Developing the Optimal Vinaigrette Dressing for Managing Blood Glucose Concentrations

by

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A Thesis Presented in Partial Fulfillment
of the Requirements for the Degree
Master of Science

Approved August 2016 by the
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ARIZONA STATE UNIVERSITY

May 2017

ABSTRACT

Background: Acetic acid in vinegar has demonstrated antiglycemic effects in previous studies; however, the mechanism is unknown.

Objective: To determine whether acetic acid dissociates in the addition of sodium chloride and describe a flavorful vinaigrette that maintains the functional properties of acetic acid.

Design: *Phase I* - Ten healthy subjects (23-40 years) taste tested five homemade vinaigrette and five commercial dressings. Perceived saltiness, sweetness, tartness, and overall tasted were scored using a modified labeled affective magnitude scale. Each dressing was tested three times for pH with a calibrated meter. *Phase II* – Randomized crossover trial testing six dressings against a control dressing two groups of nine healthy adult subjects (18-52 years). Height, weight and calculated body mass index (BMI) were performed at baseline. Subjects participated in four test sessions each, at least seven days apart. After a 10-hour fast, participants consumed 38g of the test drink, followed by a bagel meal. Capillary blood glucose was obtained at fasting, and every 30 minutes over a 2-hour period the test meal.

Results: Dressing pH reduced as sodium content increased. In the intervention trials, no significant differences were observed between groups ($p > 0.05$). The greatest reduction in postprandial glycemia (~21%) was observed in the dressing containing 200 mg of sodium. Effect size was large in both group 1 ($\eta^2=0.161$) and group 2 ($\eta^2=0.577$).

Conclusion: The inclusion of sodium into acetic acid may impair its ability to attenuate blood glucose after a meal.

This is dedicated to my parents, Russ Bonsall and Tammy Svedin. I thank you for your tremendous amount of support and patience throughout this journey. There is no doubt in my mind that I am the woman I am today because I had you both as my amazing cheerleaders. I would also like to dedicate this to the family and friends that stood by me at every moment. And, of course, those who helped house me during the poorest part of my life – Aunt Rachael & Uncle Jose, then friends Ben and Erica. You were lifesavers!

ACKNOWLEDGMENTS

Dr. Carol Johnston, I would like to send you my gratitude for being a spectacular mentor! I knew from the beginning that I wanted you on my team to help guide my way. I admire your knowledge and commitment to research., and am much appreciative for your never-ending support throughout this journey.

To my committee members, Dr. Sandra Mayol-Kreiser and Dr. Christy Lespron, thank you for providing your time and wisdom to make me better prepared and successful during this project. I feel so lucky to have the two of you as support!

I would like to thank Ginger Hook and Veronica Zamora for the endless amounts of support in the laboratory. The two of you were very helpful in purchasing all of the supplies necessary to have successful trial visits, as well as glucometer training.

Delon Altherr, Michelle Kemeny, Beca Lafarga, and Alicia Leslie – I would have not been able to do this without each of you. I value your dedication and could not have picked better colleagues for myself. I wish you the best in all of your future endeavors!

Dinorah Cornejo-Metz – Thank you very much for scheduling rooms for each of my presentations! And finally, Melanie Brown, graduate and professional student association (GPSA), thank you for all of your assistance with the grant.

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CHAPTER 1

INTRODUCTION

According to the National Diabetes Statistics Report of 2014, approximately 29.1 million Americans are currently living with diabetes.[1] This is a significant portion of the population at an increased risk for developing serious complications, such as: kidney failure, hypertension, neuropathy, retinopathy, and cardiovascular disease.[1,2] In 2012, diabetes alone contributed to roughly $245 billion dollars of medical expenses within the United States, 18% of which was a result of prescription use to combat complications from the disease and another 12% for diabetic supplies.[3]

Metabolically, individuals suffering from diabetes are incapable of producing sufficient amounts of insulin after a meal, resulting in abnormally high blood glucose levels.[1,2] The oral glucose tolerance test (OGTT) is one of the main criteria for the diagnosis of the disease, and is a dependable method of measuring blood glucose that takes place over a 2-hour span of time.[2] According to the National Diabetes Data Group, an OGTT >200 mg/dL over the course of the test is indicative of diabetes mellitus.[2]

Current treatment suggestions for maintaining normalized blood glucose in diabetics include the following: daily exercise, the use of pharmaceuticals, and following a diet plan.[2] Presently, nutrition therapy recommendations set by the American Diabetes Association do not currently mention micronutrient or herbal supplementation use, such as that of cinnamon or chromium, in the treatment of diabetes.[3] Additionally, there is no reference of consuming acetic acid in the form of vinegar to decrease blood glucose response.

Previous research has indicated that ingestion of acetic acid prior to carbohydrate consumption has a significant improvement in glucose response post-meal.[4] Though the exact mechanism is not clear, it has been proposed that the antiglycemic response may be due to decreased disaccharide activity or delayed gastric emptying by the acetic acid molecule.[5]

One study in particular evaluated the effects of acetic acid within Caco-2 intestinal cells, and identified a suppression of sucrase, maltase, lactase, and trehalase sugars.[5] Gastric emptying rate was suggested to be a mechanism in a study in which acetaminophen served as a marker and was found to be decreased in level when consumed with vinegar.[5]

In 1995, Brighenti et al. discovered that the neutralization of the molecule with sodium bicarbonate decreased its ability to attenuate the glycemic response.[6] Still, twenty years later, a randomized crossover trial has not been conducted to examine whether the dissociation of acetic acid has an effect on the antiglycemic properties of the molecule. Such information is essential to the understanding of the mechanism of acetic acid and glycemia.

The present study was performed in order to examine how the food matrix alters the antiglycemic properties of acetic acid. The purpose was to utilize the information obtained to develop a vinaigrette dressing with maximal antiglycemic effects. Such a dressing would be simple to recreate home for consumers and would provide individuals with diabetes alternative methods in managing their condition.

Purpose

The objective of the study was to investigate the dissociation of acetic acid in vinaigrette dressings and the resulting affects on postprandial glycemic response. An additional aim was to create a vinaigrette dressing that has a significant antiglycemic effect and identify comparable products that are available commercially.

Research Aim and Hypotheses

H1: Increasing the amount of sodium chloride in a vinaigrette dressing will reduce the pH of the dressing composed of vinegar and oil.

H2: A vinaigrette dressing composed of vinegar and oil will have reduced antiglycemic effects with increasing concentrations of sodium chloride in healthy adults.

Definition of terms

- Acetic Acid – Organic compound (CH_3COOH) and main constituent of vinegar (5% acetic acid)
- Antiglycemic – A reduction in blood glucose concentrations in the 2-hour postprandial period
- Glucose Tolerance Test – A measure that determines the ability of the body to utilize glucose. Normal values:
    - Fasting glucose: <110 mg/dL
    - 30 minutes: <200 mg/dL
    - 1 hour: <200 mg/dL
    - 2 hours: <140mg/dL

    - 3 hours: 70-115mg/dL
    - 4 hours: 70-115 mg/dL
- Insulin Resistance – An impaired tissue response to the action of insulin ‘

Delimitations

Participants of the study comprised of non-diabetic adult subjects between the ages of 18 to 60 years.

Limitations

Study limitations included the use of a small sample size, in addition to the potential for a break in subject adherence to the diet and fasting protocols required for accurate research outcomes.

CHAPTER 2

REVIEW OF LITERATURE

Diabetes Overview

Diabetes Mellitus is a metabolic condition in which the pancreas is incapable of producing sufficient insulin, or the action of the protein is inadequate to accommodate for blood glucose.[7] Insulin is a hormone that is synthesized by the β-cells located in clusters known as islets of Langerhans of the pancreas.[8] Secretion of insulin occurs when there is a rise in glucose concentration within the blood. Blood glucose molecules are too large to enter into the cell without the assistance of the hormone. When released into the blood, insulin binds to insulin receptors located on cell membranes.[8,9] Binding of the hormone stimulates tyrosine kinase activity, which leads to the phosphorylation of tyrosines of insulin-receptor substrate proteins (IRS).[10] IRS proteins signal the translocation of GLUT-4, glucose transporters, to the cellular membrane. Glucose molecules enter the cell through GLUT-4; thus, decreasing blood glucose levels post-meal.[7,10] Once in the cell, glucose may be oxidized to produce adenosine triphosphate (ATP), combined with other glucose molecules to be stored as glycogen, or converted into lipid.[10] In a diabetic individual, this mechanism is impaired or completely inhibited, leading to high blood glucose concentrations, a condition known as hyperglycemia.[7]

The classifications of diabetes are as follows: type 1, type 2, and gestational. Type 1 diabetes mellitus is caused by autoimmune behavior, in which the β-cells of the pancreas are destroyed and can no longer produce sufficient quantities of insulin. As stated before, this destruction of cellular function leads to the inability to effectively

transport glucose into the cell; thus, blood glucose values rise and cause hyperglycemia.[2,7]

Type 2 diabetes mellitus is characterized by insulin resistance, in which the hormone is produced, but incapable of promoting glucose transport into the cell.[2,7] Gestational diabetes mellitus is the form of diabetes that is implicated during pregnancy and affects approximately 7% of pregnant women.[11] It is defined as a glucose intolerance, and may be reversed either during or post-pregnancy.[11]

Three distinctive laboratory examinations may be utilized in the diagnosis of diabetes mellitus: fasting blood glucose (FBG) concentration, percentage of glycosylated hemoglobin A1c (HbA1c), and the oral glucose tolerance test (OGTT).[2]

Fasting blood glucose values must be obtained after the individual has underwent an 8-hour fasting period, which includes no food or beverage consumption with the exception of water.[12] An FBG value of ≥126mg/dL is concerning and indicative of diabetes.

Hemoglobin A1c blood testing evaluates the amount of glucose that is bound to the molecule.[12] The criterion for diabetes as indicated by this blood analysis is a percentage that of ≥ 6.5. Normal values are between 4-5.9% HbA1c.[12]

Lastly, an oral glucose tolerance test may be utilized in the diagnosis of diabetes. The patient must fast 12 hours prior to the examination in order to obtain an accurate reading. A 75-gram beverage containing glucose or dextrose is administered. Prior to consumption of the drink, a fasting blood glucose sample is taken.[12] Blood glucose is also evaluated over the period of the test, which may range from 2-5 hours in length.[12] A value reaching ≥200mg/dL over the course of the OGTT is indicative of diabetes. [2]

Complications associated with diabetes

Diabetes is associated with a number of micro- and macrovascular medical complications, such as: diabetic ketoacidosis, nephropathy, retinopathy, neuropathy, and cardiomyopathy.[2] In instances where diabetes is properly controlled, it is often possible to decrease the risk of developing these complications.[2]

Diabetic ketoacidosis (DKA) is a consequence of a prolonged hyperglycemic state. Without the assistance of insulin, glucose is incapable of entering the cell in order to be utilized as fuel; therefore, lipolysis occurs, and lipids become the main source of energy.[11] This results in an influx of fatty acids and ketone metabolites within the blood, lowering the pH. In cases where ketoacidosis is sustained for a long period of time, the individual may enter into a rapid deep breathing pattern, known as hyperpnea. This occurs with the intention of ridding the blood of excess carbon dioxide so that[13] the pH may normalize.[11] Ketoacidosis must be treated right away in order to prevent additional complications, such as: cardiovascular and respiratory decline, depression, coma, or even death.[11,13]

Diabetic Nephropathy may be very dangerous should it go undetected for an extended period of time. Chronic hyperglycemia attributes to the glycosylation of glomerular proteins within the kidney, meaning that the glucose within the bloodstream eventually attaches itself onto the functional groups of these proteins.[2] This action leads to a rapid increase of the mesangial cells located around kidney blood vessels, in addition to scarring of the glomeruli, and the walls of the glomerular basement membrane with increase in thickness.[2] If left untreated, diabetic nephropathy will eventually lead to more serious complications, such as chronic kidney disease and renal failure.[2] The diabetic

population is the major contributor to these two disease states. For this reason, it is imperative that individuals diagnosed with diabetes are screened on an annual basis for microalbuminuria, the indicator for the condition.[2]

Diabetic retinopathy is a microvascular complication of diabetes. This condition is a major concern, as it is the main cause for the development of blindness of the adult population in the United States.[2,14] In prolonged hyperglycemia, blood delivery to the arteries of the retina begins to decline.[2,14] Retinal pericyte detachment occurs, causing the retinal endothelial cells to degenerate and create additional changes in blood delivery to the eye.[11]

The development of an infection is very common in diabetic patients that do not maintain controlled blood glucose levels.[2] Prolonged hyperglycemia negatively affects the functionality of eosinophil, neutrophil and basophil granulocytes. T-cells will also have decreased cell function, and will not be able to effectively fight off bacterial and fungal infections.[2]

Diabetic neuropathy is a complication of diabetes that affects the nervous system, which typically attributes to loss of sensation or increased pain in individuals who are suffering from the condition.11,[15] It requires strict management of blood glucose levels, in addition to a daily foot care routine and pain management.[2] Foot care is important in the prevention of infections, due to susceptibility.[2] Topical creams, antidepressants, and anticonvulsants are medications typically prescribed to patients suffering from diabetic neuropathy in order to find comfort and lessen the symptoms associated with the condition.[2]

Cardiomyopathy is a common comorbidity amongst the diabetic population.

Hyperglycemia significantly increases production of advanced glycation end-products (AGEs). AGEs are proteins or lipids that have become glycated and tend to disable nitric oxide action. Nitric oxide is a gas within the body that has many protective roles of the endothelium, which includes: vasodilation, prevention of inflammation of the vascular wall, increased production of smooth muscle cells, and reduction of monocyte adhesion. Therefore, in an ongoing state of hyperglycemia as in uncontrolled diabetes, the patient is more likely to experience inflammation of the myocardium, as well as endothelial dysfunction.[16]

Diabetes has additional effects on the myocardium, such as an increased rate of β-oxidation. This process impairs pyruvate dehydrogenase; thus, reducing the use of glucose and pyruvate for energy. An increased rate of fatty acid oxidation may eventually lead to accumulation of lipids within the walls of the heart. The palmitic acid build-up reduces the ability for myocardial cells to properly contract and increases the rate of apoptosis.[16,17]

Pharmaceuticals

Insulin hormone injections are commonly prescribed to individuals with destroyed pancreatic β-cells, such as in type 1 diabetics, or individuals experiencing insulin resistance in which the hormone is incapable of moving glucose from the blood into the cell.[18] Appropriate injection sites are located in the anterior and lateral positions of the buttocks, thighs, and abdomen, as well as the subcutaneous tissue of the upper arm. Administration within the abdomen is the site provides the quickest absorption rate, while the thigh is most favored for overall absorption (AADE).[18,19] Insulin may not be

administered orally, as it will be destroyed during the process of digestion. The hormone for the injection may be acquired from the pancreas of a pig or human insulin developed using recombinant DNA technology.[18]

The type of insulin prescribed is dependent on the need of the patient, for each differs in the speed at which the hormone reaches the blood. Rapid-acting insulin becomes effective within fifteen minutes post-injection into the body. After an hour, the insulin reaches its peak, and may continue to be effective for an additional 2-4 hours.[19] Short-acting insulin requires a longer period of time for absorption into the blood at approximately thirty minutes; however, has an extended period of action. This form will peak at approximately 2-3 hours and will have a total duration of approximately 3-6 hours. Intermediate-acting insulin has a longer absorption time following injection of 2-4 hours. The peak is typically between 4-12 hours and is capable of lowering blood glucose levels generally 12-18 hours after administration. The long-acting form of insulin does not reach the bloodstream for several hours; however, will maintain effectiveness for a full 24 hours.[19] The onset, peak, and duration times vary depending on the brand prescribed, and may not entirely follow the aforementioned periods of effectiveness.[20]

Oral medications vary greatly within the treatment of diabetes, providing many different actions in treating and preventing hypoglycemia. Drugs currently recognized by the American Diabetes Association in the treatment of diabetes are sulfonylureas, meglitinidines, biguanides, thiazolidinediones, SGLT2 inhibitors, α-glucosidase inhibitors, bile acid sequestrants, and DPP-4 inhibitors.[19]

Sulfonylureas are oral drugs that are prescribed in order to stimulate the pancreas to release increased quantities of insulin into the bloodstream.[19] The drug targets the

sulfonylurea receptor subunits, specifically SUR1, of the ATP-sensitive potassium (K_{ATP}) channel found in pancreatic β-cells. Once targeted, the channel closes, depolarizing the β-cell membrane whilst opening the voltage-dependent Ca^{2+} channels and releasing Ca^{2+} into the intracellular membrane. Increase concentrations of Ca^{2+} within the intracellular space releases insulin out of the β-cell into the blood via active transport.[21,22] Sulfonylureas are not without side effects. Common reactions to the drug are dizziness, nausea, constipation, headaches, lethargy, skin conditions, and blurry vision. Due to its ability to lower blood glucose, hypoglycemia may occur.[20]

Meglitinides have a role in stimulating the pancreas for insulin release.[23] Repaglinides and nateglinides are both forms of meglitinides that have a mechanism of action that follows closely to that of sulfonylureas. Each acts by targeting SUR1 and closing K_{ATP} and releasing insulin from the β-cells. Repaglinides have a half-life of approximately three minutes, 90 times longer than that of nateglinides; therefore, the former tend to have a greater effect on insulin release.[24] Potential effects of meglitinides are the following: upper respiratory infection, allergies, headache, and pain of the joints, back, or chest. Similar to sulfonylureas, the drug is capable of causing the patient to develop hypoglycemia.[20,23]

Biguanides are recommended in order to lower blood glucose levels. They act by slowing the amount of glucose production by the liver.[19] Metformin is a popular biguanide that has an additional action of increasing muscle cell sensitivity to insulin for improved glucose absorption. According to treatment guidelines, Metformin is the only pharmaceutical that has been deemed suitable for the prevention of diabetes and is recommended for high-risk individuals.[24] The mechanism of action is not fully

understood; however, it has been established that biguanides inhibit complex I of the electron chain within the mitochondria. Furthermore, the drug may indirectly activate AMP-activated protein kinase (AMPK), which results in increased glucose uptake and additional suppression of glucose synthesis by the liver.[25] In addition to reducing hepatic gluconeogenesis, metformin improves insulin sensitivity. Metformin has the additional benefit of improving insulin sensitivity by increasing insulin receptor activity, as well as the release of incretin hormone glucagon-like peptide-1 (GLP-1) from the enteroendocrine L cells within the gastrointestinal tract. GLP-1 then promotes insulin secretion from the pancreas.[25] Consumption of this particular drug is accompanied by many negative side effects, such as: dizziness, chills, headache, fatigue, discomfort of the chest, heart palpitations, lack of energy, dyspnea, rashes, and flushing.[20]

Thiazolidinediones are another class of oral medication that takes a different approach in regulating blood glucose. The drug targets and binds to the peroxisome proliferator-activated receptor-γ, which increases plasma adiponectin levels. This action reduces hepatic fat, increases insulin sensitivity within the adipose and hepatic tissues. An additional effect is an increased response between β-cells of the pancreas to glucose within the blood.[26,27] Possible side effects of the drug include: headache, muscle pain, increased risk of fractures for women, in addition to sinus inflammation.[20]

Sodium-glucose transporter 2 (SGLT2) inhibitors prevent the reabsorption of glucose from the glomerular filtrate within the kidneys.[19] Type 2 diabetics tend to experience increased rates of renal glucose output, thus this medication may be beneficial.[28] SGLT2 are proteins located on the proximal tubule of the nephron and are responsible for reabsorbing glucose through the brush border of the cells back into the

blood. Approximately 90% of reabsorption from renal glucose output is accomplished by SGLT2, and the additional 10% by its co-transporter, SGLT1. Reabsorption is inhibited by the drug and glucose is then excreted into the urine.[28] Potential side effects of SGLT2 inhibitors are the following: acidosis, yeast infections, dehydration, hypoglycemia when taken in conjunction with other antihyperglycemic medications, and hypercholesterolemia.[29]

Alpha-glucosidase inhibitors (AGIs), such as acarbose, act by delaying carbohydrate absorption. Acarbose is one of the primary AGIs prescribe today.[19,30] It is derived from the bacteria *Actinoplanes* and acts by inhibiting the protein enzyme α-amylase. In doing so, the enzyme is not able to hydrolyze the alpha bonds of polysaccharides to break down into monosaccharides for proper absorption.[30] AGIs may cause diarrhea, abdominal pain, flatulence, and decreased renal function.[20]

Bile acid sequestrants have been utilized for many years in the treatment of hypercholesterolemia; however, have proved to have the capability in attenuating blood glucose levels.[31] The exact mechanism of action has not been established at this time, though it has been proposed that the action is due to activation of TGR5, a bile acid membrane receptor. Once activated, TGR5 begins the secretion of GLP-1.[31] GLP-1 then promotes insulin secretion from the β-cells of the pancreas; thus, lowering blood glucose levels.[32] Negative effects while taking the drug may consist of: drowsiness, headaches, nausea and vomiting, dyspepsia, pain, constipation, diarrhea, osteomalacia or osteoporosis, in addition to increased breakdown of thyroid hormone.[20]

Dipeptidyl peptidase-4 (DPP-4) inhibitors were only recently made available on the market within the last ten years. It acts by inhibiting the breakdown of both GLP-1

and gastric inhibitory polypeptide (GIP). The outcome is a reduction in glucagon within the bloodstream to indicate the need for glycogen breakdown, increased release of insulin, as well as delayed gastric emptying.[33] Common side effects are headaches, upper respiratory infections, or nasopharyngitis.[20]

Glucagon-like peptide-1 receptor (GLP-1R) agonists are antihyperglycemic medications that come in injectable form. It acts by binding to the GLP-1R on the pancreatic β-cells, releasing cyclic adenosine monophosphate (cAMP) and Ca^{2+} into the intracellular space; thus, resulting in exocytosis of insulin and the reduction of glucose in the blood.[35] GLP-1R has additional effect in the reduction of total hepatic glucose output.[19] Side effects comprise of nausea and constipation.[35]

Amylin analogue is an alternative injectable.[19] Amylin is a hormone that is released from the pancreatic β-cells into the blood following a meal. This action suppresses appetite and the production of glucagon, the hormone that breaks down stored glycogen into glucose molecules during periods of fasting and low blood glucose.[9,36] Diabetics often have decreased production of amylin; therefore an analogue injectable is prescribed. Common side effects while using this injection are nausea, vomiting, and anorexia.[35]

Natural Remedies

Natural remedies have been sought out to replace or supplement antihyperglycemic medications in the treatment of diabetes mellitus. Common therapies include: cinnamon, and fenugreek.

Chromium has been studied by several groups of researchers in its ability to attenuate the postprandial glycemic response. It is a mineral that may be consumed in its bioactive form, chromium III (Cr III).[36] It is a cofactor of insulin, meaning that it is required for efficient glucose uptake by the cells. The mechanism for which chromium enhances insulin activity is not fully understood.[37] It has been suggested that Cr III is included within the glucose tolerance factor and improves insulin receptor activation.[37,38] More recent studies have proposed an alternative mechanism in which Cr III is transported by the protein transferrin to inactive low molecular weight chromium binding substances (LMWCr) located within insulin-dependent cells. Cr III ions then activate the LMWCr, which, in turn, stimulate the tyrosine kinase activity of the insulin receptors.[37] Therefore, insulin activity is enhanced and blood glucose levels reduce.

In addition to chromium, researchers have evaluated cinnamon as a possibility in improving blood glucose and hemoglobin A1c levels due to its component cinnamaldehyde.[39] It is believed to increase insulin sensitivity, improve the release and disposal of insulin, as well as assist in the regulation of protein-tyrosine phosphatase 1B (PTP1B) and insulin receptor kinase.[39] A meta-analysis completed by Dr. Robert W. Allen and his colleagues concluded that there is conflicting evidence in the effects of cinnamon on glycemia. Animal studies demonstrated that cinnamon extract in a liquid-based form increased expression of peroxisome proliferator-activated receptors (PPARs), transcription factors that improve glucose metabolism and insulin sensitivity. Human studies demonstrated slight improvements in fasting blood glucose results; however, it is inconclusive whether cinnamon improves hemoglobin A1c levels.[39]

Based on the current scientific evidence, it is not advised to recommend cinnamon in managing hyperglycemia in type 2 diabetics. Side effects of utilizing the ingredient for functional purposes have not currently been established.[39]

Fenugreek is a seed that has been used by individuals in the treatment of diabetes. Gaddam et al. conducted a randomized parallel controlled trial over the course of three years to evaluate whether the seed had the capability in preventing the development of diabetes in pre-diabetics.[40] Subjects in the treatment group (n =66) consumed a total of 10g fenugreek powder per day. The fenugreek was split into two 5g doses before meals and consumed with 200ml of water. A control group (n=74) was used to compare results. At baseline and every 6 months over the duration of the year, the following measurements were performed: height, weight, BMI, waist-to-hip ratio, fasting plasma glucose (FPG), postprandial plasma glucose (PPPG), insulin, homeostastic model assessment-insulin resistance (HOMA-IR) and lipid profile. After the 3-year treatment period, there were statistically significant decreases of values ($p<0.05$) in the treatment group (n=52) for FBG and HOMA-IR.[40] Additional significant reductions were observed in PPPG and serum insulin ($p<0.01$). No significant changes were detected within the control (n=27). A multivariate regression analysis determines that the fenugreek treatment group demonstrated to be 4.2 times less likely to develop diabetes than the control after 3 years ($p<0.01$). The relative risk ratio (RRR) for fenugreek after this period was 0.6, a value that was lower than the control ($p <0.01$).[40] Based on the outcome of the study, Gaddam et al. suggests that with the consumption of 10g fenugreek powder per day results in a low-risk reduction in the development of diabetes. The seed may have

a hand in increasing insulin sensitivity, due to improved HOMA-IR and serum insulin values.[40]

Roberts et al. conducted a randomized crossover trial of six sessions to evaluate the effect of fenugreek on the postprandial glycemic response in ten healthy subjects (five males, five females).[41] Tests foods included buns and flatbread baked with wheat flour both with and without a 10% powdered form of fenugreek (50g and 20 g, respectively). In addition to the buns and flatbreads, a reference food of 50 g glucose (Glucolin^{TM}) in 250 mL of water. Blood glucose was tested after 10-12 hours of fasting, and at 15-, 30-, 45-,60-, 90-, and 120-minutes following the first intake of food. Analysis of the data showed an approximate 30% reduction in mean area-under-the-curve (AUC) in flatbreads containing 10% fenugreek powder when compared to the regular flatbread. A similar response was discovered in the bun treatments, demonstrating roughly a 39% reduction those with fenugreek when compared to regular buns. Mean values were statistically different between groups and the reference treatment ($P<0.05$).[41]

Vinegar Background

The term vinegar originates from “vin aigre”, the French word for “sour wine”.[42] It is a sour liquid of diluted acetic acid traditionally produced by a two-stage fermentation process: alcohol fermentation and acetification.[43] According to the U.S. Food and Drug Administration, however, diluted acetic acid as shown on food labels is not to be considered vinegar.[44]

Production of vinegar dates back circa 5000 B.C. and claimed to have been discovered by the Babylonians.[42,43] Greek physician, Hippocrates, encouraged its use in

the healing of wounds circa 420 B.C.[42] In the 10th century, Chinese inventor of forensic medicine, Sung Tse, added vinegar and sulfur together as a hand washing agent. This was produced with the intention of preventing the spread of infection during autopsies. Early American physicians of the 18th century reported using vinegar in the treatment of many conditions, such as: croup, stomachaches, elevated fevers, edema (also known as dropsy), and contact with poison ivy.[42,43]

Vinegar Production

Traditional vinegar is produced in a two-step process of fermentation, which may take up to one month to conclude.[43] The first step is alcohol fermentation, in which the raw materials containing carbohydrate are broken down and converted into ethanol.[43,45] The source of sugar may come from a variety of ingredients, such as: apples, grapes, barley, beer, wine, rice, and potatoes.[42,43] Starch sources must be prepped for this stage in a step known as saccharification. Saccharification is the hydrolysis of raw material by enzymes, such as α-amylase, β-amylase, β-glucanase or proteases, into fermentable sugars.[45]

Other types of raw materials do not require saccharification before fermentation. Sources with high contents of saccharine, such as honey, require dilution with water to approximately 10-15% sugar. The majority of fruit items may be pressed or introduced to pentinolytic enzymes to pull out the juice before combining with yeast.[45,46]

Once the raw material has been prepped, it is then ready to begin the alcohol fermentation process. In this stage, the sugar is converted into ethanol by adding yeast. The most common species of yeast utilized in the procedure is *Saccharomyces cerevisiae*,

more commonly referred to as “brewer’s yeast”. As the yeast is introduced to the fermentable sugars, it breaks down the sugars into monosaccharides to begin the fermentation process. Glycolysis begins, converting the glucose into two pyruvate molecules, while simultaneously transforming two coenzymes of nicotinamide adenine dinucleotide into a reduced form (NADH) by glucose electrons. Subsequently, each pyruvate is decarboxylated by the enzyme pyruvate decarboxylase, producing two acetaldehyde and two carbon dioxide molecules (C_2H_4O+ 2 CO_2). The final step in the alcoholic fermentation process is the conversion of the acetaldehyde into ethanol (C_2H_6O). A hydrogen ion is removed from each NADH produced during glycolysis, converting into the oxidized form (NAD^+), and transferred to the acetaldehyde molecules to form two ethanol molecules.[46]

The second step is the acetification process, which requires ethanol, oxygen, and the addition of acetic acid bacteria. Acetic acid bacteria (AAB) are gram-negative bacteria that are responsible for the conversion of the ethanol product into acetic acid. The strains commonly utilized in vinegar production are *Acetobacter, Gluconobacter,* and *Gluconacetobacter,* from the family *Acetobacteriaceae*.[43] The primary fermentation step is necessary before the acetification process commences, as *Acetobacter* readily oxidizes alcohol rather than glucose. AAB convert both ethanol molecules back to acetaldehyde utilizing enzyme alcohol dehydrogenase (ADH), which further converts to acetic acid by way of aldehyde dehydrogenase (ALDH).[48]

Rapid fermentation techniques are often utilized for the manufacture of industrial vinegars and are capable of being produced in one day. The difference between rapid fermentation and traditional techniques, is the method by which the bacteria obtain

oxygen. In the production of traditional vinegar, a technique known as the "surface method" is employed.[45] The AAB grow and obtain oxygen from the surface of the culture. "Submerged culture" method is generally used in industrial production of vinegar in which the liquid is oxygenated prior to the induction of AAB.[45]

Functional Uses of Vinegar

Today, vinegar is utilized for numerous purposes. It may be included as an ingredient on food for flavor and acidity, or for food processing. Pickled foods use the antimicrobial activity of the acetic acid to increase shelf life and food safety standards. Vinegar is a product often used by consumers as an antimicrobial agent in cleaning or treating ailments, such as: nail fungus, warts, lice, and ear infections.[42] Based on the research, vinegar is not recommended for any of the aforementioned treatments. Takano-Lee et al. tested six home remedies compared to a control of deionized water on active female head lice. None of the home remedies were 100% protective and the vinegar was found the least effective in active female louse mortality and reduction of fecundity.[49]

Jung et al. evaluated diluted vinegar in comparison to ofloxacin antibiotic eardrops in the treatment of chronic granular myringitis, inflammation of the tympanic membrane.[50] Every participant assigned to the dilute vinegar group (n=15) demonstrated complete resolution of otorrhoea (ear discharge) within the first three weeks of treatment; whereas the antibiotic only demonstrated effectiveness in ten out of the fifteen individuals within the antibiotic group after three weeks. After six months, there were no additional incidences of ear discharge within the vinegar treatment group. Therefore, the low pH of a diluted vinegar treatment may be used to resolve otorrhoea; however, this

method does provide potential harmful side effects, as it was reported to cause irritation and pain within the ear canal.[50]

Many types of vinegars contain polyphenols in various concentrations. Contents of phenolic compounds provide the product with antioxidant abilities that assist in defending against reactive oxygen species.

Acetic Acid

The FDA has set a standard for vinegar products, declaring that each must contain at least 4% acetic acid (4g per 100 mL) in order to be sold in the United States. Acetic acid (CH_3COOH) is the chemical compound found in vinegar. It is a weak acid (pK_a = 4.76); therefore, the molecule will not completely dissociate in water, releasing all hydrogen ions. The pH of acetic acid is low at approximately 2.4 as a 1.0M solution (see calculation below).

$$1.76 \times 10^{-5} = [H^+]^2 \div 1.0M$$

$$[H^+]^2 = 1.76 \times 10^{-5}$$

$$H^+ = 4.20 \times 10^{-3}$$

$$pH = - \log[H^+] = -\log [4.20 \times 10^{-3}] = 2.377$$

$$pH \approx 2.4$$

Acetic Acid and Antiglycemic Response

Various research trials have indicated the use of vinegar in the form of acetic acid as a method in significantly lowering the postprandial glycemic response. The literature

insinuates that the anytiglycemic action of acetic acid is comparative to that of the pharmaceutical product, acarabose.[52]

A study was published in 1987 utilizing twelve rats as the subjects randomized into two groups: cornstarch solution (10%) or the same solution with 2% acetic acid added. For two weeks, the rats were fed a 25% casein-sucrose diet then placed on a 24-hour fast prior to administration of the cornstarch solution, which provided 100 mg of starch per 100 grams of body weight.[9] Venous blood samples were collected before the solution, and at 15-, 30-, 60-, 120-, and 180-minutes post solution in order to measure blood glucose values. The results indicated that the solution containing acetic acid did not provide a typical spike and drop in blood glucose.[53]

The same investigators chose to utilize human subjects in the second segment of the study. Similar to the rat study, healthy subject volunteers (n= 7) were placed into one of two groups: (1) a treatment drink containing 60 ml of strawberry vinegar (5% acetic acid) and 50 grams of sucrose, or (2) placebo of 300 ml containing 53.6 grams of sucrose.[53] However, unlike the rat trials, this study was ran as a crossover trial; therefore, each participant received both treatments, administered one week apart. Participants were asked to fast the night prior to drink consumption the treatment drink.[53] Blood glucose and serum insulin were analyzed prior to and for 180-minutes following ingestion of the drink. Insulin values were significantly lowered in the vinegar group as compared to the control. The glycemic response upheld a similar curve in both groups.[53] In 1998, Brighenti et al. evaluated the effect of neutralized acetic acid on the postprandial response in five healthy adults (four males and one female). This randomized crossover trial tested three dressings with varying composition: neutralized acetic acid (sodium

acetate), vinegar (5% acetic acid) and olive oil, and the placebo of olive oil and sodium chloride.[6]

Two test meals were provided over the course of six sessions. The first three sessions, the participants were randomized into a treatment-dressing group and given a portion of iceberg lettuce as the test meal.[6] The second round of three sessions included the iceberg lettuce portion in addition to a slice of white bread consisting of 50 g of carbohydrate. To test the antiglycemic response of each treatment, a fasting blood sample was taken via capillary method utilizing a glucometer five minutes prior to test meal ingestion.[6] Additional blood samples were obtained immediately after meal consumption in addition to every fifteen minutes following over a 90-minute period of time. The statistical analysis indicated a significant attenuation of postprandial glycemia by approximately 30% when ingesting vinegar before a meal when compared to the placebo of olive oil and sodium chloride. The neutralization of acetic acid with sodium bicarbonate, however, created no notable changes.[6]

Three years following Brighenti and his team of researchers, Liljeberg and Björck chose to investigate acetic acid as a vinaigrette form as consumed with a starchy meal.[54] A total of ten healthy adults with a normal body mass index participated in the research. Each individual was instructed to fast overnight and consume one of two test meals in two separate sessions. All subjects were randomly assigned on the of the following test meals per session: (1) 122 grams of white wheat bread, 8 grams of olive oil, and 23 g of 10% fat cheese, (2) 122 grams of white wheat bread and 23 grams of cheese with a vinaigrette sauce made of 20 grams white vinegar, 8 grams of olive oil, and 20 grams of water.[54] Blood glucose levels were evaluated over the course of 3-hours utilizing a

glucometer and capillary blood samples. Based on the results, test meal two with the vinegar sauce produced a significant change of roughly -20% in the glycemic response as compared to test meal one.[54]

Amount and Form of Acetic Acid

In 2010, Johnson et al. investigated acetic acid and its effect on the glycemic response post-meal. In two separate randomized crossover trials, the investigators evaluated the amount of vinegar necessary to reduce the glycemic response and the validity of a vinegar pill.[55]

Healthy volunteers participated in the trial to test sufficient quantity of acetic acid to attenuate glycemia. Four treatments (placebo, 20g, 10g, and 2g of vinegar with 5% acetic acid) were given over the course of four weeks with one week between each test session. Contradictory to previous studies that utilized 20-gram vinegar doses, the results inferred that 10 grams of vinegar (5% acetic acid) have a significant effect on lowering the glycemic response post-meal when compared to the placebo, and low 2-gram dosage.[55]

To test the validity of a vinegar pill, the research team recruited individuals diagnosed with type 2 diabetes (without complications) to participate in the trial. This portion of the study was designed similarly to that of the aforementioned trial; however, it was held over the course of three weeks with three treatment groups: (1) 20g vinegar, (2) a vinegar pill containing 1.2g of sodium acetate and (3) a placebo. The vinegar pill was dissolved into water prior to administration; therefore it equated to approximately 1g of acetate. Each treatment was ingested two minutes prior to consumption of the

carbohydrate-containing test meal. Glucose values were obtained prior to test meal consumption to 120-minutes post-meal.[55]

Statistical analysis of the data demonstrated a significant change postprandial glycemic response in the diabetic individuals when acetic acid was consumed, and alternatively, no substantial evidence to support the utilization of the acetate to attenuate glycemia post-meal.[55]

These trials suggest that 10 grams of vinegar (5% acetic acid) produces a substantial attenuation of the postprandial glycemic response and does not produce this effect in acetate form. This suggests that the hydrogen must be required for acetic acid action as an antihyperglycemic agent. Therefore, a vinegar pill is not effective in reducing blood glucose levels.[55]

Timing of Acetic Acid Administration

A study trial was conducted to evaluate the timing at which the dose is to be administered in regards to the meal. Vinegar (20g, 5% acetic acid) was ingested both at two minutes and five hours prior to the test meal, and, as a comparative value; a placebo was administered two minutes prior to consumption.[55]

According to the findings, timing is imperative to the antiglycemic effect of vinegar. The consumption of acetic acid 5-hours prior to a meal did not provide any mentionable outcome in regards to lowering blood glucose. Therefore, it was proposed that individuals should consume the vinegar immediately prior to eating carbohydrate, as it has been shown to have significance as compared to the control.[55]

Acetic Acid Action on Different Forms of Carbohydrate

One particular trial observed whether the form of carbohydrate administered post acetic acid consumption made a difference in the postprandial glycemic response. The trial was ran as a randomized crossover study design, in which healthy subjects were placed into two test groups.[55] Test group A involved the consumption of either a placebo or 20g vinegar (5% acetic acid) prior to a 114-gram white bagel meal. Test group B consumed the same amount and composition of vinegar or placebo before ingestion of a dextrose drink. Blood glucose levels were obtained at 0-, 30-, 60-, 90- and 120-minutes.[55]

In the observation of these results, the only significant values were acquired from test A. As shown in previous studies, the presence of acetic acid attenuated the postprandial glycemic response as compared to the placebo. Consumption of vinegar prior to the ingestion of dextrose beverage in test B did not demonstrate any decrease in the postprandial glycemic response. On the contrary, blood glucose levels rose by approximately 90% in this trial.[55]

Acetic Acid and Hemoglobin A1c

In addition to attenuation of the glycemic response, acetic acid has been reported to reduce hemoglobin A1c values in type 2 diabetics. A 2009 pilot trial evaluated twenty-seven healthy subjects diagnosed with type 2 diabetes that were not concurrently using insulin.[56] All participants were arranges according to gender, age, and body mass index and randomly placed into one of three treatment groups: (1) vinegar pill treatment of 15 mg acetic acid, pickle group (≈ 300 mg acetic acid), or the vinegar dose containing 1400 mg of acetic acid. Over the course of two weeks, participants were instructed to consume

the treatment twice a day with no other dietary changes. Assessments occurred at baseline, week 6, and week 12, in which the participants fasting over a period of 12 hours and venous blood was obtained in order to analyze HbA1c levels.[56]

At the end of twelve weeks, the vinegar treatment demonstrated a significant improvement in hemoglobin A1c levels (0.16% overall unit decrease). This was not the case for interventions with pickles and vinegar pills, in which the overall HbA1c levels increased. A limitation within the study that may be the cause for concern is subject adherence in regards to the daily ingestion of the treatment, as well as fasting pre-data collection appointments.[56]

Acetic Acid and Glycemic Index of Meal

Two studies reported data suggesting that the postprandial antiglycemic effects of acetic acid are dependent on the glycemic index of the meal ingested. The glycemic index is an evaluative measure that is used to express the amount of which a meal or food item containing carbohydrate will increase blood glucose after consumption.[57]

In a randomized crossover trial, Johnston and Buller investigated the effect of vinegar on postprandial glycemia utilizing test meals of different glycemic indexes.[15] Eleven healthy subjects (mean age 27.9 ± 2.9 years) were required to fast prior to the session and provided one of the following three treatments: (1) control of 60 grams distilled water and 1 teaspoon of saccharine, (2) vinegar drink of 20 grams vinegar of 5% acetic acid, or (3) 60 grams distilled water and 1 teaspoon of saccharine with peanut product modifications in test meals.[58] Test meal A consisted of a bagel, butter and orange juice, with an overall glycemic index of 81. Test meal B was a chicken stir-fry with vegetables,

and had a calculated glycemic index of 48.[58] Subjects completed an overnight fast and consumed the test drink, followed by the test meal. Plasma blood glucose values were obtained before ingestion of the meal, as well as 30- and 60- minutes post-meal.

Based on the data collected, there was a statistically significant difference between the 60-minute blood glucose values of the high glycemic index meal A, and the low glycemic index meal B when consumed post acetic acid ingestion.[58]

Another group of investigators studied sixteen type 2 diabetic individuals free of diabetic complications.[59] All subjects were matched by gender and age, in addition to body mass index and hemoglobin A1c levels, and then randomized into one of two groups. Group A was fed a meal consisting of mashed potatoes and low-fat milk with a high glycemic index of 86. The meal for group B included whole grain bread, lettuce, and low-fat cheese with a low glycemic index of 38.[59] Each group consumed the meals on two occasions one week apart: once with 20 grams of white wine vinegar (6% acetic acid) prior to the meal, and another instance without vinegar. As in previous studies, the individuals were required to fast the night prior to the trial and blood glucose was utilized as a biomarker (at 0-, 30-, 60-, 90, and 120-minutes).[59] From the data obtained for meal A, there was a substantial decrease in the mean blood glucose values for the vinegar sample when compared to the control. On the contrary, consumption of the low-glycemic index meal demonstrated no meaningful attenuation of the glycemic response in the acetic acid test.[59]

Proposed Mechanisms of Acetic Acid on the Postprandial Glycemic Response

Currently, the mechanism to which acetic acid displays antiglycemic properties is unknown. Based on the literature, several potential mechanisms have been explored; yet, cannot be thoroughly explained.

One theory suggests the depression of disaccharidase enzyme activity, inhibiting the breakdown of disaccharides into smaller units. Ogawa et al. explored this particular mechanism of acetic acid on the glycemic response by evaluating its action on disaccharide activity within intestinal Caco-2 cells.[60]

The research team obtained the cells from a colonic carcinoma, which acts identically to the intestinal cells within the body. Cell cultures were contained in a microplate consisting of twenty-four wells that contained approximately 140 thousand cells each.[60] The cultures were divided into either the acetic acid test group, or the control group, which consisted of organic acid compounds (citric, succinic, maric, lactic, tartaric, and itaconic acids). Over the course of fifteen days, the cells within the test group were exposed to varying levels of acetic acid (0-5 mmol/L). Disaccharidase enzyme activity was then measured by utilizing the Dahlqvist method.[60] The investigators observed decreased sucrase activity, even at the lowest level of acetic acid administration to the cells (≈ -30%). Exposure to 5mmol/L of acetic acid resulted in an overall 50% reduction of sucrase activity when compared to the additional organic acids tested. Furthermore, maltase, trehalase, and lactase were also found to have a statistically significant decrease in activity when compared to the control.[60]

In addition to assessing the effect of neutralized acetic acid on glycemia, Brighenti and his research team simultaneously studied whether delayed gastric emptying

is a potential mechanism of the antiglycemic response. Eight healthy individuals were paired according to age and body weight.[6] Each was randomized to the group of neutralized acetic acid (sodium acetate) or acetic acid and instructed to consume a 50-gram portion of white bread immediately following the test drink. Abdominal ultrasounds were instantaneously performed to measure the width of gastric atrum openings during the process at the several time frames: pre-meal, instantly following bread ingestion, and every fifteen minutes proceeding. Gastric emptying times were calculated utilizing this data; however, it was concluded that there was no significant difference in these values when comparing the neutralized and primary forms of acetic acid.[6]

Another proposed mechanism of acetic acid a capability to restrict enteral carbohydrate absorption in order to suppress blood glucose levels. In a randomized crossover trial, Salbe et al. explored potential mechanisms for the antiglycemic properties of acetic acid, hoping to observe a reduction in glucose uptake in the repression of insulin secretion.[61]

Five healthy and non-diabetic participants were recruited and were instructed to attend four sessions over the course of four weeks, approximately one-week apart.[61] At each trial session, the subjects were given the placebo (x2) or vinegar treatment (x2), followed by a meal containing mashed potatoes (0.75 g of carbohydrate) with butter, in addition to 120 ml of sugar-free orangeade drink. The placebo consisted of 60 ml of water with 0.3 teaspoon of saccharine, and the vinegar treatment comprised of 40 ml of water with 20 ml of 5% acetic acid vinegar and 0.3 teaspoon saccharine. Test meals were consumed two minutes post ingestion of the treatment beverage.[61]

In order to suppress insulin secretion at the time of the trial, an octrecide/insulin suppression test, otherwise known as OOST, was performed. This method was sufficient in suppressing insulin production for 100 minutes; therefore, additional time after 100 minutes was invalid for the purpose of the study. In comparison to the placebo, vinegar ingestion increased blood glucose to a level that was considered to be statistically significant. Based on the results, the proposed mechanism that acetic acid restricts enteral carbohydrate absorption is void.[61]

An alternative study also considered a delay in gastric emptying as a mechanism of the antiglycemic effect of acetic acid; however, the results were contradictory to those discovered with the utilization of abdominal ultrasounds.[54] In order to test this notion, the test meal of bread was specifically produced for the study and baked with one gram of paracetamol, commonly known as acetaminophen. Serum paracetamol blood samples were obtained before the ingestion of the bread, as well as at 15-, 30-, 45-, 70- and 95-minutes thereafter. Statistical analysis of these values suggests that there is potential for acetic acid to delay gastric emptying in healthy individuals.[54]

Additional Proposed Mechanisms of Acetic Acid

Though not directly related to the antiglycemic effect, a study held in Sweden proposed another potential mechanism of acetic acid: satiety.[62] Eleven healthy subjects were provided four treatments in randomized order. All treatments included white bread that consisted of 50 grams of carbohydrates, in addition to 150 ml of water, coffee, or tea, chosen by the participant. Each meal remained consistent; however, three of the four bread meals were dipped in varying levels of vinegar containing 18, 23, or 28 grams of

6% acetic acid. The additional meal was utilized as a reference and did not contain vinegar.[62]

Participants came to the visit fasting and self-reported the level of satiety prior to ingesting the meal with the use of a rating scale (-10 to +10). Self-reported satiety measures were also obtained at 15-, 30-, 45-, 70-, 90-, and 120 minutes.
With the data collected, investigators were able to discern a statistically significant amount of difference in satiety scores between the four test meals, especially when compared to the control. As the level of acetic acid intake increased, subjects would report a higher level of fullness, which was sustained over the length of the trial (120-minutes). One of the substantial limitations of the trial was the utilization of self-reported, subjective data.[62]

Vinegar has also been proposed to increase glucose uptake in muscle cells. Eleven type 2 diabetic adults who were weight stable for two months participated in a randomized crossover trial held in Athens, Greece.[63] The aim of the study was to evaluate the potential effect of vinegar on glucose metabolism within the forearm muscle. Participants fasted prior to each of the two trial sessions.[63] Once they arrived at the hospital, each individual was catheterized within the forearm muscle and administered a treatment drink of either 30 ml of vinegar (5% acetic acid) with 20ml of water, or a placebo of 50 ml of water only. A test meal was provided and to be consumed within a 15-minute period of time. Prior to the consumption of this test meal, blood samples were taken to analyze blood glucose and insulin levels. Additionally, these values were assessed at intervals following meal consumption up to 300 minutes.[62]

Results showed that there was no significant difference in the blood glucose levels assessed pre-meal. The postprandial values, however, demonstrated that the acetic acid attenuated overall glucose values.[63] When fasting, the vinegar and placebo groups revealed a similar response in glucose uptake. Conversely, after the meal the vinegar group had an increased glucose metabolism as compared to the placebo group in the trial. Therefore, it is possible that vinegar containing 5% acetic acid has the ability to increase glucose uptake within the muscle.[63]

At the Isfahan University of Medical Sciences in Isfahan, Iran, Derakhshanseh-Rishehri and colleagues investigated the effects of honey vinegar syrup on blood sugar, post-meal. The study was designed as a randomized-controlled parallel study over the course of four weeks. Subjects included 72 healthy individuals with a normal body mass index.[64] Each participant was randomly assigned to either the control group with a normal diet (n = 36), or intervention group (n = 36), which incorporated a normal diet with the addition of 21.66 g of honey vinegar syrup throughout the duration of the study.[64] The intervention group was instructed to consume the honey vinegar syrup two times a day for the entire four weeks. It was administered as 250 ml of water with 21.6g of the syrup added. Additionally, all subjects were advised to consume a diet that included 25-30% of calories from fat, 15% from protein, and 55-60% from carbohydrate. Diet was assessed in the form of a 3-day food log at three points: baseline, week two and week four. Fasting blood glucose was tested at baseline and at week four, in addition to HDL-cholesterol, insulin, total cholesterol, and triglycerides.[64]

After data was obtained, it was determined that there is no correlation between the utilization of honey vinegar syrup and a decrease in blood glucose levels. The researcher,

however, did find that the syrup decreased HDL-cholesterol and total cholesterol values over the four-week trial.[64]

Contradictory Findings

Today, contradictory evidence to the action of acetic acid on postprandial glycemia is limited. A 2012 publication by van Dijk et al. intended to dispute the claims by conducting a randomized-crossover trial which included twelve type 2 diabetic subjects (65±1 years).[65] Trial visits took place on two separate test days at least one week apart and tested two treatments. One treatment included the consumption of 25 g white vinegar with a beverage containing 75g of glucose, and the second treatment consisted of only the 75g glucose beverage. Participants were advised to fast overnight and arrive for testing the following morning. Venous blood samples were taken to assess blood glucose and insulin at fasting and every fifteen minutes over the course of two hours following consumption of the treatment beverage. No statistically significant differences were determined between each treatment group in either glucose or insulin concentrations ($p = 0.79$ and $p=0.86$, respectively). Based on the analysis, the researchers concluded that the consumption of vinegar does not attenuate postprandial glucose in type 2 diabetics.[65]

A key difference between the Dijk et al. and the majority of aforementioned studies is the form of carbohydrate consumed by participants: simple carbohydrates versus complex carbohydrates. Research studies conducted by Dijk et al. and by Johnston et al. revealed that the acetic acid in vinegar does not appear to have an effect on simple carbohydrates, as found in dextrose beverages and may indicate increases in blood glucose when compared to a placebo.[55,65] Though the compound has proved ineffective in

the consumption of food products containing simple sugars acetic acid has demonstrated the ability to lower postprandial glycemia following a complex carbohydrate meal, as evidence by the current literature[4,5]

CHAPTER 3

METHODOLOGY

Participants

Phase I.

Ten subjects between the ages of 18-60 years were enrolled into the taste testing session. Participants were excluded if they did not meet the minimum age requirement of 18 years, were current smokers, or had a condition in which tasting ability was affected. Recruitment took place at Arizona State University, Downtown Phoenix Campus through word of mouth, posted flyers, and electronic messages. Each subject provided verbal consent after reading taste test consent letter (Appendix A).

Phase II.

Nineteen overweight non-diabetic adult subjects were enrolled into the study trial. Inclusion criteria were weight stable individuals (≤ 6.5 weight gain or loss within 3 months) between the ages of 18-60 years, non-smoking, and no history of chronic medical disease. Participants filled out a Health History Questionnaire to determine eligibility (See Appendix B). Individuals were excluded if they were pregnant or planning to become pregnant over the course of the study, as well as taking insulin or anti-glycemic medications that effect blood glucose response. Anthropometric measurements of height and weight were taken at the initial screening visit. Body Mass Index was calculated with collected height and weight data (kg/m^2). Participants were recruited from Arizona State University and the Phoenix Metro-Area community via posted flyers, word of mouth, and electronic messages. Interested individuals completed

a Survey Monkey questionnaire for screening purposes (Appendix C) and all participants provided written informed consent (Appendix D). The study received approval from the Arizona State University Institutional Review Board prior to screening.

Study Design

Research was conducted at the Arizona Biomedical Collaborative building on the Arizona State University Downtown Campus and consisted of two parts: vinaigrette dressing taste test and a placebo-controlled trial.

Phase I.

In the preliminary section of the study, five homemade vinaigrette dressings were prepared and fifteen commercial vinaigrette dressings purchased from the local grocery store. All dressings were tested for pH level using HI 99161 Waterproof pH Meter (Hanna® Instruments, Carrollton, TX 75006) (Appendix E). Homemade vinaigrette dressings were made with Star® Italian Kitchen white wine vinegar (5% acetic acid), Pompeian® extra virgin olive oil, C&H® pure cane white granulated sugar, McCormick® pure ground black pepper and Morton® granulated table salt (See Table 1). The control included RealLemon® lemon juice in the place of vinegar.

The trial portion involved a one-time taste test of ten salad dressings over the duration of 60 minutes. Taste testers were randomized into on of four groups for sequence 1 (homemade), and one of four groups for sequence 2 (commercial). Randomization determining dressing order during the taste test was determined using

QuickCalcs randomization software (GraphPad, Inc. La Jolla, CA 92037) (Refer to Table 2).

Table 1. Composition of Taste Test Dressings

HOMEMADE VINAIGRETTE DRESSINGS (2.5T ~38g)				
H1	H2	H3	H4	H5
132 calories	128 calories	128 calories	128 calories	128 calories
15.25g lemon juice	20g WWV[10]	20g WWV[10]	20g WWV[10]	20g WWV[10]
14.5g EVOO[5]	14.5g EVOO[5]	14.5g EVOO[5]	14.5g EVOO[5]	14.5g EVOO[5]
2.1 g sugar	2.1 g sugar	2.1 g sugar	2.1 g sugar	2.1 g sugar
0.43g pepper	0.43 g pepper	0.43 g pepper	0.43 g pepper	0.43 g pepper
100 mg sodium	100 mg sodium	200 mg sodium	300 mg sodium	400 mg sodium

[1]ACV = Apple Cider Vinegar
[2]CO = Canola Oil
[3]CV = Champagne Vinegar
[4]DV= Distilled Vinegar
[5]EVOO = Extra Virgin Olive Oil
[6]RW = Red Wine Vinegar
[7]RI = Rice Wine Vinegar
[8]SBO = Soybean Oil
[9]SO = Sunflower Oil
[10]WWV = White Wine Vinegar
[11]WV = White Vinegar

COMMERCIAL DRESSINGS (2.5T ~38g)				
C1	C2	C3	C4	C5
203.2 calories	50.8 calories	88.9 calories	63.5 calories	152.4 calories
19.05 g fat (23%)	3.81 g fat (5%)	6.35 g fat (8%)	6.35 g fat (17%)	13.97 g fat (18%)
CV[3](#3), RI[7](#6)	ACV[1] (#2)	RW[6] (#2)	RW[6](#4), WV[11](#5)	DV[4] (#3)
CO[2] (#1)	CO[2]/SO[9] (#3)	SBO[8]/EVOO[5] (#3)	EVOO[5](#2), SBO[8](#3)	SBO[8] (#2)
5.08 g sugar	5.08 g sugar	5.08 g sugar	<1 g sugar	0 g sugar
133.35 mg sodium	69.85 mg sodium	292.1 mg sodium	469.9 mg sodium	469.9 mg sodium

[#] Vinegar and oil products are expressed in order of appearance on the nutrition facts label

Table 2. Randomized Taste Testing Groups

SEQUENCE #1 (HOMEMADE DRESSINGS)						
	SUBJECTS	**I**	**II**	**III**	**IV**	**V**
Group #1	5, 7, 9	H1	H2	H3	H4	H5
Group #2	1, 4	H2	H3	H4	H5	H1
Group #3	2, 6, 10	H3	H4	H5	H1	H2
Group #4	3, 8	H4	H5	H1	H2	H3

SEQUENCE #2 (COMMERCIAL DRESSINGS)						
	SUBJECTS	**I**	**II**	**III**	**IV**	**V**
Group #1	3, 6, 7	C1	C2	C3	C4	C5
Group #2	5, 9	C2	C3	C4	C5	C1
Group #3	2, 8, 10	C3	C4	C5	C1	C2
Group #4	1, 4	C4	C5	C1	C2	C3

Each participant was given five minutes to taste and evaluate each dressing in four areas: overall taste, saltiness, tartness, and sweetness. Following the five minutes, the subjects were not permitted to sample that particular dressing a second time. Ten scoring sheets (one sheet per dressing) were provided to the participants in order to rate the samples and leave comments (Appendix F). Scoring sheets were removed from the tasting space after each sequence; therefore, the participants were able to revisit H1-H5 testing sheets during sequence 1 and C1-C5 throughout sequence 2. Between each tasting, testers were provided water and Kroger unsalted thin and crispy saltine crackers as a palate cleanser. A 10-minute break was held between sequence 1 and sequence 2. Subjects were advised not to discuss dressings with other individuals during data collection. Dressings included in the taste test were the five dressings made by the researcher (H1-H5), in addition to the following commercial products: Brianna's ® Champagne Caper Vinaigrette (C1), Annie's® Lite Raspberry Vinaigrette (C2), Wishbone® Red Wine Vinaigrette (C3), Kraft® Greek Vinaigrette (C4), and Bernstein's® Restaurant Recipe Italian dressing (C5) (Table 1).

Phase II.

The secondary portion of the study was designed as a single-blind, placebo-controlled randomized crossover trial to evaluate the effect of the dissociation of acetic acid on the postprandial response. All subjects were randomized into treatment groups using a 4x4 block design and randomization determined by QuickCalcs randomization software (GraphPad, Inc. La Jolla, CA 92037). Each was asked to participate in a total of four sessions (Refer to Table 3 for randomization of treatment groups). Sessions were

held over the course of four weeks, at least seven days apart. Each testing session lasted two hours.

Table 3. Randomized Treatment Groups

GROUP #1

	SUBJECTS	I	II	III	IV
A	22, 24, 31	H1	H2	H3	C4
B	21, 23, 25	H2	H3	C4	H1
C	11, 13	H3	C4	H1	H2
D	12, 14, 15	C4	H1	H2	H3

GROUP #2

	SUBJECTS	I	II	III	IV
A	16, 18, 28	H1	H4	H5	C5
B	27, 29	H4	H5	C5	H1
C	17, 19, 30	H5	C5	H1	H4
D	20, 26	C5	H1	H4	H5

Seven dressings were tested: five homemade vinaigrette dressings (H1-H5), two commercial dressings (C4, C5), and one placebo. Commercial dressings included were Kraft® Greek Vinaigrette (C4) and Bernstein's® Restaurant Recipe Italian dressing (C5). The commercial dressings were selected due to high sodium content. Refer to Table 1 for treatment dressing composition.

In each trial session, the subject consumed the assigned test dressing, immediately followed by a bagel meal (See Table 4 for composition). Blood glucose was tested at fasting and 30-, 60-, 90-, and 120-minutes post-meal in order to examine glycemic response.

Table 4. Test Meal Composition

	Serving Size (g)	Calories	Carbohydrate (g)	Fat (g)	Protein (g)
Bagel	114	260	54	1	10
Butter	14	100	0	11	0
Apple Juice	245	120	29	0	0

The day preceding each session, the subjects were advised to eat a bagel along with the evening meal and begin fasting 12 hours before the trial time. All subjects were to refrain from participating in any light, moderate, or rigorous exercise 24 hours before testing.

Anthropometric Measurements

Body weight and height of each subject was measured at the initial screening visit. Height was measured using the Seca 213 stadiometer (Seca GmbH & Co, Chino, CA 91710), and weight using a TBF-300A Body Composition Analyzer (Tanita, Arlington Heights, Illinois 60005). Body mass index was calculated with the height and weight measurements and reported as kg/m^2. Values were reported as mean ± standard error.

Blood Analyses

Blood glucose levels for Phase II were taken at fasting pre-meal, and at 30-, 60-, 90-, and 120-minutes post-bagel meal for placebo and each vinaigrette dressing. These values were collected utilizing 28G, 1.25mm purple Capiject® safety lancets (Terumo

Medical Supplies, Somerset, NJ 08873) and a calibrated Accu-chek Aviva Plus glucometer (Indianapolis, IN 46256) (see Appendix G). The glucometers were calibrated once per week, and each subject was assigned the same glucometer throughout the four trial visits.

Statistical Analyses

All results were analyzed by the utilization of Statistical Package for Social Sciences (SPSS 23.0 for Mac; SPSS, Inc., Chicago, IL). Spearmen rho correlations were performed to assess relationships between variables. An independent t-test was utilized to determine difference in means at baseline between groups, and a general linear model for repeated measures ANOVA tested to evaluate significant treatment effects. Significant values were expressed as $p \leq 0.05$. All data were reported as means ± standard error of mean (SEM).

CHAPTER 4

RESULTS

Phase I: Dressing pH

Each of the homemade and commercial dressings was tested three times for pH levels to evaluate acidity based on the amount of sodium present within the product. Mean pH was calculated for every dressing (See Table 5). Results of pH testing determined that commercially manufactured dressings are similar in acidity, regardless of salt content (mean = 3.11). On the contrary, the homemade recipes containing vinegar in addition to 100 mg, 200 mg, 300 mg, and 400 mg of sodium demonstrated reductions in pH with the addition of salt (4.39, 3.12, 2.89, and 2.56, respectively). Star® white vinegar had the highest acidity at 2.65.

Table 5. Dressing Sodium Content and pH

Dressing[a]	Sodium content (mg)[c]	pH[c]
H1	100	4.27
H2	100	4.39
H3	200	3.12
H4	300	2.89
H5	400	2.56
C2	69.85	3.17
C1	133.35	3.08
C3	292.1	2.73
C4	469.9	3.28
C5	469.9	3.3
Star® White Vinegar (undiluted)	0	2.65

[a]Dressings listed in increasing order of sodium content
[b]Sodium content for ~38 grams of dressing
[c]Data expressed as mean of three collected measurements.

Phase I: Taste Test Descriptive Characteristics

The taste test was conducted in order to determine the likability of each dressing. Data was obtained from ten participants (3 males and 7 females). Table 6 represents the baseline characteristics of the taste test cohort.

Table 6. Baseline Characteristics of Taste Test Subjects

Gender (M/F)	Age (years)	Height (cm)	Weight (kg)	BMI (kg/m^2)
3M/7F	32.5±5.4[a] (23-40)[b]	176.5±13.8[a] (164.2-209.4)[b]	81.7±14.9[a] (60.6-110.6)[b]	26.4±4.8[a] (16.84-32.4)[b]

[a]Data are expressed as Mean ± SD
[b]Range from lowest to highest value

Taste Test Outcomes

Kraft® Greek Vinaigrette (C4) was established to be the most well liked of all dressings (score 68.3±8.1 SE). Subjects provided comments on the dressing, stating: "Nice balance with a hint of citrus" and "Great herb flavor- my favorite so far. (I would definitely buy this)." In addition to overall rankings, the Kraft® dressing received the top scores for saltiness (65.9±5.9 SE) and tartness (68.4±7.2 SE). Of the homemade vinaigrettes, dressing H3 containing 200 mg of sodium had the highest overall taste rating (58.6±6.7 SE), followed by the control dressing (57.9±8.6 SE). Overall, H4 was deemed the least desired by the subjects (41.55±6.5). Moreover, the dressing maintained the lowest ratings for saltiness, tartness, and sweetness (44.7±6.8 SE, 45.75±6.7 SE, and 52.7±4.6 SE, respectively). It was considered, "too salty – very salty aftertaste," and stated "This one definitely has the salt, but no other flavor was present." Generally, there

were no differences between overall ratings of commercial and homemade dressings (Refer to Figure 1).

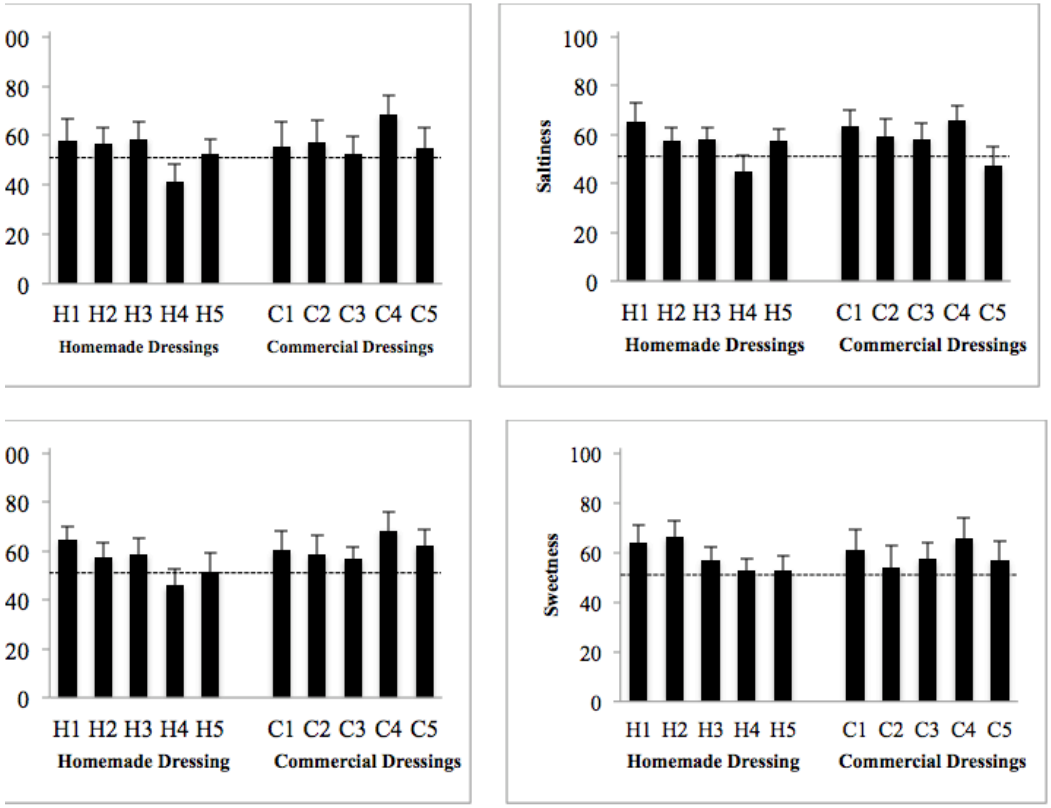
Three Spearman's rho correlations were performed on the collected data (Table 7). The first was completed in order to assess whether overall likeability of a dressing is associated with the sodium content. The test demonstrated a slight negative correlation between overall taste rating and sodium content ($r = -0.3$), suggesting that as sodium content of the dressings increased, overall likability decreased. Analysis determined that the relationship between the two variables was weak and not statistically significant ($p>0.05$).

The second correlation test was used to evaluate the ability of the subjects to rate saltiness in relation to sodium content of each dressing. The association between the variables was small and not statistically significant ($r = -0.3$, $p>0.05$). This suggests that the subjects who participated in the taste testing trial likely do not have trained palates and are not fully able to distinguish between differences in sodium content based on taste alone. In the final correlation, it was demonstrated that overall rating had a negative correlation to saltiness rating ($r = -0.733$, $p<005$).

Table 7. Correlation Between Sodium Content of Dressings and Perception of Likability and Saltiness[a]

Variable	*n*	*r*	*p*-value
Overall Rating vs. Sodium Content	10	-0.262	0.464
Saltiness Rating vs. Sodium Content	10	-0.305	0.392
Overall rating vs. Saltiness Rating	10	-0.733	0.016

[a]Variables in correlation with sodium content of dressings Spearman rho correlation



Taste Test Scores for Overall Taste, Saltiness, Tartness and Sweetness of Homemade and Commercial Dressings. re determined by measuring the likert scale on taste test scorecard (0-102) in millimeters with a double-edge ruler. as follows: 102 = Greatest Imaginable Like, 51 = Neither like nor Dislike (represented by dotted line in charts), 0 = naginable Dislike. Overall Taste is the average likability of the dressing (tartness + saltiness + sweetness) Data are as mean ± SE.

Phase II: Vinaigrette Trial Visit Descriptive Characteristics

The vinaigrette trial visits were conducted in order to determine the effect of each dressing on postprandial glucose. Data was collected from a total of twenty participants. Subject #14 dropped out of the study after the first visit due to pregnancy. Results for subject #26 were omitted as a result of pre-diabetic fasting blood glucose values of >100mg/dL and 2-hr postprandial glucose values >140mg/d; therefore, statistical analysis was completed on 18 subjects (4 males and 14 females), nine individuals in each group. Table 7 represents the baseline characteristics of group 1 and group 2. Fasting blood glucose was within the normal range of 70-110 mg/dL within both groups 1 and 2 (92.9±5.3 and 88.2±5.3, respectively). Independent t-tests were performed to determine Mean age, height, weight, and BMI did not vary greatly between each cohort ($p>0.05$).

Table 8. Baseline Characteristics of Vinaigrette Trial[a,b]

	Group 1	Group 2	*p*-value
Gender (M/F)	2M/7F	2M/7F	-
Age (years)	26.9±10.1 (18-52)	25.3±6.2 (18-33)	0.72
Height (cm)	166.3±5.9 (158-175)	169.1±10 (157-191)	0.483
Weight (kg)	70.2±8.9 (59.6-86.9)	71.3±16.7 (46.5-93.8)	0.872
BMI (kg/m^2)	25.3±2.4 (23.1-29)	24.8±5.2 (18.2-29.3)	0.788
Fasting glucose	92.9±5.3 (81.5-101)	88.2±5.3 (79.3-93.8)	0.08

[a]Data are mean ±SD
[b]Range from lowest to highest value

Phase II: Postprandial glucose response

During this phase of the study, each individual was evaluated for blood glucose concentrations via capillary fingerstick at fasting, 30-, 60-, 90-, and 120 minutes post-consumption of test dressing and bagel meal. Incremental area-under-the-curve (iAUC) was calculated for each dressing using the trapezoidal rule. Table 8 displays postprandial blood glucose values for each treatment prior to calculation of iAUC. Statistical analysis using a general linear model for repeated measures ANOVA was processed separately for group 1 (H1, H2, H3, C4 dressings) and group 2 (H1, H4, H5, C5 dressings) (Tables 9 and 10). All 120-minute blood glucose data were omitted from the final analysis, as the curve levels off after 90-minutes.[66]

Table 9. Average Postprandial Blood Glucose Values

Group 1	H1[a]	H2[a]	H3[a]	C4[a]
Fasting	96.2±3.2	90.6±2.7	91.2±2.0	93.4±3.0
30 mins	130.0±7.2	120.6±6.6	125.9±4.8	121.8±5.4
60 min	119.7±6.6	102.7±3.1	105.3±4.8	114.4±6.6
90 min	106.4±3.3	105.1±3.2	108.0±2.8	103.6±2.4
120 min	105.2±3.6	107.3±3.2	100.8±3.0	102.6±2.7
Group 2	**H1[a]**	**H4[a]**	**H5[a]**	**C5[a]**
Fasting	89.1±2.5	89.3±2.3	86.4±2.1	87.9±3.2
30 mins	121.2±2.6	123.9±2.9	125.3±5.0	127.7±8.1
60 min	117.9±3.7	112.7±2.7	113.9±3.0	119.6±4.9
90 min	104.2±6.2	105.7±2.1	103.3±2.2	112.7±5.0
120 min	106.1±4.5	105.0±2.4	105.6±3.0	105.0±3.8

[a]Data are mean ± SEM

Treatment H2 (100 mg sodium) was the most effective of all dressing treatments, demonstrating an approximate 21% reduction in postprandial glucose when compared to the control. Of the commercial dressings, the Kraft® Greek Vinaigrette (C4) was more effective in lowering blood glucose than Bernstein's ® Restaurant Recipe Italian dressing (C5) at 54.4mg/dL±12.62 and 83.8mg/dL±5.3, respectively (Figures 3 and 4). Additionally, commercial dressing C5 was the least effective in the attenuation of blood glucose post bagel meal. No statistically significant changes between dressings were observed in either group 1 or group 2 ($p>0.05$) for iAUC at 90 minutes. Effect size was large in both group 1 ($\eta^2=0.161$) and group 2 ($\eta^2=0.577$).

Table 10. Group 1 Incremental Area-Under-Curve of Postprandial Glucose Following Dressing and Bagel Meal[a,b,c]

	Control (H1)	H2	H3	C4	*p*-value
iAUC Blood glucose	62.3±7.5	49.4±9.9	57.2±8.8	54.4±12.6	0.769

[a]Data are mean ±SEM
[b]Incremental-area-under-the-curve calculated using trapezoidal rule for capillary fingerpricks at fasting, and 30, 60, and 90 minutes postprandially
[c]Randomized 4x4 block design using QuickCalcs software (*n*=9)

Table 11. Group 2 Incremental Area-Under-Curve of Postprandial Glucose Following Dressing and Bagel Meal[a,b,c]

	Control (H1)	H4	H5	C5	*p*-value
iAUC Blood glucose	68.4±10.6	66.1±7.7	74.8±6.5	83.8±5.3	0.137

[a]Data are mean ±SEM
[b]Incremental-area-under-the-curve calculated using trapezoidal rule for capillary fingerpricks at fasting, and 30, 60, and 90 minutes postprandially
[c]Randomized 4x4 block design using QuickCalcs software (*n*=9)

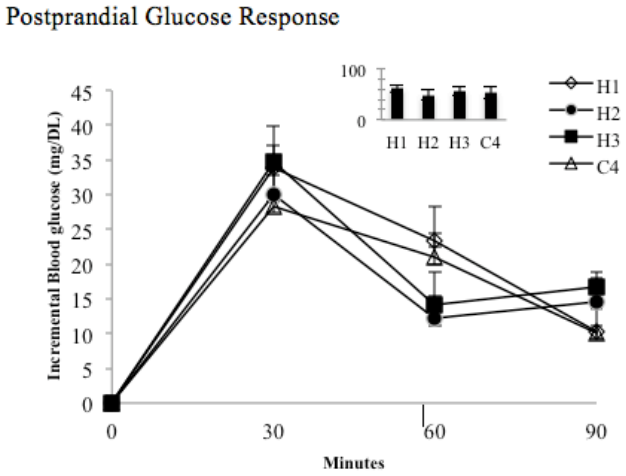


Figure 2 – Group 1 postprandial incremental blood glucose concentrations following bagel meal using H1, H2, H3, and C4 treatment dressings. Chart inlay depicts incremental area-under-curve (iAUC, trapezoidal rule). Values are expressed as means ± SEM.

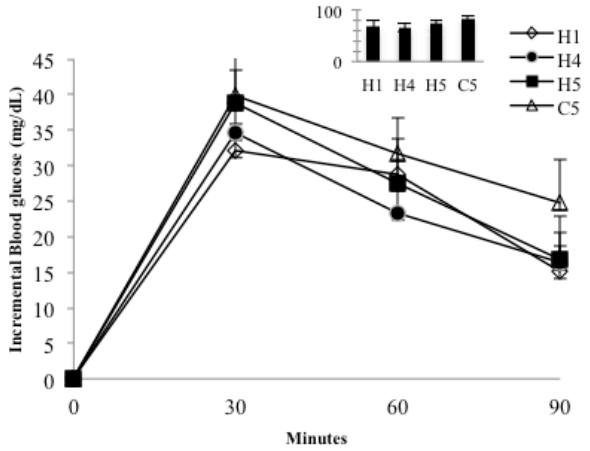


Figure 3 – Group 2 postprandial incremental blood glucose concentrations following bagel meal using H1, H4, H5, and C5 treatment dressings. Chart inlay depicts incremental area-under-curve (iAUC, trapezoidal rule). Values are expressed as means ± SEM.

CHAPTER 5

DISCUSSION

The present study examined the effects of sodium chloride (table salt) on the ability of acetic acid to attenuate the postprandial glycemic respond following a bagel meal. And additional aim was to develop a flavorful vinaigrette that is beneficial in reducing the hyperglycemic effect of food. As of today, several studies have observed the antiglycemic effects of acetic acid; however, there does not appear to be any research performed to examine the compound in the form of vinaigrette and its dissociation by sodium chloride.[6,52,54]

Phase I (vinaigrette pH testing and tasting trial) established that pH tended to decrease with the addition of sodium. The Spearman's rho correlation analysis of the taste testing data suggests that the participants involved within the study did not have trained palates, although each denied taste inhibitions during consent. This denotes that within the present trial, the individuals may may not be capable of accurately discerning the amount of sodium content in each dressing.[67] Saltiness did not have a direct correlation to sodium content of the dressing, nor did the preference in dressings correlate directly with sodium content ($p = 0.464$ and $p = 0.392$, respectively). Additionally, the overall taste rating and overall saltiness ratings demonstrated a negative correlation ($p = 0.016$), suggesting that the more salt the dressing contained, the less the consumer appeared to like the product. Therefore, if the average consumer is not able to efficiently perceive sodium content of a food product, it may be beneficial to omit the ingredient entirely from the dressing. By doing so, it is less likely that the pH will decrease, and thus

less prone to interfering with the antihyperglycemic properties of the acetic acid molecules.

Kraft® Greek Vinaigrette dressing (C5) received the highest scores in overall taste, saltiness, and tartness categories . This particular dressing contains garlic, black olives, feta, cucumber juice, dried onions and other spices for flavor. Future studies should add herbs and spices, such as in the Kraft® product, to the homemade test dressings in order to determine if it is likely improve the flavor profile; hence, producing a dressing that is more desirable to consumers while excluding salt. It is important to note that the Kraft® dressing contained 469.9mg of sodium in 38g (~2Tbsp), which may have an enhancing effect on the flavor. Therefore, the new formula must be taste tested both with and without the inclusion of salt and evaluate desirability.

In phase II it was expected that the antiglycemic effect and sodium content would be inversely related. Incremental area-under-the-curve calculations revealed this pattern marginally within the homemade dressing group. H2 (100mg) had the lowest iAUC at 49.4mg/dL±9.9, followed by H3 (200 mg) at 57.2mg/dL±8.8, H4 (300 mg) at 66.1mg/dL±7.7, and H5(400mg) with the highest at 74.8mg/dL±6.5.

When compared to the control, H2 was the dressing that demonstrated the highest reduction in blood glucose at roughly 21%; however, none of the treatments demonstrated a statistically significant reduction in blood glucose levels ($p>0.05$). Based on the statistical significance, the intervention phase did not deliver sufficient evidence to support either a homemade or commercial dressing that is optimal for the reduction of postprandial glycemia.

Conversely, a large effect size was observed within group 1 (η^2=0.161) and group 2 (η^2=0.577). This suggests that sodium content may negatively effect the antiglycemic mechanism of acetic acid, similar to the effects of sodium bicarbonate within the study by Brighenti et al., in which the molecule neutralized the acetic acid and was not effective in lowering blood glucose levels.[6] The sodium chloride is potentially removing the hydrogen ions from the acetic acid; thus, transforming the molecule into its base, acetate. As previously discussed, Johnston et al. demonstrated that the acetate form was ineffective in attenuating postprandial glycemia; therefore, the hydrogen ion may be important piece to the currently unknown mechanism.

A limitation within the trial phase was sample size (n=9 per group). Sample size calculations determined that at least eleven participants were required in each intervention group in order to achieve significant results (Appendix H). The appropriate power was not achieved due to attrition and the exclusion of data from participant #26. An additional limitation was the control dressing selected for the study. Earlier research has established 20 grams of vinegar (5% acetic acid) as efficient in reducing postprandial glycemia.[58] Based on this evidence, it may have been beneficial to use 20 grams of undiluted vinegar as a control, rather than H1. The control employed in the present study was selected for it was expected to be difficult for the test subject to discern from a treatment containing vinegar or lemon juice. This is due to the acidity of the ingredient, as well as the addition of olive oil to create a vinaigrette-like dressing. Additionally, there is no current evidence to claim lemon juice has an effect on the glucose response; therefore, it was deemed an acceptable ingredient to employ as a control. Moreover, the acetic acid content of commercial dressings C1-C5 was not tested and is unknown.

Consequently, it is undetermined whether 38 grams of commercial test dressings contained 20 grams of acetic acid. Dressings C4 and C5 contained the same amount of sodium (469.9mg); however, C4 was more effective in lowering blood glucose levels (iAUC 54.4±12.6), while C5 spiked the blood glucose level higher than that of the control (83.8±5.3). If the acetic acid content was identified, the reason for the variance in values may have become clearer.

CHAPTER 6

CONCLUSION

At this time, it is not recommended to consume a vinaigrette dressing containing sodium to attenuate blood glucose levels. There is not enough evidence to support the consumption of a dressing to manage hyperglycemia.

The present study serves as a step in the direction of producing an optimal vinaigrette dressing. Prospective studies may evaluate homemade dressings that exclude sodium as flavoring component, utilizing spices and herbs within its place. An additional taste test dressings could be performed including dressings both with and without sodium in order to analyze the association between groups and determine if sodium is favored in dressings or may be omitted completely.

Additional modifications to incorporate into future study designs include the utilization of 20 grams of vinegar containing 5% acetic acid as a control, rather than a lemon juice and oil concoction. Testing acetic acid content of all commercial dressings prior to conducting an intervention trial would also provide added benefit to the conduction of studies. This will allow the research team to provide at least 20 grams of acetic acid per treatment and complete a proper analysis of the data.

Today, the benefits of vinegar are not discussed by the American Diabetes Association for the treatment of hyperglycemia in diabetic individuals. Continuation of this research may have the potential of validating the effects of acetic acid, as well as produce an effective vinaigrette for the attenuation of postprandial glucose; an act that will positively alter the future for diabetics.

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APPENDIX A

TASTE TEST CONSENT LETTER

DEVELOPING THE OPTIMAL VINAIGRETTE DRESSING FOR MANAGING BLOOD GLUCOSE CONCENTRATIONS

I am a graduate student under the direction of Dr. Carol Johnston, Professor, in the Nutrition Program at Arizona State University. I am conducting a research study to develop the optimal vinaigrette dressing recipe to aid the diabetic condition.

I am inviting your participation, which will involve tasting 10 vinaigrette dressings over the course of one hour. You will have 5 minutes to assess each dressing and complete a scoring measure; a 10 minute break will occur after the first 5 assessments, and water with a no salt saltine will be used between testing to cleanse the palate. Finally, your height and weight will be recorded. We will ask you if you wish to consider participating in a follow-up study. No names will be recorded; however, if you wish to be considered for the follow-up study, we will ask you for your email address in order to reach you later. You have the right not to answer any question, and to stop participation at any time.

You must be 18 or older to participate in the study. Your participation in this study is voluntary. If you choose not to participate or to withdraw from the study at any time, there will be no penalty. You will receive a $10 Target card at the end of the testing session.

Although there is no non-monetary benefit to you for participating in this taste testing, your participation will help us determine the most effective vinaigrette dressing recipe for managing blood glucose. There are no foreseeable risks or discomforts to your participation.

Your responses will be anonymous. We will record your email address if you wish to participate in the follow-up study. The results of this study may be used in reports, presentations, or publications but your name will not be used.

If you have any questions concerning the research study, please contact the research team at: carol.johnston@asu.edu. If you have any questions about your rights as a subject/participant in this research, or if you feel you have been placed at risk, you can contact the Chair of the Human Subjects Institutional Review Board, through the ASU Office of Research Integrity and Assurance, at (480) 965-6788. Please let me know if you wish to be part of the study

Thank you for your time and commitment to research at Arizona State University.

APPENDIX B

HEALTH HISTORY QUESTIONNAIRE

HEALTH HISTORY QUESTIONNAIRE

ID#_____

Height _____ft. ____in.	Weight: _____lbs.	Waist: _____ins.	To be completed by investigator

Age: _____

Gender: □ Male □ Female

Smoker: □Yes □ No

1. Have you been diagnosed with pre-diabetes or diabetes? Y N

2. Have you been diagnosed with other chronic diseases (such as heart disease, neurological disease, autoimmune disease, or cancer)? Y N

3. Do you take any medications regularly? Y N
Please list what kind and how frequently:

Medication	Dosage	Frequency

4. Do you currently take supplements (vitamins, minerals, herbs, etc.)? Y N
If yes, what supplements and how often?

_____
_____
_____
_____
_____

5. Do you have medical conditions that you see a physician for on a regular basis? Y N
Please explain:

_____
_____
_____
_____
_____

OVER →

6. Do you have any food allergies? Y N
If yes, please describe _____
_____

7. Do you follow a special diet? (weight gain/loss, vegetarian, low-fat, etc.) Y N
If yes, please describe
_____

_____

8. Will you have any problems fasting for 12 hours prior to testing sessions? Y N

9. Do you have any difficulty chewing or swallowing? Y N

10. Are you OK with eating bagels with different spreads? Y N

11. Will you have a problem (such as fainting) providing blood samples? (5 finger pricks per test day) Y N

12. If you drink alcohol or caffeine, will you be able to abstain from these beverages for the 24-hour periods prior to test days? Y N

13. If you exercise regularly, will you be able to not exercise (other than basic walking and work activity) for the 24-hour period prior to testing? Y N

14. Please circle the number of times you did the following kinds of exercises for **more than 15 minutes** last week.

Mild exercise (minimal effort):
Easy walking, golf, gardening, bowling, yoga, fishing, horseshoes, archery, etc.

Times per week: 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14+

Moderate exercise (not exhausting):
Fast walking, easy bicycling, tennis, easy swimming, badminton, dancing, volleyball, baseball, etc.

Times per week: 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14+

Strenuous exercise activities (heart beats rapidly):
Running, jogging, hockey, football, soccer, squash, basketball, cross country skiing, judo, roller skating, vigorous swimming, vigorous long distance bicycling, etc.

Times per week: 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14+

Please describe any other conditions or medical reasons that may affect your participation below:

_____
_____
_____
_____

APPENDIX C

TRIAL VISIT SURVEY MONKEY QUESTIONNAIRE

ASU Nutrition Professor Carol Johnston and her Master's student, Amber Bonsall, are inviting your participation in this screening process, which will consist of answering questions regarding health history, demographics, and scheduling availability. You have the right not to answer any question, and to stop participation at any time.

Your participation in this survey and study is voluntary. If you choose not to participate or to withdraw from the study at any time, there will be no penalty. Your responses to this survey will be confidential. If you meet the criteria for this study, you will be contacted to schedule an in-person appointment at the downtown campus of Arizona State University.

If you have any questions concerning the research study, please contact the research team at carol.johnston@asu.edu. If you have any questions about your rights as a subject/participant in this research, or if you feel you have been placed at risk, you can contact the Chair of the Human Subjects Institutional Review Board, through the ASU Office of Research Integrity and Assurance, at (480) 965-6788.

By completing the survey below you are agreeing to be screened for this research (*The Vinaigrette Dressing Study*) and be contacted by investigators (via e-mail) to schedule an appointment, should you qualify.

* 1. Please provide your email address:

* 2. Are you between the age of 18 and 60 years old?

- Yes
- No

* 3. Has your body weight been stable (plus or minus 6.5 pounds) over the past 3 months?

- Yes
- No

* 4. Are you healthy and free of chronic disease?

- Yes
- No

* 5. Has your physician diagnosed you with either pre-diabetes or diabetes?

- Yes
- No

* 6. Do you have any condition that might affect your ability to taste?

- Yes
- No
- Unsure

* 7. Do you currently smoke, or have smoked in the past 6 months?

- Yes
- No
- Unsure

* 8. Do you have any food allergies, or are there foods you cannot eat?

- Yes
- No
- If yes, please list.

* 9. Are you willing to fast for 10 hours and then eat vinaigrette dressing, a bagel, and juice at the test site?

- Yes
- No

* 10. Would you be willing and able to commit to four, 2--hour visits one week apart for this study?

- Yes
- No

* 11. Would you be willing and able to tolerate five finger pricks on test days for this study?

- Yes
- No

Thank You!

APPENDIX D

TRIAL VISIT CONSENT FORM

DEVELOPING THE OPTIMAL VINAIGRETTE DRESSING FOR MANAGING BLOOD GLUCOSE CONCENTRATIONS

INTRODUCTON

The purposes of this form are (1) to provide you with information that may affect your decision as to whether or not to participate in this research study, and (2) to record your consent if you choose to be involved in this study.

RESEARCHERS

Dr. Carol Johnston, a Nutrition professor at Arizona State University Downtown Campus, and Amber Bonsall, a MS student in nutrition, have requested your participation in a research study.

STUDY PURPOSE

Scientific evidence has shown that vinegar ingestion at meal-time reduces blood glucose concentrations in healthy individuals and in those with prediabetes or type 2 diabetes. The purpose of this research is to develop the optimal vinaigrette dressing recipe to aid the diabetic condition.

DESCRIPTION OF RESEARCH STUDY

You have indicated to us that you are 18-60 years of age and generally healthy. You have not been diagnosed with diabetes, and (if female) you have not recently been pregnant or lactating. Participants will be asked to maintain their usual diet and physical activity level throughout the trial with the exception of the day prior to testing. This study will initially involve the completion of a brief medical history questionnaire to demonstrate the absence of medical conditions that may impact the study. Your weight, height, and girth will be measured at this time. This first meeting will take <30 minutes. There are four additional visits (e.g., the test days) that will last about 2 hours each and scheduled a week apart. The procedures on test days are identical. On the day prior to testing you are asked to avoid exercise (normal activities such as walking to work or walking the dog is ok). You will be asked to eat a breakfast and lunch of your choice. The evening before the test day, you will eat a bagel with your dinner. Bagels will be given to you. Following dinner, you will fast overnight and not consume any food or beverage with the exception of water. On test days, you will travel to ASU (the Nutrition labs at the ABC1 Building on the ASU Downtown campus) early in the morning. Your finger will be pricked for a drop of blood. You will sit down and consume the dressing and a test meal (bagel and juice). Your finger will be pricked 4 more times over the next 2 hours. You may drink water during these two hours but you are not to consume any food. Otherwise, you may read, study, or work on the computer at the test site. Once testing is complete, you may proceed with your normal activities. About 20 subjects will participate in this study.

Finger pricks will be conducted under sterile conditions using disposable, retractable lancets. Blood samples will be analyzed for glucose.

RISKS

Bruising of the skin or a feeling of faintness is possible during the finger pricks

BENEFITS

There is no direct benefit for participating in this trial. If desired, you will be provided with study results and your personal blood data at the end of the study.

NEW INFORMATION

If the researchers find new information during the study that would reasonably change your decision about participating, then they will provide this information to you.

CONFIDENTIALITY

All information obtained in this study is strictly confidential unless disclosure is required by law. The results of this research study may be used in reports, presentations, and publications, but your name or identity will not be revealed. In order to maintain confidentiality of your records,

Dr. Johnston will use subject codes on all data collected, maintain a master list separate and secure from all data collected, and limit access to all confidential information to the study investigators. Plasma from blood samples will be stored for 5 years in freezers in the laboratories of the Nutrition Program at Arizona State University Downtown Campus after which time they will be disposed of as biohazard waste.

WITHDRAWAL PRIVILEGE

You may withdraw from the study at any time for any reason without penalty or prejudice toward you. Your decision will not incur negative treatment to you by the researchers.

COSTS AND PAYMENTS

The all test foods will be given to you during the study free of charge. You will receive a $5 Target card at test visits 1 and 2 and a $10 Target card at test visits 3 and 4.

COMPENSATION FOR ILLNESS AND INJURY

If you agree to participate in the study, then your consent does not waive any of your legal rights. However, in the event of harm, injury, or illness arising from this study, neither Arizona State University nor the researchers are able to give you any money, insurance coverage, free medical care, or any compensation for such injury. Major injury is not likely but if necessary, a call to 911 will be placed.

VOLUNTARY CONSENT

Any questions you have concerning the research study or your participation in the study, before or after your consent, will be answered by Dr. Carol Johnston; 500 N. 3rd Street Phoenix, AZ 85004; 602-827-2265.

If you have questions about your rights as a subject/participant in this research, or if you feel you have been placed at risk, you can contact the Chair of the Human Subjects Institutional Review Board, through the ASU Research Compliance Office, at 480-965 6788.

This form explains the nature, demands, benefits and any risk of the project. By signing this form you agree knowingly to assume any risks involved. Remember, your participation is voluntary. You may choose not to participate or to withdraw your consent and discontinue participation at any time without penalty or loss of benefit. In signing this consent form, you are not waiving any legal claims, rights, or remedies. A copy of this consent form will be given to you.

Your signature below indicates that you consent to participate in the above study.

_____	_____	_____
Subject's Signature	Printed Name	Date

_____	_____
Contact phone number	Email

INVESTIGATOR'S STATEMENT

"I certify that I have explained to the above individual the nature and purpose, the potential benefits, and possible risks associated with participation in this research study, have answered any questions that have been raised, and have witnessed the above signature. These elements of Informed Consent conform to the Assurance given by Arizona State University to the Office for Human Research Protections to protect the rights of human subjects. I have provided the subject/participant a copy of this signed consent document."

Signature of Investigator_____ Date_____

APPENDIX E

PH METER INSTRUCTIONS

HANNA INSTRUMENTS® HI99161 PH METER INSTRUCTIONS

Hanna Instruments ® HI99161 pH meter is a portable food and dairy meter. It directly measures pH of food products with an electrode that has a conical shape capable of penetrating solids, semi-solids, and emulsions for accurate readings.

pH Measurement:

1. Calibrate the meter (see below in *Meter Calibration* section)

2. In the event that the electrode is dry, place a sufficient amount of HI 70300 storage solution into a clean small beaker to fully immerse the tip of the electrode.

3. Immerse the electrode in the HI 70300 solution for 60 minutes and gently wipe down the tip with a dry paper towel.

4. Insert the tip of the electrode into the sample.

5. Wait for the stability indicator to turn off on the LCD, and the pH measurement will then appear on the screen.

6. Clean the probe between test samples to prevent cross-contamination.

Meter Calibration:

1. Press the ON/OFF/MODE button on the LCD until “OFF” on the screen is replaced with “CAL”. Release.

2. The LCD will then display “pH 7.01 USE”

3. Place a sufficient amount of the 7.01 pH buffer solution into a clean small beaker to fully immerse the tip of the probe.

4. Insert the tip of the electrode into the pH buffer solution. Meter will display “OK”.

5. For a two-point calibration, follow steps 1-4, then the display will state “pH 4.01 USE”

6. Place a sufficient amount of the 4.01 pH buffer solution into a clean small beaker to fully immerse the tip of the probe.

7. Insert the tip of the electrode into the pH buffer solution. Meter will display “OK” and return to regular mode for pH testing.

APPENDIX F

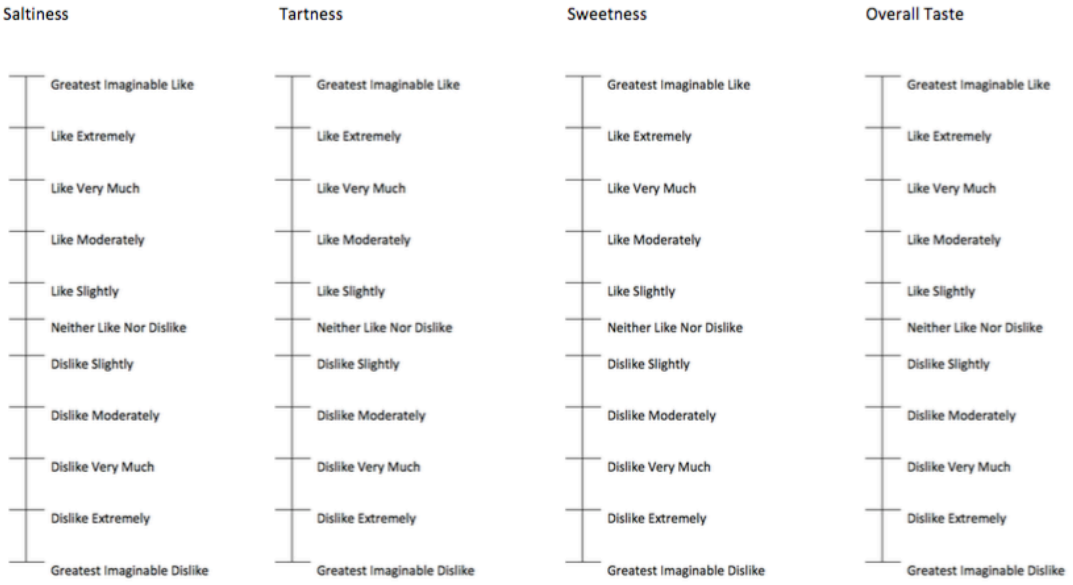
TASTE TEST SCORECARD

DEVELOPING THE OPTIMAL VINAIGRETTE DRESSING FOR MANAGING BLOOD GLUCOSE CONCENTRATIONS
Taste Test

ID:

Dressing:

Please place an 'X' anywhere on the line to rate the following:



Comments:

The taste test scale created for the present study is a modified version of the labeled affective magnitude scale (LAM) developed by Schutz and Cardello in 2000.[68,69] The scale was originated from the 9-point hedonic scale that is often utilized in food science to receive feedback on food products by consumers The LAM scale was developed to create a ratio scale, rather than the gradation method of the 9-point scale.[69] Additionally, the LAM scale was noted to be easy to use by consumers, and was deemed just as effective as the hedonic method.[68,69] The limitation to this method, is that it can only be utilized for within-group comparisons and not across-group comparisons.[68] For the present study, participants were permitted to mark anywhere on each line to express thoughts in regards to each test dressing. Markings were measured in millimeters. Scale was as follows: 102 = greatest imaginable like, 51 = neither like nor dislike, and 0 = greatest imaginable dislike.

APPENDIX G

GLUCOMETER INSTRUCTIONS

ACCU-CHEK® AVIVA PLUS INSTRUCTIONS

Accu-check® Aviva Plus is a small and portable glucometer. It measures blood glucose values within five seconds after a blood sample is placed on the test strip. The blood sample size required is 0.6µl.

Blood Glucose Test:

1. Put on gloves.
2. Hold on/off button of the glucometer to make certain that the display is working.
3. Have the patient wash hands with soap and water. Completely dry hands.
4. Prepare Capiject® safety lancet by removing white tip.
5. Insert test strip into the device, arrow-side up. Meter will turn on.
6. Check that the test strip code number and the number on the display match.
7. A blood drop sample will flash to indicate readiness for blood sample.
8. Use the Capiject® safety lancet to obtain blood by pushing the device firmly against the patient's skin on the side of the desired fingertip.
9. Gentle squeeze the patient's finger to encourage blood flow and touch the drop of blood to the yellow tip of the test strip.
10. An hourglass will blink on the screen in order to indicate sufficient amount of blood on the test strip. In the instance that the hourglass does not flash, wait five seconds and apply an additional drop of blood to the strip.
11. The result will appear on the display.

APPENDIX H

SAMPLE SIZE CALCULATIONS

	Author	Year	Change	SD	n per group	Calculated n	Age Range (years± SEM)	Subject State	Test
1	Brighenti et al.	1995	2886	900	5	10	37±3	Healthy	Blood glucose (randomized crossover)
2	Johnston et al.	2005	1.2	1.3	11	21	27.9±2.9	Healthy	Blood glucose (randomized crossover)
3	Ostman et al.	2005	23.2	11.2	12	6	22.9±0.5	Healthy	Blood glucose (randomized crossover)
4	Johnston et al	2010	38	21	10	7	35±4	Healthy	Blood glucose (randomized crossover)

According to the sample size calculations completed utilizing a calculation tool by Dr. David A Schoenfeld.[70] Average *n* per group within the four studies is 9.5. The average calculated *n* is 11.5; therefore, in order to obtain 80% power and achieve a significant outcome ($p<0.05$), it would be beneficial to recruit approximately 12 subjects per group for the present study.