Vinegars Effects on Hemoglobin A1c and Postprandial Glycemia in Individuals at Risk for Diabetes

by

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ABSTRACT

Objective: Vinegar consumption studies have demonstrated possible therapeutic effects in reducing HbA1c and postprandial glycemia. The purpose of the study was to closely examine the effects of a commercial vinegar drink on daily fluctuations in fasting glucose concentrations and postprandial glycemia, and on HbA1c, in individuals at risk for Type 2 Diabetes Mellitus (T2D).

Design: Thirteen women and one man (21-62 y; mean, 46.0±3.9 y) participated in this 12-week parallel-arm trial. Participants were recruited from a campus community and were healthy and not diabetic by self-report. Participants were not prescribed oral hypoglycemic medications or insulin; other medications were allowed if use was stable for > 3 months. Subjects were randomized to one of two groups: VIN (8 ounces vinegar drink providing 1.5 g acetic acid) or CON (1 vinegar pill providing 0.04 g acetic acid). Treatments were taken twice daily immediately prior to the lunch and dinner meals. Venous blood samples were drawn at trial weeks 0 and 12 to measure insulin, fasting glucose, and HbA1c. Subjects recorded fasting glucose and 2-h postprandial glycemia concentrations daily using a glucometer.

Results: The VIN group showed significant reductions in fasting capillary blood glucose concentrations ($p=0.05$) that were immediate and sustained throughout the duration of the study. The VIN group had reductions in 2-h postprandial glucose (mean change of -7.6±6.8 mg/dL over the 12-week trial), but this value

was not significantly different than that for the CON group (mean change of 3.3±5.3 mg/dL over the 12-week trial, p=0.232). HbA1c did not significantly change (p=0.702), but the reduction in HbA1c in the VIN group, -0.14±0.1%, may have physiological relevance.

Conclusions: Significant reductions in HbA1c were not observed after daily consumption of a vinegar drink containing 1.5 g acetic acid in non-diabetic individuals. However, the vinegar drink did significantly reduce fasting capillary blood glucose concentrations in these individuals as compared to a vinegar pill containing 0.04 g acetic acid. These results support a therapeutic effect for vinegar in T2D prevention and progression, specifically in high-risk populations.

DEDICATION

This is dedicated to my wonderful parents, Olga and Dan, who had me grow up believing in myself and realizing I can accomplish anything I put my mind to. Thank you for always being supportive and caring, as I have pursued my adventures and journeys in life. I love you very much.

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Chapter 1

INTRODUCTION

Type 2 Diabetes Mellitus (T2D) is a growing epidemic: 26 million Americans have T2D and another 79 million are pre-diabetic and at high risk of developing T2D (1). Worldwide incidences of T2D dramatically escalate to an estimate of 366 million individuals having T2D by 2030 in comparison to 171 million in 2000 (2). Sedentary behaviors, low levels of physical activity, and poor diet quality are major factors influencing this epidemic (3-7). Complications arising from diabetes mellitus include blindness, amputations, and kidney problems, as well as the development of cardiovascular disease (CVD) and stroke (1, 8, 9).

Many trials and studies have investigated how to ameliorate complications associated with T2D, but there is a need to develop primary prevention plans aimed at the high risk or pre-diabetic state to slow the progression of pre-diabetes to diabetes. Postprandial glycemia (PPG), the rise in blood glucose after eating, and glycoslyated hemoglobin (HbA1c) are two risk markers that predict development of T2D. The American Diabetes Association (ADA) defines pre-diabetes as fasting plasma glucose $\geq$ 100 mg/dL and $<$ 126 mg/dL and HbA1c $\geq$ 5.7% and $<$ 6.4% (10).

Many costly treatments and prevention plans are structured to lower the high blood glucose concentrations commonly found in diabetes. Various studies indicate that insulin or oral hypoglycemic medications are effective in reducing blood glucose concentrations and treating diabetes (11, 12). Although these

treatments have shown to be effective in various ways, the costs from medical expenses and the drug related side effects reduce quality of life (1, 13). More research is needed to develop inexpensive, alternative prevention plans to help control of blood glucose surges after meals.

In current studies, vinegar consumption has been proposed as an inexpensive, safe, alternative therapy showing effects comparable to current diabetic medications (9). These promising results include reductions in PPG and HbA1c in individuals with T2D (14-16). Additionally, healthy and pre-diabetic subjects may also benefit from reducing PPG, as this rapid surge in blood glucose following meal consumption is a strong predictor of T2D and CVD risk (11, 17, 18). Hence, if vinegar research continues to demonstrate anti-glycemic effects, many adults would benefit – not just those with diabetes or at risk of developing diabetes.

Vinegar's health benefits are believed to be attributed to acetic acid, the defining component of vinegar, and include disrupting digestion of some carbohydrates by hindering disaccharidase activity in the small intestine and decreasing the rate of gastric emptying (17-22). These beneficial effects associated with vinegar ingestion have been demonstrated in numerous labs in different countries. However, a long-term vinegar-feeding trial involving individuals at high risk or with pre-diabetes has not been conducted.

At risk individuals would benefit greatly from a simple diet intervention to reduce blood glucose concentrations and lower HbA1c since it may reduce their risk for developing T2D. Not only can risk for T2D be affected, but also the risks

and complications that those with pre-diabetes or diabetes face for other diseases such as heart disease. Therefore, a clinical trial examining the effect of the medicinal use of vinegar is essential in at risk or pre-diabetic subjects. If the use of vinegar is demonstrated to be an effective and preventive measure slowing down and/or stopping the progression into T2D, vinegar may act as an inexpensive, alternative therapy for individuals to use worldwide.

Purpose

The purpose of this randomized, parallel arm study was to examine the effects of vinegar consumption (2 tablespoons for 12 weeks) on blood glucose concentrations in adults at risk for diabetes.

Hypothesis

It was hypothesized that the daily ingestion of vinegar (2 tablespoons or 1.5 g acetic acid) would lower HbA1c, fasting and postprandial blood glucose concentrations in at risk individuals for diabetes as compared to the placebo treatment (daily ingestion of 2 commercially available vinegar pills containing 0.04 g acetic acid).

Definitions

- Acetic acid: main component of vinegar; vinegar is typically 5% acetic acid (CH_3COOH)
- Type 2 Diabetes: characterized by high blood glucose resulting from an impairment of insulin production, the action of insulin, or both (1). ADA's diagnosis criterion includes the following blood values: fasting plasma glucose ≥ 126 mg/dL, casual plasma glucose ≥ 200 mg/dL, and 2-h post load glucose of ≥ 200 mg/dL (seen after an oral glucose tolerance test).
- Fasting state: no food or drink consumed other than water for > 8 hours.
- Hemoglobin A1c (HbA1c): glycosylated hemoglobin measures the average blood glucose concentrations over the past 3 months.
- Postprandial glycemia: blood glucose concentrations at 2 hours following meal ingestion.

Limitations

- The study is limited by small sample size.
- Study duration is 12 weeks, which may not be adequate time to see significant effects.
- Possible subject non-compliance regarding instructions not to change physical activity during or diet the 12-week trial.
- Possible subject non-compliance with protocols regarding glucometers and self –reporting of daily fasting and postprandial glucose concentrations.

Delimitations

- Subjects were from campus population and other communities around the area; hence, results may not be applicable to individuals in other geographical locations or regions.
- Subjects were normoglycemic adults and pre-diabetics who were not on medications for diabetes therefore this study might not be generalizable to a diabetes population or individuals on diabetic medication.
- This study was confined to subjects having a minimum age of 20 years of age or older and may not be applicable to adolescents or children.

Chapter 2

REVIEW OF LITERATURE

Diabetes Mellitus: The disease state

American Diabetes Association describes Diabetes Mellitus as different disease states that have higher blood glucose concentrations and are affected by insulin function and/or secretion (23). Type 2 Diabetes (T2D) makes up 90-95% of diabetes cases found within individuals, and disease rates in the general population are estimated to substantially increase by 50% over a 25-year span (15, 23). Typically individuals with T2D have some form of dysfunction with insulin, resulting in a degree of insulin resistance and higher levels of fasting and postprandial glucose concentrations. When insulin concentrations are measured in type 2 diabetics, these values are elevated because the beta-cells in the pancreas increase insulin secretion to counter the increased glucose concentrations (23).

Individuals who have T2D may experience acute symptoms along with the development of chronic diseases over time. The T2D symptoms include polyphagia (excessive hunger), polyuria (excessive urination), polydipsia (excessive thirst), impaired vision, blurriness, dizziness, and feeling more fatigued (23). The high blood glucose concentrations, which are the hallmark of T2D, contribute to these symptoms. Chronic diseases such as hypertension, CVD, and kidney issues may also derive from the dyslipidemia or high glucose concentrations commonly associated with T2D (23). Therefore, it is important to try to normalize blood glucose concentrations and lipids in T2D.

The costs associated with T2D involve medications, hospitalization, medical visits, disabilities, or even mortalities (1). The American Diabetes Association stated that the prevalence of physician and clinic facility visits, and the use of hospital care and pharmaceuticals, was higher in individuals with T2D as compared to healthy adults (24). Other diabetes costs come from the symptoms and chronic diseases associated with the progression and development of T2D. In 2007, the direct and indirect costs associated with these events are estimated to be around $174 billion (1, 24).

Contributions to Diabetes Mellitus

As evident by the increase of newly diagnosed cases in the year 2010, estimated to be 1.9 million of people, the magnitude of the epidemic continues to rise steadily (1). It is important to examine what some of the major contributors are to the development of T2D. Sedentary behaviors and a lack of exercise are behaviors that drive the growth of the T2D epidemic. Oftentimes, insulin resistance and excess weight gain are accompanied to these behaviors (17). It is suggested that exercise with increased weight loss may be sufficient to manage glucose in T2D (23).

Having a quality diet is another aspect that influences the rise of T2D incidences. O'Keefe et al. (17) discusses how dietary strategies can play a role in postprandial concentrations of glucose and lipids, which directly affect chronic disease risk such as T2D and CVD. Currently, many dietary patterns are high in processed foods or low nutrient dense foods that provide high calories, but low

nourishment to an individual. Consuming meals that are high in fiber, fruits, vegetables, protein dense plant sources, and whole grain complex carbohydrates can beneficially influence the parameters involved with T2D markers, such as PPG (17). These meals provide antioxidants, vitamins, minerals, and other substances, which assist with chronic disease prevention.

Complications of Diabetes Mellitus

T2D complications include the symptoms individuals may experience and have elevated risks for developing other chronic diseases such as cardiovascular disease or coronary heart disease, cancers, and stroke. These diseases have been related to the hyperglycemic conditions seen in fasting and postprandial state along with high concentrations of blood insulin.

Ceriello et al. (8) discusses several studies that show associations between the 2-h post load of glucose in an oral glucose tolerance test and CVD development. The results indicated that mortality from CHD, CVD, and all-cause was significantly predicted and associated with the 2-h post glucose values and postprandial glycemia that were examined in different cohort studies. The study suggests that postprandial hyperglycemia may have detrimental effects on the vasculature involving endothelial function and oxidative stress; hence increase risk for CVD or CHD. In agreement, a meta-analysis showed that postprandial glycemia increases in risk of mortality from CVD or all-cause, along with greater risks for myocardial infarctions and stroke (9).

The carotid intima-media thickness (IMAT) was identified as an area that indicates atherogenic stage and progression, which is important for identifying risk for stroke and CVD state (18). One meta-analysis analyzed how glycemic parameters can affect the IMAT in type 2 diabetics and individuals who have impaired glucose tolerance. The results found that PPG or the 2-h post glucose load were the most prominent indicators of the atherogenic development, leading to possible cardiovascular and cerebral incidents. (18). This suggests that aiming to improve these markers may be beneficial in reducing risk for detrimental occurrences such as a stroke.

The common characteristics of having some degree of insulin resistance and hyperglycemia, as in T2D, also increase risk for different forms of cancer including pancreas, breast, and colon (25). Insulin may influence cancer risk by promoting cell growth and proliferation while hindering cell apoptosis (25); hence, it is important to improve insulin sensitivity and lower blood glucose concentrations through the use of different interventions such as exercise, consumption of healthy meals, or oral hypoglycemic agents and medications specified for risk reduction and management of T2D.

Pre-diabetes: The preceding disease state to Diabetes Mellitus

Pre-diabetes is the disease state that occurs prior to and can progress to T2D. Usually, individuals with pre-diabetes have elevations in their blood glucose concentrations in either the fasting or post load glucose state. These individuals

face not only the complications of developing T2D, but are also at high risk of other chronic diseases such as CVD and hypertension.

The diagnostic criterion for pre-diabetes includes fasting glucose concentrations, glucose tolerance, and glycosylated hemoglobin A1c values. The elevated glucose concentrations found in pre-diabetics can be referred to as impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) (23). The IFG values are typically between 100-125 mg/dL and IGT values are 140-199 mg/dL (23). IFG can be measured after a fasting period of 8-12 hours with no food or drink (except water) being consumed. Individuals diagnosed with pre-diabetes may also have HbA1c percentages that are considered normal or above normal, but are not elevated enough to be diagnostic for diabetes. Typically, HbA1c values for pre-diabetes fall between $\geq 5.7\%$ and $< 6.4\%$ (10).

Treatment and management for type 2 diabetes and pre-diabetes

As the incidences of individuals with diabetes are estimated to more than double by the year 2030, prevention is key for slowing the progression of this epidemic (2). Current treatments for T2D and pre-diabetes vary from medications to lifestyle interventions. These therapies have beneficial effects, but it is necessary to find therapies that target a pre-diabetic population to slow the growth and progression into T2D. Medications for T2D include insulin and oral hypoglycemic medications.

Some of the oral hypoglycemic medications include alpha-glucosidase inhibitors (acarbose), biguanides (metformin), and thiazolidinediones

(rosiglitazone), which lower blood glucose concentrations through various mechanisms (11, 12). Alpha-glucosidase inhibitors such as acarbose or miglitol inhibit the enzyme, glucosidase, which will slow down the carbohydrate digestion and breakdown, and possibly lead to a lowered peak in postprandial blood glucose concentrations (12, 18). In the STOP-NIDDM study, which was a randomized, placebo-controlled trial in IGT subjects, acarbose (~ 194±87 mg per day) was used as a treatment aiming to stop or reduce glucose intolerance to diabetes development. The results displayed significant improvements in blood glucose tolerance ($p<0.001$) and lowered risk for T2D by 25% (26).

Another class of medications, called biguanides, act to lower the production of glucose in the liver (12). An example of a biguanide medication is metformin, which also increases insulin sensitivity enabling glucose to be taken out of the blood and utilized in the muscles (12). In the US Diabetes Prevention Program, metformin (850 mg twice per day) had a 31% risk reduction in pre-diabetic subjects by delaying the progression into T2D compared to the placebo group (1, 4, 26). Biguanides, such as metformin, have shown to be effective oral treatments that are targeted in the risk reduction of T2D.

Similar to biguanides, the class of medications called thiazolidinediones reduces hepatic glucose production and enhances insulin function in muscle and adipose tissues (12, 27). Medications like rosiglitazone also enhance flow-mediated dilation (FMD) and endothelial function, which are both important markers in CVD risk. An experimental trial performed on healthy subjects showed favorable increases in FMD (7.8±2.6%) after one day of consuming

rosiglitazone (4 mg, twice per day), although there were no significant statistical differences in insulin sensitivity or blood glucose concentrations (28).

Although oral hypoglycemic medications exhibit effective and useful treatments in lowering the high blood glucose concentrations commonly seen in T2D, there have been reported adverse and unfavorable side effects. Alpha-glucosidase inhibitors may produce abdominal problems characterized by flatulence and diarrhea (26). In the same way, Biguanides have reported side effects with flatulence, diarrhea, and vomiting (4, 12). Thiazolidinediones, on the other hand, may cause increased liver problems or heart risks such as heart failure or even heart attack (12). Commonly, many of the adverse effects seen with the oral hypoglycemic agents have influences on the gastrointestinal tract.

Other therapies, such as lifestyle interventions that involve eating a more healthy diet and having increased exercise can be effective alternatives to oral medications or insulin for pre-diabetes and reducing risk for T2D. The Diabetes Prevention Program (DPP) study was a randomized, placebo controlled trial that enrolled 3,234 subjects who were all considered to be at high risk for T2D by having both IFG and IGT (4). Subjects were randomly assigned to one of three groups: placebo, metformin (850 mg twice per day), or the lifestyle intervention group (≥ 150 minutes/wk). The metformin group began with a single dosage of 850 mg plus one placebo tablet, and then increased dosage by taking metformin twice in a single day at 850 mg each. The lifestyle group received counseling that focused on eating healthy, increasing physical activity to ≥ 150 minutes/wk, and overall goal of losing approximately 7% of baseline body weight measures. The

DPP study results depicted risk reductions of T2D by 58% for the lifestyle group and 31% by metformin comparing to the placebo group (4). These outcomes revealed that the lifestyle intervention was beneficial for reducing risk for T2D than the oral hypoglycemic drug, metformin.

Another randomized, clinical trial conducted on pre-diabetic subjects with coronary artery disease examined the effects of using an exercise intervention or the oral hypoglycemic medication, rosiglitazone, on endothelial function and the vasculature (27). The subjects were randomized into three groups: control, rosiglitazone (8 mg), or the exercise intervention (30 min/day plus 2, 1-hour group session/wk). The results illustrated that the exercise significantly improved FMD in comparison to rosiglitazone, which was previously shown to favorably effect FMD, and the control group (27).

The Pritikin program is another intervention that employs intensive exercise and diet modification. Diets that are high fiber/low fat and high complex carbohydrates and exercises use moderate intensity, aerobic training, which occurs daily (29). The program was used in a clinical, 3-week trial on 652 subjects diagnosed with T2D (30). The outcomes of the study were significant, reporting that out of the 197 individuals who were previously taking oral hypoglycemic medications at baseline, 71% withdrew use of any of the medications by the end of the 3 weeks. In addition, another 212 individuals who were on insulin at the beginning of the trial, 39% also terminated insulin use after 3-weeks of the lifestyle intervention (30). The results indicate lifestyle interventions composed of healthy diet options plus exercise conditioning are

important and beneficial for the prevention of T2D, which can be significant for individuals at high risk such as pre-diabetics. In spite of this, lifestyle interventions that are as rigorous or as intensive may be harder to adhere and apply into daily life of some individuals.

Therapies including pharmacological agents and lifestyle interventions have shown to produce effective changes on blood glucose concentrations, but an inexpensive, alternative therapy is needed to slow the maturation process into T2D. Vinegar is a treatment that has been exhibited in past literature as a possible alternative therapy that may also slow the progression into T2D. Vinegar may beneficially influence T2D, but may also reduce complications associated with pre-diabetes and diabetes.

History of Vinegar

Vinegar has been widely used throughout the millenniums. History records dated as far back as 3000 B.C. show the Babylonians making vinegar from raw materials used to make alcoholic beverages, have been exposed to air (31). Historical traces of vinegar use have been found throughout the world in geographical locations such as Egypt (3000 B.C), China (1200 B.C), and Greece (400 B.C) (31). Well-known and influential leaders also used vinegar for numerous uses. Hippocrates (c. 400 B.C) utilized vinegar to treat common cold symptoms such as coughs whereas the Carthaginian general, Hannibal (c 218 B.C) used vinegar (the soldiers' sour wine that they drank) as a tool to help make paths through the Alps so his army and pack animals could make their way to

invade Italy (31). Vinegar uses has also been in the treatment and healing of wounds and infections (c. 10th century), as practiced by Sung Tse, the founder in forensic medicine (32).

Vinegar Production

Vinegar derives from the French word "vin aigre" which translates to "sour wine" (32). Sour wine stems from wine or other substances being exposed to air, making it become sour/fermented and thus creating the product vinegar (31). Vinegar can be produced from various carbohydrate sources including apples, grapes, molasses, malt, syrups, wine, and grain (33) leading to an influx of the variety of vinegars that were used over the past 200 years (31). When the alcohols ferment, acetic acid is made, souring the taste.

Whether the vinegar process is the slow or quick method, a bacteria culture is used to allow the fermentation process to occur (32). The slow, or more traditional methods, involve acetic acid bacteria growing on the surface of the vinegar where the oxygen content is high; this film referred to as the "mother of vinegar" (34). The semi-continuous or quick method involves submerging the acetic acid bacteria with oxygen for a more swift fermentation process (34).

Acetic acid gives the classifying characteristic of bitter smells and taste to vinegar. Acetic acid accounts for a minimum 4% of vinegar (33) and therefore is not the same as vinegar itself. The amount of acetic acid will vary depending on what type of vinegar is being produced. Distilled vinegar is approximately 4-7%

acetic acid whereas wine vinegar is less acetic acid (5-6%) making the distilled vinegar one of the strongest sour vinegars (32).

Vinegar Practices

Throughout history, vinegar has been used as a component of foods, a medicine, and even a cleaner. Legendary folklore has discussed the various ways vinegar has been utilized including improved appetite, fatigue reduction and nutrient absorption (35). Although the folklores state these variations in use, there has not been much research establishing and verifying these uses.

Vinegar, as a constituent of different foods, has been part of different cultural traditions. Looking at European traditions, Italy has used utilized homemade balsamic vinegar as a dressing and in Asia, rice wine vinegars are commonly produced (32, 34). Vinegar is also commonly used in the pickling and preservation of foods such as cucumbers, asparagus and other vegetables. One study showed that by inoculating pickled asparagus (by use of vinegar) with Escherichia coli, Listeria monocytogenes, and Salmonella enterica, the vinegar (with varying acetic concentrations) was able to provide an antibacterial protection against the bacteria strains (36).

The extensive medicinal use of vinegar has grown though the ages emerging from influential leaders such as Hippocrates and Cleopatra and was used in the Old and New Testament of the bible (31). Vinegar phenolic content has been proposed as a mechanism against oxidative stress, which can slow disease progression such as cancer, cardiovascular disease, and T2D (37). In

addition, vinegar has demonstrated antihypertensive effects, driven primarily by acetic acid, which significantly decreased rennin and angiotensin II (38). Although treatments and various uses have been ascribed to vinegar, there are conflicting reports on vinegar use and more research needs to be done.

Vinegar acting as a disinfective agent has been used in an assortment of situations. Vinegar was shown to be an effective cleaning agent for cleaning surfaces. One study illustrated that vinegar was as effective as other cleaning tools such as bleach in disinfecting surfaces against influenza A virus (39). The father of modern medicine, Hippocrates, used vinegar as an anti-infective therapy for cleaning ulcers and sores (32). Vinegar was also was effective in cleaning dentures as a better option than a bleach mixture (32).

Vinegar Uses: Evidenced based research

Research studies have demonstrated that vinegar consumption has beneficial effects on glucose and insulin concentrations (as evidence by reduced postprandial glycemia and HbA1c and increased insulin sensitivity) in diverse populations (5-7). Both Johnston et al. (7) and Liatis et al. (14) found significant reductions in the glucose response and insulin concentrations after a high carbohydrate meal was consumed with the addition of vinegar (20 g apple cider vinegar- 5% acetic acid and 20 g wine vinegar- 6% acetic acid) in both healthy and type 2 diabetic subjects. One randomized, crossover study examined the effects of vinegar intakes in subjects who were insulin sensitive, pre-diabetic, or type 2 diabetics (15). In the pre-diabetic subjects, insulin sensitivity was increased

by 34% along with reductions in glucose and insulin concentrations. These studies are in agreement suggesting that the addition of vinegar in differing patient populations favorably impacts postprandial glycemia and insulin concentrations.

Several studies examined the anti-glycemic effects of vinegar for different carbohydrate sources (given as a meal). A randomized, controlled trial conducted in thirteen healthy subjects (19-32 years) used a reference meal of white bread and examined the results when a vinaigrette sauce (containing 28-mmol acetic acid) was added with cold potatoes (3). The study found a significant reduction in postprandial glucose and insulin responses (GI=96 and II=128) suggesting that vinegar was effective for managing glucose following potato ingestion (3). Another study in ten healthy subjects showed similar results when vinegar was ingested with white wheat bread (40). The vinegar contained 18-mmol per test meal and significantly reduced ($p<0.05$) postprandial glucose and the insulin response when taken with the starchy load (GI=64 and II= 65) (40).

In a 12-week randomized, parallel arm trial 27 individuals (T2D, 20-80 years) were randomized into three groups: vinegar drink, vinegar pills, or pickle group (19). The results found the greatest reduction in the group who consumed the vinegar drink (2 T) and decreased HbA1c by 0.16% whereas the other two group HbA1c values rose. The study conveyed how 2 tablespoons of vinegar can be an effective treatment for lowering HbA1c. In addition, a recent randomized, crossover trial showed vinegar amounts as low as 2 teaspoons to be effective in reducing PPG by 23-28% when consumed with a complex carbohydrate loaded meal (11). In contrary, this study also demonstrated that vinegar consumption was

not effective with a meal composed only of monosaccharide carbohydrate sources such as dextrose or corn syrup. Results indicated a rise in mean PPG 90% larger in the vinegar treatment group in comparison to the placebo group after consuming the monosaccharide drink made of dextrose ($p= 0.059$) (11).

In addition, vinegar was shown to be ineffective for meals with low GI in comparison to meals with high GI when examining PPG and insulin responses. Liatis et al. (14) stratified and divided sixteen T2D participants into two groups: group A (high GI mixed meal) and group B (low GI mixed meal), some of which included and did not include the addition of wine vinegar (20 g and 1.2 g acetic acid). Results indicated glucose and insulin responses significant for the high GI meal ($p= 0.04$, $p= 0.056$), but did not significantly affect glucose and insulin responses to the low GI meal when consumed with wine vinegar ($p=0.56$, $p=0.98$) (14). Another study examined postprandial glycemic responses in eleven healthy subjects for two tests meals (high GI and low GI meal) when consumed with vinegar, peanuts, or the control (15). Results were significant for the meals that contained a high GI with vinegar or peanuts ($p<0.05$) and lowered postprandial glycemia by approximately 55% when compared to the control (15).

Postprandial glycemia and fasting glucose concentrations are important factors for improving HbA1c. A randomized, double blind, placebo controlled trial illustrated how influential both postprandial glycemia and fasting glucose is to HbA1c (41). The controlled study was carried out on 495 subjects (35-70 years) for 24 weeks. The 5 arms of the study involved different doses of acarbose (25, 50, 100, and 200 mg) and placebo. The results showed that postprandial

glucose and fasting glucose decreased from baseline for the acarbose groups and HbA1c was reduced by 0.4% (at 25 mg t.i.d. acarbose) to 1.09% (at 200 mg t.i.d. acarbose) (41). This confirms that postprandial and fasting glucose concentrations influence HbA1c and may be an appropriate objective to focus on in future research studies when the aim is to improve HbA1c.

Vinegar's Mechanisms

The mechanisms by which vinegar promotes the anti-glycemic effects have been proposed, but the evidence is still inconclusive. These mechanisms include slowing the gastric emptying rate, inhibiting disaccharidase enzymes in the small intestine, and increasing glycogen storage rates in different tissues such as the liver and skeletal muscle. These effects are promoted by the acetic acid content of the vinegar solutions.

Research evidence from several studies has shown that vinegar ingestion slows the gastric emptying rate (GER), which may be a realistic treatment for aiding postprandial glycemia. A randomized crossover trial conducted on ten subjects (Type 1 diabetics with diabetic gastroparesis, 57-79 years) consumed 200 ml of 5% apple cider vinegar for two weeks before consuming their breakfast (2). GER was slowed after consumption of vinegar, as evidenced by a GER of 17% after the additional of vinegar compared to a GER of 27% after the consumption of the breakfast meal only (2). Other studies link a reduction GER by acetic acid with decreases in postprandial glycemia (25-35%) and increases in satiety (2

times greater) (5, 17). The enhanced satiety may also lead to reductions in energy intake and hunger (5, 7, 21).

Recent studies suggested that acetic acid taken with cinnamon enhanced the satiety and postprandial glycemic effects of acetic acid (6, 21). The potential increases in satiety levels were analyzed in a randomized trial where twenty-seven participants completed 4 different trials comprising of: a control meal, control meal with the addition of 4 g of cinnamon, control meal with the addition of 28 mmol of acetic acid, or a combination of the control meal with the 4 g of cinnamon and 28 mmol of acetic acid (6). At 15 minutes and 30 minutes following meal ingestion, the combined effect of both the cinnamon and acetic acid was significant in comparison to the control meal ($p=0.024$) (6). The cinnamon (4 g) or the acetic acid (1.68 g) given alone was not effective in lowering blood glucose concentrations. The study suggested that the combination of cinnamon and acetic acid did improve postprandial glycemia and increased the satiation levels in the subjects. In addition, the researchers also explained that the effect the addition of vinegar along with slowing of gastric emptying was evident and explained in the research literature.

In contrast to the other studies, one randomized trial on twelve healthy subjects (24-56 years) showed decreases in the glucose response after meal with either the addition of acetic acid or acetate, but no statistically significant effects were seen in GER (22). Also, although a randomized trial studying effects of vinegar and sodium acetate with test meals containing white bread did show glucose and insulin responses being attenuated with the use of the vinaigrette

sauce, the difference in gastric emptying between both interventions were the same (42). The study ruled out the possibility that a gastric emptying mechanism was the driving force to the result seen.

Another study showed that a lower concentration of 16 mmol of vinegar was efficacious in reducing glucose response to a meal higher in carbohydrates (white bread) by 30% (42). This study also suggested that the low acidic pH might be driving the reduction of glucose responses. In addition, another study suggested that the fermentation process of certain foods such as breads could produce specific acids that may be beneficial to improving glucose concentrations after a consumption of a meal (22).

In cultured Caco cells, acetic acid was demonstrated to inhibit disaccharidase enzymes, which can be found in the small intestine. Commonly when carbohydrates are digested, once the substances pass the stomach, their main site for absorption is the brush border in the epithelial of the small intestine (43). This location in the body is where enzymes such as disaccharidase inhabit, breakdown, and absorbs the consumed food items. If vinegar treatments containing specific amounts of acetic acid inhibit the glucose absorption process, this will affect glucose concentrations in the blood and may be a tool for lowering both fasting and postprandial glucose concentrations (43).

This in vitro experiment demonstrates why vinegar is not an effective anti-glycemic agent with monosaccharide, but is effective for starchy carbohydrates that contain disaccharides that are broken down by varying disaccharidases. The disaccharidases that were analyzed were sucrase, maltase, trehalase, and lactase

by incubating the enzyme with the appropriate substrate and testing to see how much glucose was released after the incubation period. The results indicated that acetic acid inhibited sucrase and maltase by 40% whereas lactase and trehalase were inhibited almost entirely in comparison to the control group (43). These results signify that the acetic acid treatment did inhibit the disaccharidase activity, as proposed, at the cellular level in cell culture.

Other studies proposed glucose uptake might be another mechanism where vinegar attenuates the glycemic responses. Fusjimi et al. (35) hypothesized that dietary acetic acid at levels varying 0.1, 0.2, 0.4 g/100 g of the intervening diet can increase glycogen repletion in the skeletal muscle and liver in rats by inhibiting the glycolysis pathway and increasing expression of glucose-6-phosphate (G6P) (35). G6P is important for blood glucose control as it retains glucose in the skeletal muscle. The rats (Male, Sprague-Dawley, 5-weeks old) were put into 4 different intervention groups: control, 0.1 g, 0.2 g, or 0.4 g acetic acid plus each group got 2 g of the experiment diet. They were able to show how the feeding of acetic acid did indeed increase the glycogen stores found in both the liver and skeletal muscle. For instance, the soleus muscle had increases of 60% in the acetic acid intervention groups than the control group (35).

In agreement with Fushjimi et al. (35) a pilot trial conducted on type 2 diabetics examined vinegar consumption at bedtime and the effects on fasting glucose (16). The results indicated a significant reduction, measured in the morning hours, for fasting glucose (0.26 mmol/l, $p=0.033$), which was largest in the vinegar groups (4% reduction). The results were consistent that the acetic acid

in vinegar may have a mechanism that relates to the glucose regulation and glycogen cycles occurring in the liver.

Vinegar Safety

Vinegar has widely been accepted and used in food practices and therefore, it is considered safe to consume. The Food and Drug Administration states that acetic acid consumption is safe when the vinegar products are manufactured with proper protocols and purity (44). Vinegar also needs to have correct labeling to ensure safest and non-deceptive marketing of the vinegar product that includes acid strength and what is used as a dilutant (33).

There have been a few cases in research where adverse effects have been noted for vinegar consumption. One woman reported esophageal injury characterized by pain and swallowing issues following consumption of apple cider tablet (45). Researchers examined different traits of the tablets that include the pH, acid percentage, and size of each tablet and how it varied between brands. The results indicated the acid content, pH, and labels varied greatly between the products, which make some of the tablets considered dangerous or toxic at the indicated acid amount on the labels (45). Another individual reported that a family member suffered spasms within the larynx of the throat after accidently swallowing vinegar containing cucumbers (46). The adverse events that have been reported suggest vinegar may be best consumed in a food matrix or as a component of another food such as in vinaigrette dressing on a leafy salad. Although adverse effects connected to vinegar are scarcer in the literature, each

study conducted on vinegar consumption should take in account any possible harmful effects that may result.

Conclusion

The various studies discussed signify the importance for the development of an inexpensive, primary prevention therapy that targets a high risk or pre-diabetic population in the endeavor to slow or stop the progression of T2D. Vinegar has potential and beneficial effects in minimizing and reducing blood glucose concentrations, as evidence by postprandial glycemia and HbA1c. Both PPG and HbA1c are prominent and important indicators for T2D and other chronic disease risk. An intervention trial testing these two markers, with the use of vinegar as an intervention, on an at risk population for diabetes is necessary. Currently there are no investigations on long-term vinegar intervention in individuals with pre-diabetes. If vinegar proves and establishes a significant anti-glycemic effect in the at risk individuals, vinegar may be an alternative therapy that can be used globally.

Chapter 3

METHODOLOGY

Subjects

Healthy, non-smoking individuals (20-75 years) considered non-diabetic who were not prescribed insulin or oral hypoglycemic medications for their condition, and, if applicable, have stable medication use and no unresolved medical conditions, were recruited from a campus population and surrounding communities via advertisements and list serves. Recruitment began in December 2011. All human subjects who were included in the study after the initial survey and screening provided written informed consent. At the consenting visit, subjects were given a description of the study including the length of the trial, possible harms and benefits, how data will be collected and protected, and contact information regarding the researchers. Investigators were present to answer any questions and provide detailed information about the study. The study received approval by the Arizona State University Institutional Review Board.

Study Protocol

The study was conducted as a 12-week, randomized, parallel arm trial. The subjects (n=14) were stratified by height, weight, waist circumference, age, gender, and HbA1c concentrations, and block randomized into two groups: vinegar (VIN) or control (CON). Each subject was given instructions to take the study treatment, either VIN (8 ounces vinegar drink) or CON (1 vinegar pill), twice each day with their lunch and dinner meals. The commercially available

vinegar pill (Apple Cider Vinegar tablets, General Nutrition Corporation, Pittsburgh, PA) contains 0.04 g acetic acid, and the apple cider vinegar drink (Bragg Apple Cider Vinegar, Cinnamon, Calorie-free, Santa Barbara, CA) contains 2 tablespoons vinegar or 1.5 g acetic acid. Participants were asked to maintain their usual diet and physical activity level throughout the 12-week trial.

Prior to the start of the trial, participants arrived at the test site to provide written consent and to have body weight and girth measured. Each subject was assigned a calibrated glucometer (ACCU-CHEK, Avia meter system, Indianapolis, IN) to use throughout the trial. Glucometers were calibrated by insertion of a chip that corresponded with the assigned glucose strips. Subjects measured blood glucose twice daily, once at fasting upon waking and once at 2-h post-meal ingestion. For the at home finger sticks, subjects were provided with retractable lancets and instructions for sterile conditions. Calendars were also given to each subject to record the daily waking and 2-h post-meal ingestion blood glucose concentrations to help increase adherence to the study protocol. Subjects began the trial 1-week later. At trial weeks 0 and 12, fasting blood samples were collected and analyzed for insulin, glucose, and HbA1c. At weeks 6 and 12, glucometers were collected from subjects to download the daily glucose information. Breath samples were collected from subjects at week 12 by having the subject breath fully through a mouthpiece into a collection bag.

Blood Analysis

Venous blood draws were performed at weeks 0 and 12. Blood was drawn from each subject after a fasting period of 12 hours (19). A trained nurse collected the blood samples and put them in Lavender cap Vacutainer (Becton Dickinson, Franklin Lakes, NJ) containing the anticoagulant EDTA. The blood sample was centrifuged for 15 minutes at 3000 RPM and frozen at -80ºC until further analysis. Glucose was examined through the use of a Gray cap Vacutainer containing sodium fluoride and then analyzed using a Cobas C111 chemistry analyzer (Roche Diagnostics, Indianapolis, IN). Plasma insulin concentrations were measured using the radioimmunology (RIA) kit (Millipore Corporation, Billerica, MA) (7, 11). HbA1c was determined using the DCA Vantage Analyzer (Siemens Healthcare Diagnostics, Tarrytown, NY).

Statistical Analysis

All data was entered into Social Sciences (SPSS, Chicago, IL) v.19 for statistical analysis including descriptive and inferential analyses. Values were reported as ± SE. The outcome variables were checked for normality and transformed if necessary. If the data are normally distributed, parametric tests were used otherwise nonparametric tests were used. Change data (week 12 – week 0) was calculated and compared using independent t tests. Daily fasting and postprandial glucose concentrations were plotted over the 12 weeks in separate curves. Average concentrations for weeks were compared to average concentrations in the week preceding the start of the trial using repeated measures

ANOVA for interaction. Within- and between-group differences were expressed as mean percent difference (95% CI). Differences were considered significant at $p \leq 0.05$.

Chapter 4

DATA ANALYSES AND RESULTS

Descriptive characteristics

Fourteen subjects (1 male, 13 females) participated in the 12-week randomized, parallel arm trial examining the effects of vinegar consumption on blood glucose concentrations and HbA1c. Initially, fifty-six individuals were surveyed on www.surveymonkey.com. Out of the fifty-six surveyed individuals, forty-eight people qualified to be in the study. Of those individuals, twenty-three voluntarily enrolled to participate in the study. Seven individuals dropped from the study due to loss of response during follow-ups, medical issues, time commitments, and study compensation. Two individuals were excluded at the consenting visit and visit 3 of the study due to current diabetic state and new inclusion of glucose controlling medications. The fourteen subjects that had completed the study were considered to be in a normal blood glucose or pre-diabetic state. Original inclusion criteria regarding subjects being diagnosed by a doctor or physician as a pre-diabetic were modified and eased to also include subjects having normal blood glucose concentrations, due to low response rates and enrollment into the study.

At the consenting visit, subjects were assigned a glucometer and instructed how to use the device daily throughout the duration of the study. The subjects reported back one week later to start the study and received test foods (vinegar drink or pills) and instructions on how to consume the study test foods. At week 0, subjects were stratified by height, weight, waist circumference, age, gender,

and HbA1c concentrations, and block randomized into two groups: vinegar (VIN) or control (CON). At weeks 0 and 12, venous blood samples were collected to determine HbA1c, glucose, and insulin concentrations. At weeks 6 and 12, glucometers were collected and data was downloaded. Subjects received compensation via gift cards for voluntarily participation and completion of the study at weeks 6 and 12.

Table 1 depicts descriptive characteristics of the fourteen subjects who had completed the study. The data was reported by treatment group and recorded at baseline. Fasting blood glucose concentrations, insulin, and HbA1c were obtained from venous blood samples of each subject at weeks 0 and 12. None of the characteristics reported significant differences between treatment groups ($p>0.05$).

Figure 1 visually depicts treatment compliance by the number of times the treatment was consumed daily throughout the 12-week study duration. In the drink group, subjects complied with consuming the treatment two-times per day (89%), once a day (4%), and zero times a day (7%). In the pill group, treatment compliance decreased to 77% of the subjects consuming the treatment two-times per day, increased to 16% consuming treatment once a day, and stayed the same at 7% of the study duration the treatment was not consumed.

Table 1—Descriptive characteristics of subjects *.

	Drink (n= 7)	Pill (n= 7)	p value
Gender, M/F	0/7	1/6	ND
Age, y	48.1±5.2	43.8±6.2	0.237
Weight, lbs	167.6±13.6	168.9±16.4	0.954
Waist circumference, cm	35.7±2.1	36.3±2.3	0.869
Height, inches	63.4±0.9	65.1±1.5	0.689
BMI	29.2±2.2	27.6±2.0	0.515
Body fat percentage	34.3±5.33	33.8±3.6	0.819
Fasting glucose, mg/dL	100.3±5.1	96.1±5.5	0.589
Insulin, mIU/mL	17.2±2.9	20.7±5.1	0.575
HbA1c, %	5.6±0.2	5.7±0.1	1.000

*** Data are mean ± SEM. P values represent independent t-test results. Baseline fasting glucose, insulin, and HbA1c collected from venous blood sample.**

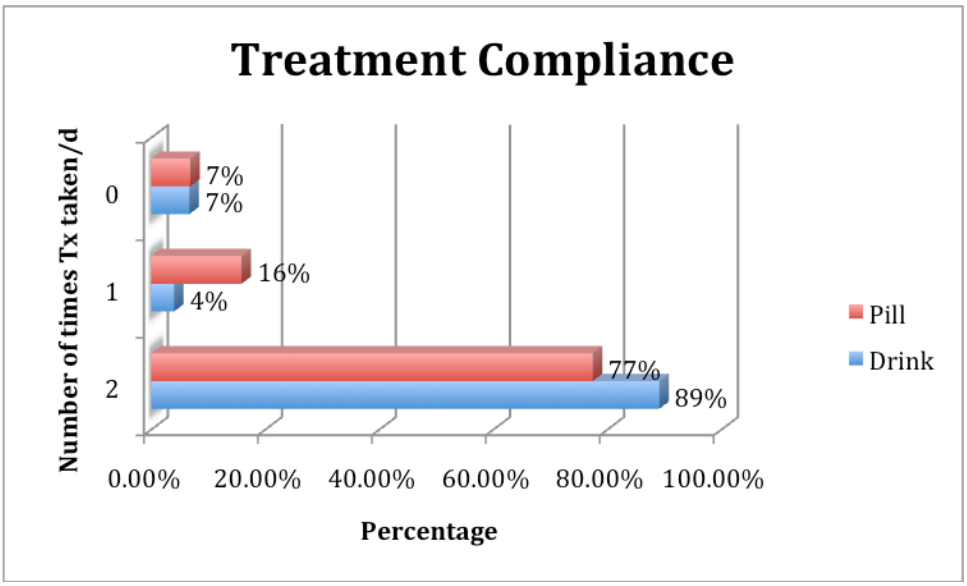

Figure 1—Treatment compliance of subjects under two experimental conditions: vinegar drink or placebo (pill). Values represent treatment compliance percentage daily through the 12-week study duration.

Changes in parameters

Throughout the study, at the consenting visit and weeks 0, 6, and 12 anthropometric measurements were determined and recorded. HbA1c, fasting glucose, and insulin were also measured at week 0 and 12 from venous blood samples. These parameters were analyzed for change throughout the 12-weeks of the study and significance was not found ($p>0.05$), although reductions were observed. Also, HbA1c change was not found to be significantly different between treatment groups; though it is expected when examining the baseline HbA1c averages which were below 6 and having a small sample size (n=14) (Table 1). Table 2 provides information regarding change in the descriptive parameters throughout the 12-week period.

Table 2—Change in descriptive parameters *

	Drink (n= 7)	Pill (n= 7)	Effect size	p value
Weight, lbs	-1.7±1.3	0.2±1.3	0.083	0.316
Waist circumference, cm	0.1±0.4	-0.1±0.4	0.016	0.668
Body fat percentage	2.2±3.3	2.5±3.3	0.000	0.960
Fasting glucose, mg/dL	0.00±.5	1.7±2.5	0.031	0.547
Insulin, mIU/mL	-2.0±1.1	-2.4±1.8	0.002	0.873
HbA1c, %	-0.14±0.1	-0.07±0.1	0.013	0.702

*** Data are mean ± SEM. P values represent univariate test results. Fasting glucose, insulin, and HbA1c collected from venous blood sample.**

Fasting blood glucose response

Fasting blood glucose concentrations were measured daily throughout the 12-weeks with a glucometer. At weeks 0, 6, and 12 these concentrations were downloaded and evaluated for change over the 12-week study period. A repeated measures ANOVA analysis observed a significant F value ($p=0.05$) with an effect

size of .84, representing a significant reduction and change of fasting glucose over the 12-weeks. Independent t-tests showed the treatment groups differed significantly at week 4 ($p=0.047$) and week 5 ($p=0.025$). The effect size was also determined revealing that the treatment predicted 29% of the variance at week 4 and 35% of the variance at week 5. Although results were not considered significant ($p>0.05$) for the other 10 weeks, there were suggestive trends at weeks 3, 6, 7, and 9. The average change for fasting glucose produced no significant change ($p=0.066$), but the effect size was also observed showing that the treatment predicted 25% of the variance. Table 3 displays the fasting blood glucose change data for each of the 12-weeks of the study.

Figure 2 demonstrates the fasting blood glucose change for the vinegar drink or the pill (placebo) treatments continued to decrease throughout each of the 12-weeks. The average fasting glucose change for the drink group (-16.3±4.9 mg/dL) is lower, but is not significantly different ($p=0.066$) than the pill treatment group (-4.5±3.1 mg/dL) (Table 3 and Figure 2). The mean point for the fasting glucose change is revealed in Figure 2, demonstrating the vinegar drink and vinegar pill treatment group final points.

Figure 3 reveals each subject's average fasting glucose change through the 12-week duration. Subjects were ranked according to their HbA1c concentrations and divided per group. Figure 3 shows subjects who were responders and non-responders to the treatment (drink or pill) they were randomized to consume throughout the study. It was visually shown that all subjects in the vinegar drink group decreased average fasting glucose change compared to the pill group where

subjects had increases and decreases in their average fasting glucose change over the 12-weeks. It was also observed that the subjects with highest baseline HbA1c values (%) tended to respond the most to the treatment consumed.

Table 3—Change in fasting glucose *.

	Drink (n= 7)	Pill (n= 7)	Effect size	p value
Week 1	-16.1±5.9	-6.3±2.7	0.158	0.159
Week 2	-16.2±5.8	-8.7±4.0	0.086	0.309
Week 3	-17.3±6.2	-4.0±3.7	0.216	0.094
Week 4	-16.5±5.2	-4.0±2.0	0.290	0.047
Week 5	-17.7±5.6	-0.7±3.4	0.353	0.025
Week 6	-17.3±3.9	-1.6±7.0	0.239	0.076
Week 7	-19.8±5.3	-8.3±2.9	0.229	0.084
Week 8	-14.8±4.8	-8.3±3.3	0.108	0.298
Week 9	-16.3±5.3	-2.4±5.0	0.263	0.088
Week 10	-17.3±4.8	-7.9±4.1	0.177	0.174
Week 11	-14.9±5.1	-2.3±4.7	0.244	0.103
Week 12	-12.5±8.7	-0.9±2.8	0.129	0.277
Mean	-16.3±4.9	-4.5±3.1	0.254	0.066

*** Data are mean ± SEM. P values represent independent t-test results. Fasting glucose measured from glucometer. Significance (p=0.05 and effect size= 0.84) observed from Repeated measures ANOVA test results.**

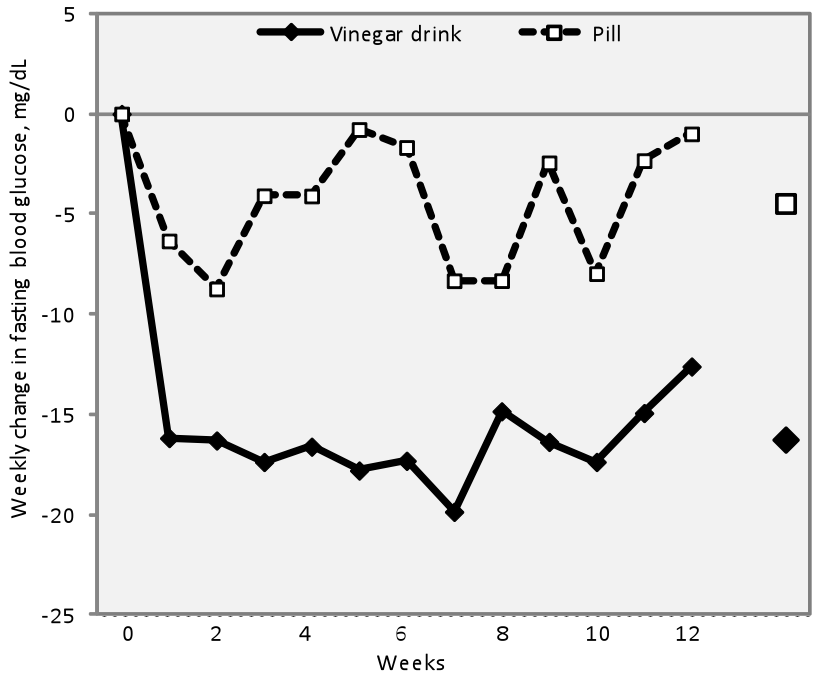

Figure 2—Weekly change of fasting blood glucose concentrations under two experimental conditions: vinegar drink or placebo. Final point represents averages of weekly change. Values are mean±SEM.

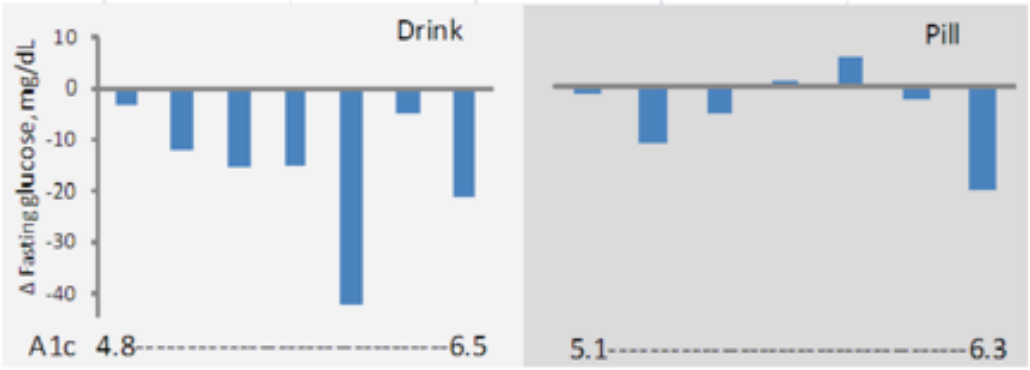

Figure 3—Change of fasting blood glucose concentrations under two experimental conditions: vinegar drink or placebo. A1c represents baseline measures. Values are mean±SEM.

Postprandial blood glucose response

Postprandial blood glucose concentrations were measured daily, with the use of a glucometer, throughout each of the 12-weeks of the study. Specifically, postprandial glucose was recorded 2-h after each subject's largest meal of the day. At weeks 6 and 12, the glucometers were also downloaded for the postprandial glucose information and was examined for weekly change. Analysis was performed with independent t-tests, revealing no significant differences in change between the vinegar drink and pill treatment groups, but reducing 2-h postprandial glucose concentrations were observed in the drink group in comparison to the pill group ($p>0.05$, table 4).

Figure 4 displays the change in the weekly 2-h postprandial glucose by each treatment group. The data demonstrates decreases in 2-h postprandial glucose for the vinegar drink group whereas in the vinegar pill group, increases and decrease in 2-h postprandial glucose concentrations were observed throughout each week of the study. The mean change final point for each group is visually depicted (Figure 4), but is not significantly different between the vinegar drink (-7.6±6.8 mg/dL) and the vinegar pill group (3.3±5.3 mg/dL) ($p=0.232$) (Table 4).

Table 4—Change in 2-h postprandial glucose *.

	Drink (n= 7)	Pill (n= 7)	Effect size	p value
Week 1	-8.0±5.0	0.8±5.9	0.098	0.275
Week 2	-8.3±6.6	5.6±4.3	0.208	0.101
Week 3	-2.7±9.1	-7.7±6.1	0.017	0.672
Week 4	-3.2±8.3	1.4±5.8	0.018	0.664
Week 5	-4.7±8.8	0.7±9.3	0.017	0.685
Week 6	-5.3±6.4	5.7±7.5	0.104	0.282
Week 7	-5.5±4.0	-1.5±2.5	0.065	0.423
Week 8	-5.2±9.9	4.5±9.9	0.050	0.507
Week 9	-3.3±8.8	1.2±6.6	0.018	0.694
Week 10	-7.2±8.4	8.2±11.8	0.101	0.314
Week 11	-10.8±14.3	-5.3±6.7	0.012	0.738
Week 12	-4.9±14.7	-7.4±7.9	0.002	0.894
Mean	-7.6±6.8	3.3±5.3	0.117	0.232

*** Data are mean ± SEM. P values represent independent t-test results. 2-h postprandial glucose measured from glucometer.**

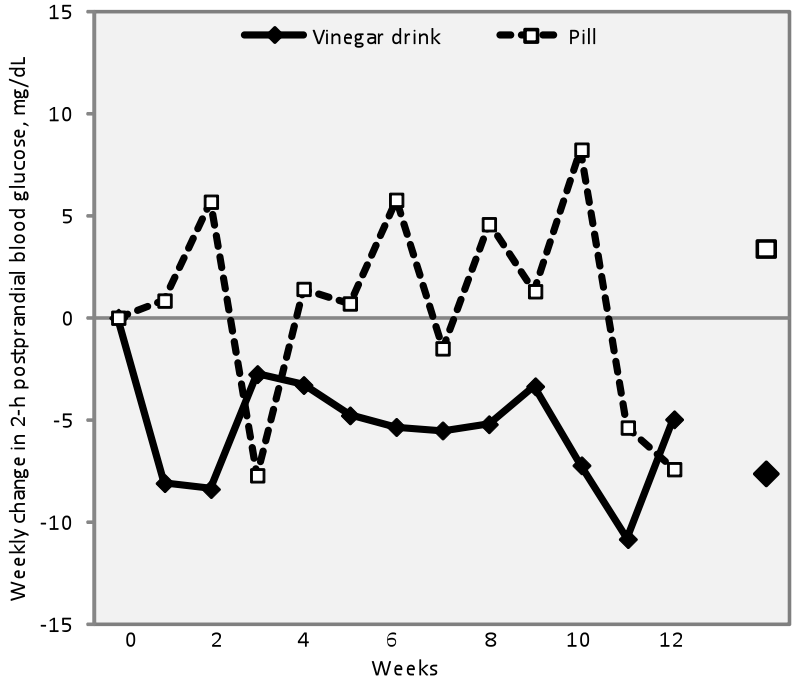

Figure 4—Weekly change of 2-h postprandial blood glucose concentrations under two experimental conditions: vinegar drink or placebo. Final point represents averages of weekly change. Values are mean±SEM.

Chapter 5

DISCUSSION

Research evidence indicates that vinegar ingestion has beneficial effects on HbA1c and postprandial glycemia in diabetic patient population. The present vinegar intervention study is the first to track a subject population at high risk for diabetes daily for 12 weeks. Both fasting and 2-h postprandial glucose concentrations were recorded each day during the trial. After analysis, a clear and significant reduction in fasting capillary blood glucose concentrations was observed in this small sample of individuals at high risk for diabetes. The reduction in fasting glucose concentrations was immediate and sustained throughout the 12-week duration when compared to the controls. The average reduction in fasting glucose concentrations over the 12-week trial for the VIN group was 16.3±4.9 mg/dL, a remarkable change in terms of physiological health. One study found a significant reduction (4%, $p=0.033$) in fasting glucose when 2 tablespoons of apple cider vinegar was ingested at night before bedtime with a standardized meal. This reduction in fasting glucose is related to and is important for reducing risk for T2D and other conditions such as heart disease or cancer.

The venous fasting blood concentrations for insulin, glucose, and HbA1c before and after the study trial were suggestive of a change, but the differences did not attain significance. Mean baseline insulin values were 18.9±2.9 mIU/mL, which demonstrates some insulin resistance, but may not be high enough to show change. The subjects also reported average HbA1c at baseline of 5.6±0.1%, which may be too low to observe significant effects from the vinegar treatment as

evidence by the change in HbA1c, reporting reductions of 0.14±0.1% (drink group) and 0.07±0.1% (pill group) (p=0.702); normal ranges for HbA1c are <5.7% (10). An observation was made that the subjects, in either treatment group, that had the higher HbA1c values, tended to have the largest changes to their treatment regimen. Including individuals that are high risk or diabetic according to their HbA1c values, who are also only being treated with diet and exercise alone may be viable qualities to use for a target population.

The American Diabetes Association states that the normal range for fasting plasma glucose is <100 mg/dL (10). The subjects revealed having baseline venous fasting blood glucose concentrations of 98.2±3.6 mg/dL, which did not achieve a change that reached statistical significance and may not be likely to fall since it was normal at baseline. Venous blood glucose and capillary blood glucose concentrations may vary and will differ depending on the amount and rate at which glucose is being utilized by the tissues and muscles in the body (47).

Since there may be variation, capillary blood glucose concentrations were also measured throughout the duration of the study twice daily. Fasting capillary blood glucose attained significant reductions immediately and were continuous through the 12-week trial. Capillary blood was also checked daily for 2-h postprandial glucose concentrations after the main meal of the day via glucometers. The data did not show significant differences at any time points in the 12-week duration, nor was the average 2-h postprandial glucose change significant (p=0.232), but sustained reductions in 2-h postprandial glucose in the drink group were observed. The 2-h time mark to measure the glucose may not be

the best measure; however measuring at 30 minutes or 1-hour post-meal period may be a better option in this population. In a study conducted on healthy, insulin resistant, and diabetic population, significance was observed for postprandial plasma glucose and insulin in the insulin resistant population at 30 min and at 60 min (15). Likewise, in another study conducted on healthy subjects, significance was seen in post-meal glucose and insulin at the 30-minute time interval (5).

Since this study had promising results, future trials should select subject populations that are insulin resistant, pre-diabetic or diagnosed with diabetes who are likely to have higher HbA1c values. Given that having insulin resistance accompanies T2D development, this is a key risk factor that can be evaluated to further help at risk individuals (17). Current options for these high-risk individuals include medications such as oral hypoglycemic medications and insulin or lifestyle alterations such as healthy dietary changes and increased physical activity. Although some of these options are effective in reducing risk for T2D, there is a need for a more simple and cheap dietary strategy that would be beneficial for these individuals.

Although this study presented promising findings, it was noted that the study was not adequately powered with a small sample size of fourteen subjects. The study was powered 11% for HbA1c and 40% for fasting glucose. It is a necessity to power the trial at 80% to detect and observe significance or difference. Each treatment group had seven subjects consuming the treatment that they were randomized to (drink or pill). It would be expected to see significant changes from the treatment in the target population from a larger sample size

calculated from past literature. A 20% dropout rate of the subjects was expected, but with having a small sample size from the beginning of the study (n=23), the study had a dropout rate of 40%, ending with fourteen subjects completing the study. So with a larger sample size completing the study and an expected 20% dropout rate, there would be enough subjects to power the study at 80% to detect significance.

This study did have good compliance to treatment protocols, which can have affects on the trial results. Compliance data revealed that VIN subjects complied on 89% of the trial days (75/84 days) and CON subjects complied on 77% of the trial days (65/84 days). Compliance could be improved by changing the bottle type given to the participants from glass to plastic so it is easier to carry around when the subjects are doing daily tasks. Another way to increase compliance would be to send out daily reminders to the subjects in ways that they prefer such as text message, email, phone call, or a calendar reminder.

More research is needed to see if vinegar, with varying amounts of acetic acid, reduces fasting and postprandial glucose and insulin responses in individuals that are considered high risk for T2D. These individuals may be targeted according to their HbA1c and how they are currently being treated for their medical state, with the use of medications or lifestyle and diet alone.

Chapter 6

CONCLUSIONS

To summarize, this study observed significant reductions for fasting capillary blood glucose concentrations that were immediate and continuous over the 12-week duration after consuming the vinegar drink (8 ounces vinegar drink providing 1.5 g acetic acid) twice daily when compared to the vinegar pill (1 vinegar pill providing 0.04 g acetic acid). Changes were also suggestive in the comparisons of venous fasting blood samples before and after the study in addition to reductions being observed in 2-h postprandial glucose concentrations.

Future research should examine populations that have baseline HbA1c that are considered high risk pre-diabetic or diabetic, since this study's subjects had HbA1c that were already low at baseline; therefore making it more difficult to observe significant reductions or changes over time. In addition, vinegars effects on glucose and insulin responses by comparing venous to capillary blood samples should be analyzed to see if levels vary or are more sensitive depending on where the blood is drawn from. Measuring postprandial responses should also be conducted at 30 and 60-minute intervals as well 120 minutes to more accurately measure the post-meal response in this population. Overall, it would be ideal for further studies to examine populations that are within parameters that indicate pre-diabetic or diabetic at baseline and measure venous and capillary blood samples for glucose, insulin, and HbA1c changes over a duration that extends 12-weeks. Future studies then can further confirm vinegar's use as an alternate intervention that can stop or slow the progression into T2D.

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APPENDIX A

CONSENT FORM

12-WEEK VINEGAR TRIAL IN HEATHLY ADULTS WITH PRE-DIABETES

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 Samantha Quagliano and Serena Loeb, nutrition graduate students, have requested your participation in a research study.

STUDY PURPOSE

The purpose of the research is to examine the effects of vinegar consumption on blood glucose concentrations.

DESCRIPTION OF RESEARCH STUDY

You have indicated to us that you are minimum age of 20 years of age, a non-smoker, generally healthy, and have stable medication use over the last 3 months. You have been diagnosed with pre-diabetes but you do not take insulin. Participants will be asked to maintain their usual diet and physical activity level throughout the trial. This study will initially involve the completion of a brief medical history questionnaire to demonstrate the absence of medical conditions (aside from pre-diabetes) that may impact the study. Your weight, height, and girth will be measured at the start and at the end of the trial. This first meeting will take ~30 minutes. This research study will last 12 weeks. At the start of the study and at trial weeks 6 and 12, you will travel to ASU (the Nutrition labs at the ASU Downtown campus) early in the morning to meet investigators for some testing: breath sampling at 0, 6, and 12 weeks, and a blood draw (<1/2 Tbsp/day) at 0 and 12 weeks. For the blood draw, you will fast overnight (12 hours). These visits will last <45 minutes.

You will be randomly assigned to the 'apple cider vinegar drink' group or to the 'apple cider vinegar tablet' group. During the 12-week trial you are asked to consume 8 oz vinegar drink or 1 vinegar pill (depending on group assignment) twice daily with the lunch and dinner meals (a total of 16 oz vinegar drink or 2 vinegar pills per day). You will be given a glucometer to use daily to measure blood glucose at waking and at 2-hs post meal ingestion after your largest meal. You will mark your blood glucose reading on a 12-wk calendar to be posted at home or office. During the 12-week trial, you are asked to not change your typical diet or activity patterns. If you deviate from your routine diet, or if you begin taking medications, at any time between during the 12-wk trial, you are to notify the investigators of the study. About 40 subjects will participate in this study.

A research nurse will draw blood using standard, sterile techniques. Blood samples will be analyzed for biomarkers that are associated with blood glucose control such as glucose, insulin, and hemoglobin A1c.

RISKS

Bruising of the skin or a feeling of faintness is possible during the blood draws. For the at home finger sticks, subjects will be provided with disposable retractable lancets, strips, and glucometers as well as instructions for sterile conditions.

BENEFITS

This study will provide information regarding the usefulness of vinegar for controlling blood glucose and insulin concentrations in individuals with pre-diabetes.

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 affect you in any manner.

COSTS AND PAYMENTS

During the experimental periods, you will be provided with free test beverages and supplements. You will also receive one $10 and one $15 gift certificate during the study.

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 B

IRB APPROVAL

ASU Knowledge Enterprise Development

Office of Research Integrity and Assurance

To:	Carol Johnston ABC 132
From:	Carol Johnston, Chair Biosci IRB
Date:	01/23/2012
Committee Action:	**Amendment to Approved Protocol**
Approval Date:	01/23/2012
Review Type:	Expedited F12
IRB Protocol #:	1112007155
Study Title:	Therapeutic value of vinegar for adults classified as pre-diabetic.
Expiration Date:	12/06/2012

The amendment to the above-referenced protocol has been APPROVED following Expedited Review by the Institutional Review Board. This approval does not replace any departmental or other approvals that may be required. It is the Principal Investigator's responsibility to obtain review and continued approval of ongoing research before the expiration noted above. Please allow sufficient time for reapproval. Research activity of any sort may not continue beyond the expiration date without committee approval. Failure to receive approval for continuation before the expiration date will result in the automatic suspension of the approval of this protocol on the expiration date. Information collected following suspension is unapproved research and cannot be reported or published as research data. If you do not wish continued approval, please notify the Committee of the study termination.

This approval by the Biosci IRB does not replace or supersede any departmental or oversight committee review that may be required by institutional policy.

Adverse Reactions: If any untoward incidents or severe reactions should develop as a result of this study, you are required to notify the Biosci IRB immediately. If necessary a member of the IRB will be assigned to look into the matter. If the problem is serious, approval may be withdrawn pending IRB review.

Amendments: If you wish to change any aspect of this study, such as the procedures, the consent forms, or the investigators, please communicate your requested changes to the Biosci IRB. The new procedure is not to be initiated until the IRB approval has been given.

Please retain a copy of this letter with your approved protocol.

APPENDIX C

SAMPLE SIZE CALCULATIONS

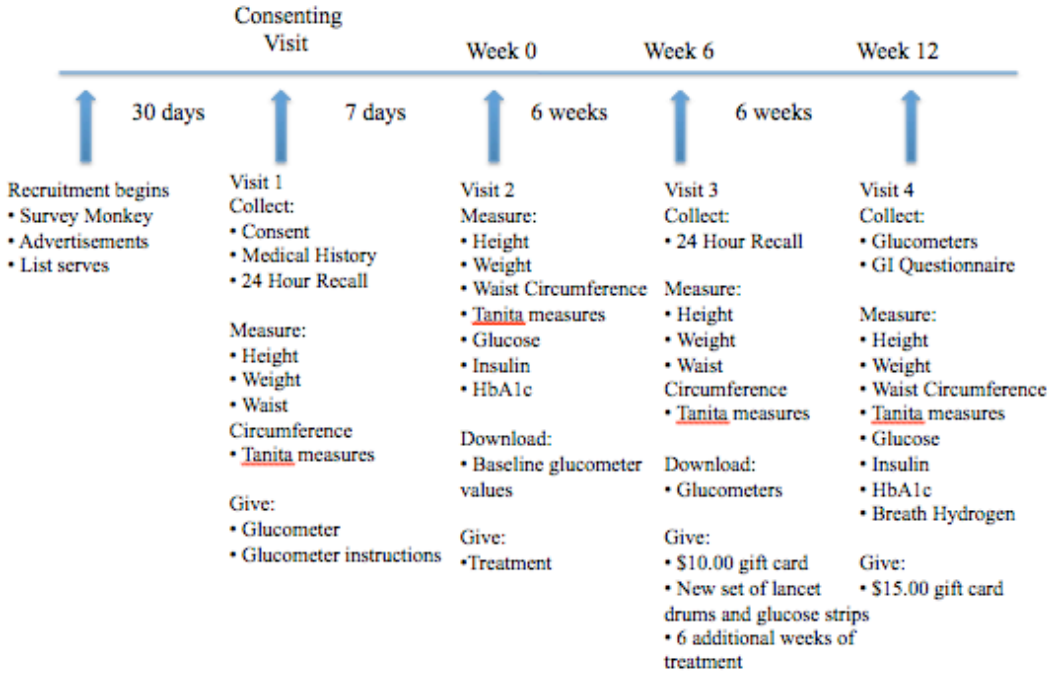
Articles	Significant Δ	Standard Deviation (SD)	Sample Size (n)	Calculated n
Beer (Obara et al., 2009)	.14	.18 (Difference)	23 (Pre-diabetics)	27 (54/2)
Lifestyle (Kang et al., 2010)	.15	.64 (Difference)	25 (Pre-diabetics)	287 (574/2)
Almonds (Cohen et al., 2011)	.30	.57 (Difference)	8 (Diabetics)	58 (116/2)
Vinegar (Johnston et al., 2009)	.16	.51 (Difference)	27 (Diabetics)	161 (322/2)
Summary:	.19	.48	21	133

Sample size. A parallel arm study of vinegar in diabetic adults provided data for sample size calculations (19). The assumption was made that a 0.06% change (increase) in HbA1c in the 12-week treatment period would occur in the placebo group, while a 0.16% change (decrease) was expected in the experimental group. The alpha error level for the primary end point was set 0.05 and the beta error level at 0.2 (power of 80% to detect a difference as large as 10%). A 20% dropout/unevaluable rate was anticipated. The estimated sample size was 40, with a 1:1 randomization ratio.

APPENDIX D

FLOW CHART

FLOW CHART


Consenting Visit
Week 0
Week 6
Week 12
30 days
7 days
6 weeks
6 weeks
Recruitment begins
• Survey Monkey
• Advertisements
• List serves
Visit 1
Collect:
• Consent
• Medical History
• 24 Hour Recall
Measure:
• Height
• Weight
• Waist Circumference
• Tanita measures
Give:
• Glucometer
• Glucometer instructions
Visit 2
Measure:
• Height
• Weight
• Waist Circumference
• Tanita measures
• Glucose
• Insulin
• HbA1c
Download:
• Baseline glucometer values
Give:
•Treatment
Visit 3
Collect:
• 24 Hour Recall
Measure:
• Height
• Weight
• Waist Circumference
• Tanita measures
Download:
• Glucometers
Give:
• $10.00 gift card
• New set of lancet drums and glucose strips
• 6 additional weeks of treatment
Visit 4
Collect:
• Glucometers
• GI Questionnaire
Measure:
• Height
• Weight
• Waist Circumference
• Tanita measures
• Glucose
• Insulin
• HbA1c
• Breath Hydrogen
Give:
• $15.00 gift card

APPENDIX E

MEDICAL QUESTIONNAIRE

MEDICAL 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? Y N

2. How long have you had pre-diabetes? _____

3. Do you take insulin to treat pre-diabetes? Y N

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

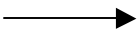
Medication	Dosage	Frequency

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

_____
_____
_____
_____

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

_____
_____
_____
_____
_____

OVER 

7. Do you have any food allergies?
Y N

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

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

12. Do you have dentures?
Y N

12. Do you have any swallowing issues?
Y N

13. Will you have any problem drinking an apple cider drink (8 oz with meals twice daily)?
Y N

14. Will you have any problem swallowing an apple cider vinegar pill (2 per day)?
Y N

15. Will you have a problem providing venous blood samples? (3 samples during the study)
Y N

16. Will you have a problem pricking your own fingers daily to provide blood drops for analyses? (2 pricks per day)
Y N

17. 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

18. If you exercise regularly, will you be able to not exercise (other than normal activity) for the 24-hour periods prior to testing?
Y N

19. Do you consume vinegar (flavorings, dressings, pickled foods) on a regular basis? If yes, please describe how often and in what form:
Y N

_____
_____
_____
_____

20. Will you have a problem reducing vinegar consumption to no more than the assigned treatment per week during the study duration (up to 12 weeks)?
Y N

21. Please describe any other medical conditions that may affect your participation below (i.e. pregnancy, infections, allergies, etc):

_____
_____
_____
_____

APPENDIX F

GLUCOSE ASSAY

Blood glucose was measured with a QuantiChrom Glucose Assay Kit (BioAssay Systems, Hayward, CA).

Procedure using 96-well plate:
1. Dilute standard in distilled water as follows.
Set up 1.5-mL centrifuge tubes. Transfer 5 µL diluted standards and samples to appropriately labeled tubes. Transfer 500 µL Reagent to each tube. Close the tubes tightly and mix. Store diluted standards at -20°C for future use.
2. Place the tubes in a tube holder and heat in a boiling water bath or heat block for 8 min. Cool down in cold-water bath for 4 min.
3. Transfer 200 µL in duplicate into a clear bottom 96-well plate. Careful: avoid bubble formation. Read optical density at 620-650nm (peak absorbance at 630nm).

Procedure using cuvette:
1. Dilute standards and transfer 12 µL water blank, Standards and samples to appropriately labeled tubes. Transfer 1200 µL Reagent to each tube. Close the tubes tightly and mix.
2. Place the tubes in a tube holder and heat in a boiling water bath for 8 min. Cool down in cold- water bath for 4 min.
3. Transfer 1000 µL reaction mixture into cuvette. Read optical density at 620-650nm (peak absorbance at 630nm) against blank.
Note: 1. If the Sample OD is higher than the Standard OD at 300 mg/dL, dilute sample in water and repeat the assay. 2. To determine low glucose concentrations, use 50 µL sample and standards (instead of 5 µL) per 500 µL Reagent.

Calculation
Subtract blank OD (water, #5) from the standard OD values and plot the OD against standard concentrations. Determine the slope using linear regression fitting. The glucose concentration of Sample is calculated as
= (ODSAMPLE – ODBLANKS)/ Slope (mg/dL)
ODSAMPLE and ODBLANK are optical density values of the sample and sample “Blank” (water or buffer in which the sample was diluted). Typical serum/plasma glucose values: 70 - 110 mg/dL. Conversions: 1mg/dL glucose equals 55.5 µM, 0.001% or 10 ppm.

APPENDIX G

INSULIN ASSAY

Blood insulin was measured with the Lispro Insulin RIA Kit (Millipore Corporation, Billerica, MA).

Assay set-up, Day 1
1. Pipette 300 µl assay buffer into the non-specific binding (NSB) tubes (#3, 4) and 100 µl assay buffer into the references tubes (#5, 6) and sample tubes (#25 through the end).
2. Pipette sequentially 100 µl standards and quality controls in duplicate into tubes #7 to 24.
3. Pipette sequentially 100 µl samples in duplicate into tubes #25 to the end. If sample volume is less than 100 µl, see notes in the Flow Chart.
4. Pipette sequentially 100 µl Matrix solution-PS into tubes #5 to 24. See notes in the Flow Chart.
5. Pipette 100 µl hydrated Lispro Insulin label into all tubes.
6. Pipette 100 µl Lispro Insulin antibody into all tubes except total count tubes (#1, 2) and NSB tubes (#3, 4).
7. Vortex, cover tubes, and incubate for 20-24 hours at room temperature.

Day 2
8. Add 1.0 ml precipitating reagent to all tubes except total count tubes (#1, 2).
9. Vortex and incubate 20 minutes at 4°C.
10. Centrifuge tubes at 3000 xg at 4°C for 25 minutes.
11. Immediately decant supernatant and drain the tubes for 20 to 30 seconds.
12. Count pellets in a gamma counter following the manufacturer's instructions.
13. Calculate results according to Section IX.