen phosphorylase (PHOS) activation and flux, 2) increased allosteric activation of glycogen PHOS

via increased free ADP and AMP (energy status of the cell) levels, and 3) higher intramuscular temperature during exercise when dehydrated. Since our participants were sedentary throughout the entire study, possibility two and three would not be a factor.

In conclusion, participants began the trial in a euhydrated state; they were then infused with a hypertonic or isotonic saline solution for 2hrs. After the infusion stopped and OGTT was administered there was a spike in carbohydrate oxidation and copeptin in the blood. Even though participants are in a euhydrated state, the copeptin response is triggering a dehydrated response by reducing insulin sensitivity. This may indicate that the elevated copeptin is associated with elevated glucose levels, and carbohydrates as the primary fuel source. This finding is supported in other papers as well, specifically Roussel et al. group who conducted a community-based cohort in which copeptin demonstrated an increase risk diabetes by affecting insulin sensitivity. The study also suggested that increased water intake or vasopressin antagonist, could help improve metabolic status. This could be a novel finding in helping with identifying risks for the development of diabetes. Future studies could examine these effects in other BMI categories as well examining the effects of substrate utilization and metabolism with participants on controlled diets (ie. Mediterranean, ketogenic, vegan diets)

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UNIVERSITY OF ARKANSAS

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.