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The Effect of Hypertonicity on Glucose Regulation in Healthy Adults

Alison L. Schroeder
University of Arkansas, Fayetteville

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Alison Leigh Schroeder

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ABSTRACT

Impaired glucose tolerance is the key defining characteristic of diabetes, a condition which affects nearly 30% of Americans[2]. Hydration status and the physiological effects of poor hydration have been linked to compromised glucose tolerance in animal models, but has not been thoroughly investigated in humans.

PURPOSE: The purpose of this investigation was to observe the effect of altered hydration status on a healthy male adult's response to an oral glucose tolerance test (OGTT). **METHODS**: Four healthy adult men (28.4±1 y, 23.6±1.2 kg/m^2, HbA1C 5.6±0.2%, non-smoking, non-athletes) participated. Subjects took OGTT's in both a hypohydrated and euhydrated state. The euhydrated state was achieved by infusing an isotonic solution of 0.9% NaCl at a rate of 0.1 mL/min/kg for 120 minutes via venous catheter, while the hypohydrated state was achieved by infusing a hypertonic solution of 3% NaCl at the same rate. Blood samples of 15 mL each were taken before infusion and every 30 minutes during infusion. The OGTT commenced after 120 minutes of infusion and blood samples were taken after 5, 30, 60, 90, and 120 minutes. **RESULTS**: Plasma osmolality increased during the hypertonic infusion (to maximum 300 mmol/kg) and remained normal during the isotonic infusion (284-288 mmol/kg). Plasma volume increased during infusion in both conditions, though more significantly in the hypertonic condition (maximum 16.5% increase from baseline) than in the isotonic condition (maximum 5.6% increase from baseline). Both insulin and glycemic responses to the OGTT showed a greater increase in the hypertonic condition (insulin peak 56.0 μU/mL; glucose peak 138 mg/dL) than in the isotonic condition (insulin peak 41.7 μU/mL; glucose peak 133 mg/dL). Three measures of insulin response–homeostatic model

assessment for insulin resistance (HOMA IR), Matsuda index for insulin sensitivity, and quantitative insulin sensitivity check index (QUICKI)–indicated decreased insulin sensitivity and increased insulin resistance in the hypertonic condition (HOMA IR 0.93; Matsuda 12.4; QUICKI 0.8) compared to the isotonic condition (HOMA IR 0.85; Matsuda 12.67; QUICKI 0.9). However, none of the measured results reached the thresholds for diagnosis of diabetes. **CONCLUSION**: Infusion of hypertonic saline solution is a valid means of simulating hypohydration. Acute hypertonicity elicits an acute, negative effect on glucose tolerance and insulin sensitivity in healthy men. Though these results were only measured in an acute sense, there may be implications that chronic proper hydration could be an effective and inexpensive means of delaying or preventing the onset of type II diabetes in those with a high risk of developing the condition.

INTRODUCTION

Diabetes is the seventh leading cause of death among Americans, and as of 2012 nearly 3 million Americans had diabetes, with an additional 86 million adults showing symptoms of prediabetes[2]. The prevalence of diabetes in the United States cost approximately $245 billion in 2012[2]. Inadequate glucose regulation due to insulin resistance is the defining characteristic of type II diabetes mellitus (T2DM). In a healthy adult, fasting plasma glucose concentration is typically less than 100 mg/dL, and 2 hours after an oral glucose tolerance test (OGTT) is less than 140 mg/dL[1]. Diabetes is diagnosed when one's fasting plasma glucose concentration is greater than 126 mg/dL and is over 200 mg/dL after an OGTT or at any given time[1]. Untreated hyperglycemia can have several damaging effects on the body, including cardiovascular disease, neuropathy, nephropathy, problems with the eyes, poor blood circulation, and infection[6].

In a healthy, euhydrated condition, glucose levels are expected to rise immediately following ingestion of high amounts of carbohydrates, but due to the regulatory effects of the hormones glucagon and insulin, plasma glucose levels should fall over time. Therefore, a higher concentration of glucose in the blood, or one that takes longer to subside after ingestion is an indication of poor glucose tolerance. Insulin is released into the bloodstream in response in increased plasma glucose concentrations and binds to cells allowing glucose to enter them from the bloodstream, thereby reducing the amount of glucose in the blood[5]. In cases of T2DM, this process is altered and the body is unable to properly regulate the blood's glucose concentration, leading to hyperglycemia[5].

The efficacy of insulin has been directly linked to hydration status, though the mechanisms and extent of this relationship have not been adequately investigated[11].

Hydration status has been found to effect glucose tolerance both in animals and in humans [7, 10]. Poor hydration increases the risk of hyperglycemia and leads to compromised plasma glucose regulation in healthy adults[7] and in those already diagnosed with T2DM[3]. In diabetics, dehydration leads to increases in plasma glucose by reducing the amount of insulin, triggering gluconeogenesis, and increasing the amount of glucagon, a hormone with the opposite effect of insulin[3]. In camels, dehydration has been found to alter the response to glucose loading, causing plasma glucose to increase more and stay elevated longer when in a dehydrated state than when the same amount of glucose is introduced to a euhydrated individual[10].

Vasopressin, also known as antidiuretic hormone (ADH), is released in response to an increase in plasma osmolality and acts to increase water retention by the kidneys. Vasopressin has also been linked to glucose tolerance, with studies observing that a chronic increase in vasopressin can lead to decreased glucose tolerance and even hyperglycemia[8]. High levels of copeptin, a marker of vasopressin, contribute to insulin resistance and can be used to predict the risk of developing T2DM[4]. In this investigation, vasopressin was not measured, because it is well known and confirmed by many studies that the hormone is released due to increases in plasma osmolality[9]. We can infer that any results of this study may be related to a rise in vasopressin, or any other effect of dehydration.

The purpose of this investigation was to observe the effects of increased plasma hypertonicity and vasopressin on glucose tolerance in healthy adults. In the first phase of the study, hypertonic saline infusion was used to increase the osmotic concentration of the blood, simulating dehydration. During this period, plasma volume and blood

osmolality were measured periodically. The second phase consisted of an OGTT after 2 hours of hypertonic saline infusion in order to measure various components of glucose tolerance, including plasma glucose concentration and insulin levels. The OGTT involves subjects drinking a highly concentrated glucose drink and subsequent blood draws to monitor glucose tolerance. OGTT results are commonly used in the diagnosis of T2DM and prediabetes[1]. In this investigation, data regarding glucose tolerance was compared between subjects after dehydration by hypertonic saline infusion and after a control condition using isotonic saline infusion.

Glucose tolerance was measured by plasma insulin concentration, plasma glucose concentration, and three measures of insulin tolerance. The homeostatic model assessment for insulin resistance (HOMA IR) measures resting insulin resistance. The Matsuda index measures whole body insulin sensitivity, the inverse of insulin resistance, during an OGTT. The quantitative insulin sensitivity check index (QUICKI) measures hepatic insulin sensitivity.

METHOD

Institutional Review Board (IRB) approval was given from the University of Arkansas IRB (project IRB#: 14-12-360). The subjects for this investigation were four healthy adult males ages 18 to 45 who demonstrated sedentary lifestyles. Exclusion criteria for the subjects included obesity, diabetes, liver or kidney disease, cardiovascular disease, recent operations, smoking, insulin therapy, and drastic weight change. Each subject's fluid and food intake were regulated 12 hours before the beginning of the experiment. Participants were given a standardized dinner and 45mL/kg of water to

consume 12 hours prior to their arrival, and they were asked to refrain from caffeine, alcohol, and exercise in the 24 hours prior to each visit. Subjects were asked to consume a high-carbohydrate diet for the three days leading up to the study in order to reduce the body's glucose production due to low levels of dietary carbohydrates.

Each subject visited the Human Performance Laboratory three separate times over the course of at least two weeks. The first visit was a screening visit to determine the subject's eligibility and ability to complete the study. Also during the screening visit, body composition, body weight, and glycated hemoglobin (HbA1C) were measured. HbA1C measures average blood glucose over the previous 2-3 months. Subjects' HbA1C had to be less than 5.7%, the standard for diagnosing prediabetes[1]. The following two visits were experimental days during which each subject experienced one euhydrated condition and one hypertonic condition. The two experimental days were separated by at least one week but no more than four weeks.

Upon arrival, subjects were euhydrated and fasted. Urine samples and body weights were used to confirm hydration status, then the subjects rested for a 30 minute equilibration period before two venous catheters were inserted into the antecubital veins. One catheter was used for periodic blood draws, while the other was for the infusion of saline solution. The infusion solution was 3% NaCl for the hypertonic condition and 0.9% NaCl for the isotonic condition, and was administered at a rate of 0.1 mL/min/kg for 120 minutes. Blood samples of 15 mL each were drawn from the catheter before the infusion and at +30, + 60, +90, and +120 minutes from the start of the infusion. At the end of the infusion period, the subject provided a second urine sample and was allowed to

equilibrate for another 30 minute period, at the end of which another 15 mL blood sample was drawn.

After the equilibration period the OGTT commenced with the subject drinking a standardized glucose drink containing 75 g of glucose. The purpose of the OGTT is to stimulate insulin and glucose responses following regulated consumption of dietary carbohydrates. The subject sat at rest for the next 2 hours, and blood draws of 15 mL each were taken at +5, +30, +60, +90, and +120 minutes from the start of the OGTT.

Time (min)	**-180**	**-150**	**-120**	**-90**	**-60**	**-30**	**0**	**5**	**30**	**60**	**90**	**120**
	Rest	Begin Infusion				Rest	Begin OGTT					
Blood sample		X	X	X	X	X		X	X	X	X	X

Table 1. Protocol and periodic blood draws

Each of the blood samples was analyzed for hemoglobin and hematocrit immediately following extraction to measure plasma volume shift. The remaining blood was centrifuged to yield plasma, which was analyzed for plasma osmolality by freezing point depression. Additionally, glucose and insulin were measured by colorimetric assay and radioimmunoassay, respectively, by Quest Diagnostics, a commercial laboratory.

RESULTS

Four healthy, non-smoking males aged 28.4±1 years were observed in this study. Their BMI was 23.6±1.2 kg/m^2, which is widely considered a healthy range. HbA1C for the subjects was 5.6±0.2%, which falls in the normal range, according the the American Diabetes Association[1].

During the first phase of the protocol, the hypertonic or isotonic saline infusion, plasma osmolality and plasma volume were recorded with each blood draw. Plasma volume is calculated by measuring the shift in hematocrit (hct), which indicates the proportion of red blood cells. These data were measured throughout the rest period and the second phase as well. The results indicate that plasma osmolality increases significantly in the hypertonic condition and remains higher even up to 2 hours after the infusion has stopped. The increase in plasma osmolality is well known to lead to an increase in vasopressin, among other effects. Plasma volume increased slightly in the isotonic condition during saline infusion but more significantly increased in the hypertonic condition.

	Time (min)	-150	-120	-90	-60	-30	0	5	30	60	90	120
Osmolality (mmol/kg)	**0.9% NaCl**	284	286	286	286	286	286	287	288	288	287	287
	3% NaCl	284	290	293	298	300	300	299	300	299	298	297

Table 2. Average plasma osmolality

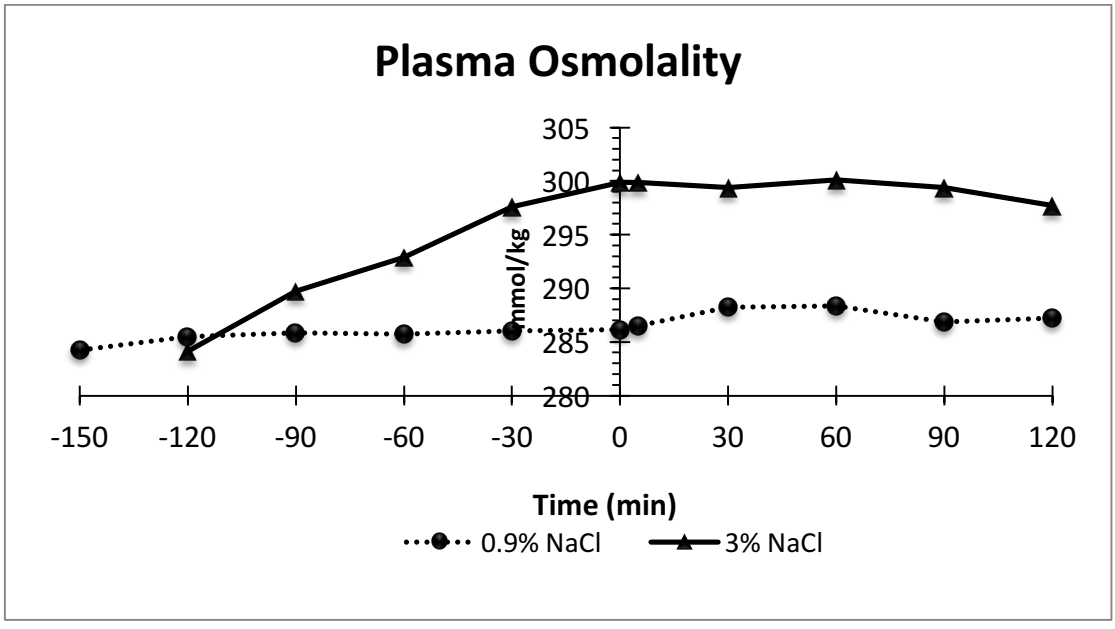

Figure 1. Average Plasma Osmolality

	Time (min)	-150	-120	-90	-60	-30	0	5	30	60	90	120
Plasma Volume Shift (% baseline)	**0.9% NaCl**	0	2.7	5.6	2.0	2.8	-1.8	0.1	-0.8	1.0	3.8	2.6
	3% NaCl	0	7.3	13.6	16.0	16.5	12.6	12.1	10.2	9.8	8.5	9.0

Table 3. Average Plasma Volume

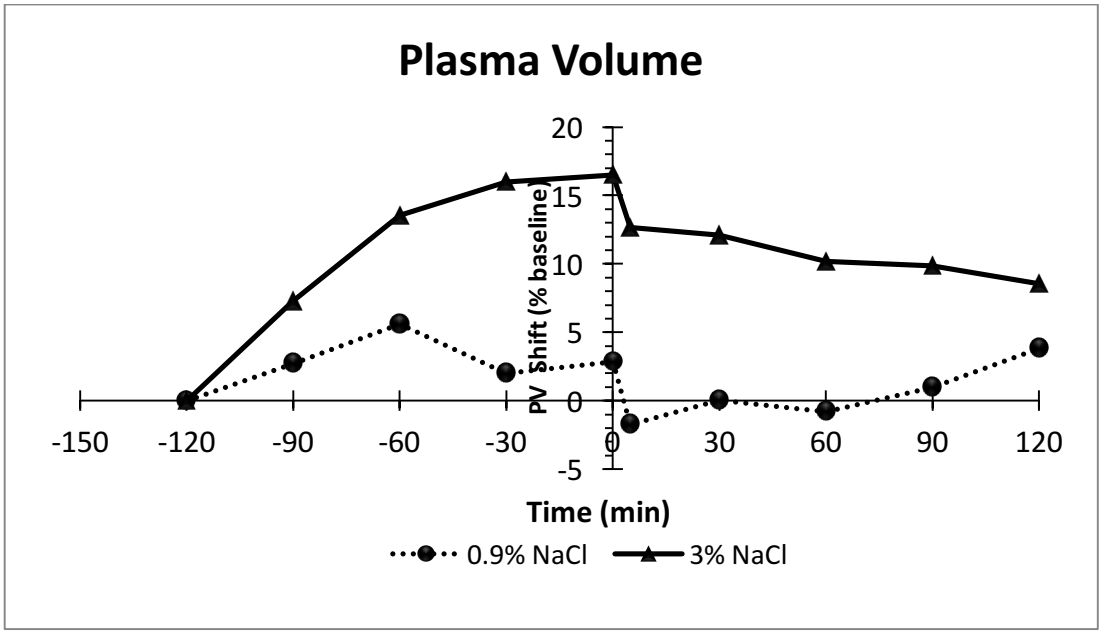

Figure 2. Average plasma volume

In the second phase of study the OGTT commenced at time t=0, and insulin and glucose concentrations were measured with each subsequent blood draw. Both the insulin and glycemic responses show the form of a typical response. After consumption of large amounts of carbohydrates, plasma glucose is expected to rise for a period of time, then return to the fasting state, with insulin levels following a similar curve in response to the increased glycaemia. The results of this investigation indicate an increased rise in both insulin and glycaemia in the hypertonic condition compared the the isotonic condition.

	Time (min)	-150	-30	0	5	30	60	90	120
Insulin	0.9% NaCl	4.4	3.6	3.7	12.0	36.5	41.7	26.4	27.9
µU/mL	3% NaCl	5.0	3.2	4.2	8.6	33.4	56.0	34.2	26.1

Table 4. Insulin response to OGTT

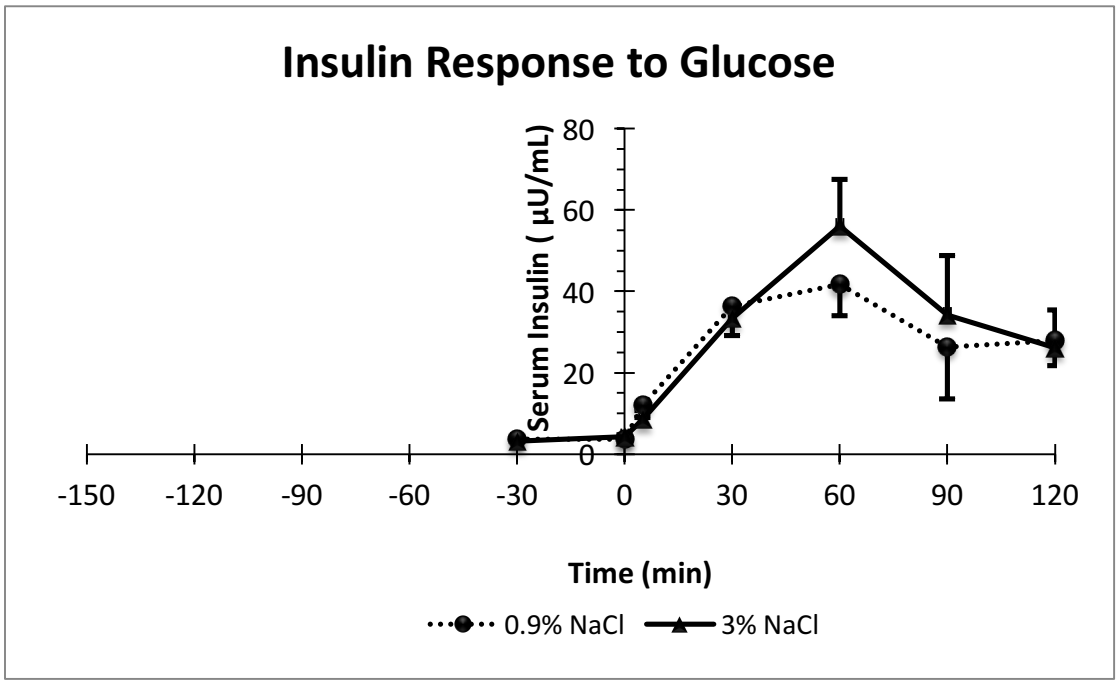

Figure 3. Insulin response to OGTT

	Time	-150	-120	-90	-60	-30	0	5	30	60	90	120
Glucose mg/dL	**0.9% NaCl**	87	87	88	88	88	90	90	128	133	120	112
	3.0% NaCl	89	87	88	87	90	89	95	127	138	115	106

Table 5. Glycemic responses

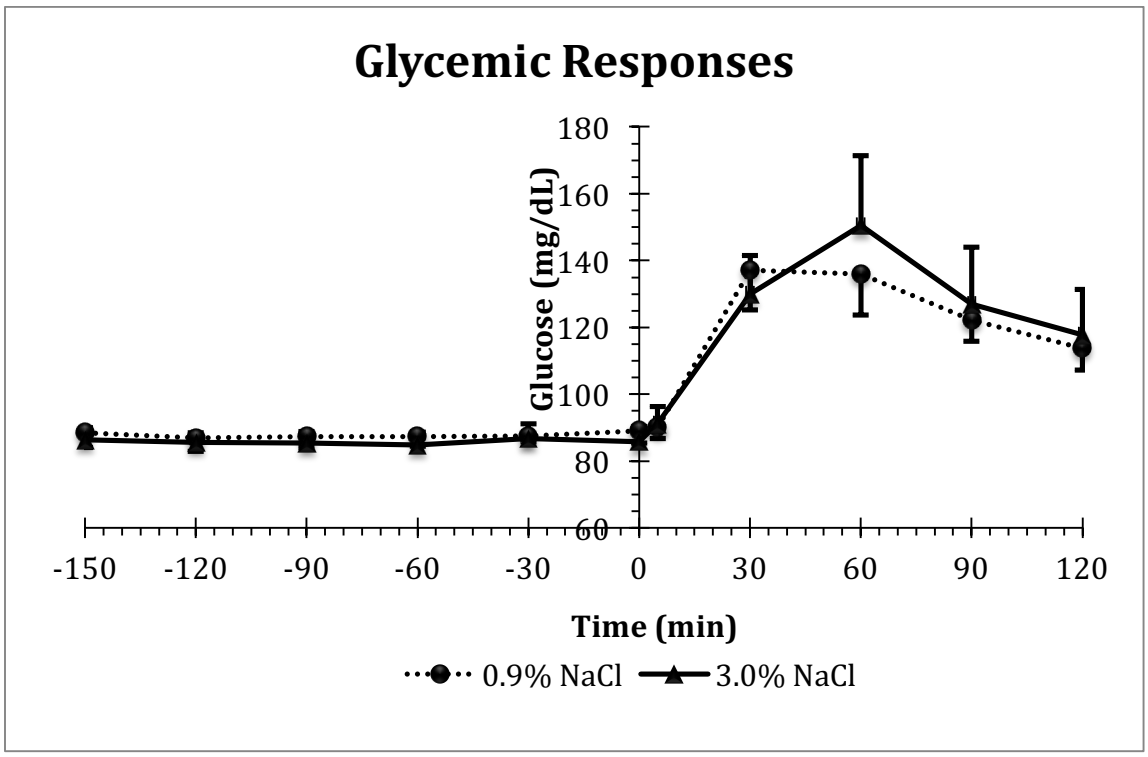

Figure 4. Glycemic responses

Utilizing the insulin and glucose concentrations, HOMA IR, Matsuda, and QUICKI indices were calculated. Matsuda, and QUICKI, which indicate insulin sensitivity, were greater in the isotonic condition. HOMA IR measures insulin resistance and indicated the opposite results, since resistance is the inverse of sensitivity. These findings suggest that the subjects were more sensitive to insulin when euhydrated than when dehydrated.

	HOMA IR	**Matsuda**	**QUICKI**
0.9% NaCl	0.85	12.67	0.9
3% NaCl	0.93	12.4	0.8

Table 6. Measures of insulin resistance and sensitivity

HOMA IR =	(fasting glucose × fasting insulin) / 405
Matsuda =	10,000 / $\sqrt{}$ (fasting glucose × fasting insulin) × (mean OGTT glucose × mean OGTT insulin)
QUICKI =	1 / [*log*(fasting insulin) + *log*(fasting glucose)]

Table 7. Calculating measures of insulin resistance and sensitivity

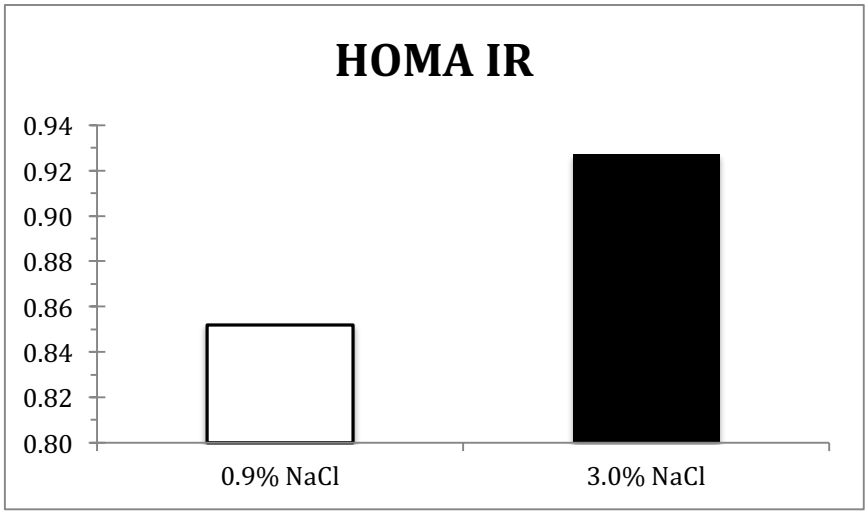

Figure 5. Homeostatic model assessment for insulin resistance

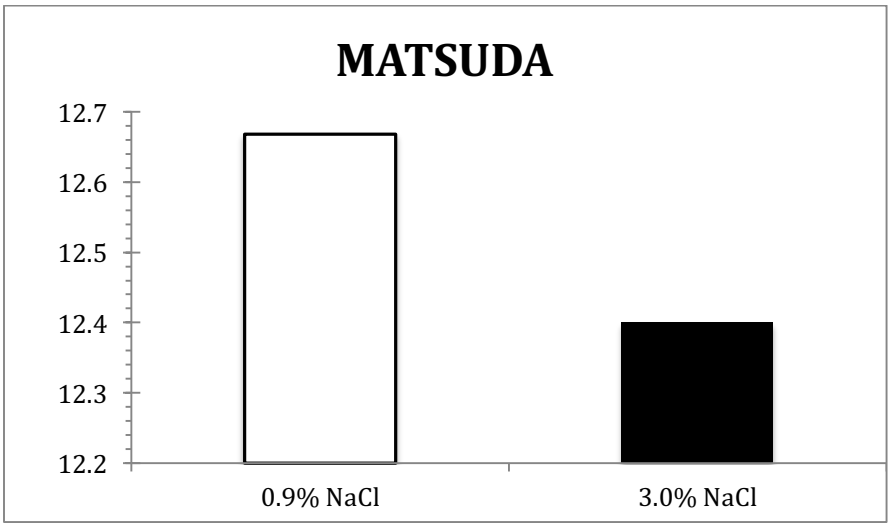

Figure 6. Matsuda measure of insulin sensitivity

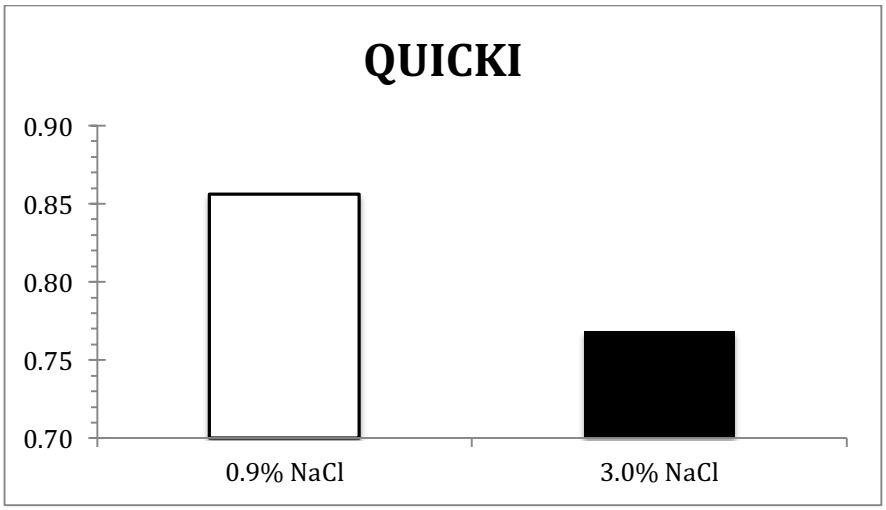

Figure 7. Quantitative insulin sensitivity check index

DISCUSSION

The goal of this investigation was to observe differences related to the infusion of isotonic and hypertonic saline solutions and their potential effects on glucose tolerance. The preliminary findings validated the practice of saline infusion as a method to simulate dehydration and found that dehydration negatively effects glucose tolerance. The hypertonic infusion led to an increase in plasma osmolality, due to the increase of solutes entering the bloodstream during the infusion. This in turn caused an increase in plasma volume as water diffused into the bloodstream from nearby cells in an effort to maintain blood osmotic homeostasis.

Preliminary studies have suggested a correlation between poor hydration and compromised glucose tolerance, which the present investigation supports[3, 4, 7, 8]. The prolonged rise in both insulin and glycaemia observed in the hypertonic condition are evidence of poor glucose tolerance. The data from the HOMA IR, Matsuda, and QUICKI all agree with one another and indicate compromised insulin sensitivity in the hypertonic condition. Though these changes are notable, they do not prompt immediate concern

about an increased risk for diabetes. None of the subjects observed had unusual resting plasma glucose concentrations, nor did theirs ever surpass 200 mg/dL, the threshold for diagnosis of diabetes at any random time. Further investigation is necessary to determine whether long-term reductions in hydration status can lead to symptoms of diabetes or prediabetes in healthy humans, as was observed by Taveau and colleagues in rats[8]. The results of this study only confirm that acute dehydration temporarily impairs glucose tolerance.

Limitations of this study include the small sample size and restriction to male subjects. This investigation also did not observe whether similar trends would occur in children or the elderly. A larger, more broad sample population would give further insight into the relationship between hydration status and glucose tolerance.

Having confirmed that dehydration impairs glucose tolerance in healthy adults, we can speculate that there may be implications for the prevention of hyperglycemia and delay of the onset of diabetes by improving hydration status. This would suggest that a simple increase in fluid intake of a person at risk for diabetes might be a valid recommendation to help improve their glucose tolerance, thus reducing their risk of developing diabetes, as suggested by Taveau and colleagues[8]. Roussel and colleagues also suggest increased water intake as a potentially valid means of reducing the risk of hyperglycemia in healthy adults with nearly no additional cost to the individual or institution[7]. Future research should be performed on a wider subject base and over longer periods of time to determine the importance of the effect hydration status has on glucose tolerance and the implications for the management and prevention of diabetes.

REFERENCES

1. American Diabetes Association. Diagnosing Diabetes and Learning About Prediabetes. American Diabetes Association [Internet]. 2014 Jun 9 [cited 2016 Mar 29]. Available from: http://www.diabetes.org/diabetes-basics/diagnosis/
2. American Diabetes Association. Statistics About Diabetes. American Diabetes Association [Internet]. 2016 Feb 1 [cited 2016 Mar 29]. Available from: http://www.diabetes.org/diabetes-basics/statistics/
3. Burge MR, Garcia N, Qualls CR, Schade DS. Differential effects of fasting and dehydration in the pathogenesis of diabetic ketoacidosis. Metabolism. 2001 Feb;50(2): 171–177.
4. Enhörning S, Wang TJ, Nilsson PM, Almgren P, Hedblad B, Berglund G, Struck J, Morgenthaler NG, Bergmann A, Lindholm E, et al. Plasma Copeptin and the Risk of Diabetes Mellitus. Circulation. 2010 May 18;121(19): 2102–2108.
5. Henquin J-C, Dufrane D, Kerr-Conte J, Nenquin M. Dynamics of glucose-induced insulin secretion in normal human islets. American Journal of Physiology: Endocrinology And Metabolism. 2015 Aug 11;309(7): E640–E650.
6. Mayo Clinic. Hyperglycemia in diabetes. Mayo Clinic [Internet]. 2015 Apr 18 [cited 2016 Apr 1]. Available from: http://www.mayoclinic.org/diseases-conditions/hyperglycemia/basics/definition/con-20034795
7. Roussel R, Fezeu L, Bouby N, Balkau B, Lantieri O, Alhenc-Gelas F, Marre M, Bankir L. Low Water Intake and Risk for New-Onset Hyperglycemia. Diabetes Care. 2011 Dec;34(12): 2551–2554.
8. Taveau C, Chollet C, Waeckel L, Desposito D, Bichet DG, Arthus M-F, Magnan

C, Philippe E, Paradis V, Foufelle F, et al. Vasopressin and hydration play a major role in the development of glucose intolerance and hepatic steatosis in obese rats. Diabetologia. 2015 Jan 27;58(5): 1081–1090.

9. Thornton SN. Thirst and hydration: Physiology and consequences of dysfunction. Physiology & Behavior. 2010;100(1): 15–21.
10. Yagil R, Berlyne GM. Glucose loading and dehydration in the camel. Journal of Applied Physiology: Respiratory, Environmental, and Exercise Physiology Environmental, and Exercise Physiology. 1977 May;42(5): 690–693.
11. Yatabe T, Tamura T, Kitagawa H, Namikawa T, Yamashita K, Hanazaki K, Yokoyama M. Preoperative oral rehydration therapy with 2.5 % carbohydrate beverage alleviates insulin action in volunteers. Journal of Artificial Organs. 2013 Aug 6;16(4): 483–488.