The Effects of Dietary Vinegar on Salivary pH and Dental Erosion

by

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ABSTRACT

Vinegar is gaining popularity as a natural and proven treatment for common diseases and conditions ranging from high blood pressure to diabetes. While the evidence to support the benefits of vinegar is growing, few studies have considered possible negative consequences. One concern relates to the effect of vinegar on saliva pH and dental erosion. The aim of this study is to explore this relationship as well as unsubstantiated claims that vinegar, although acidic, has an alkalizing effect on the overall body, specifically looking at its effect on resting saliva pH. Healthy adults aged 18-45 were recruited for this trial. Twenty-two participants completed this eight-week, parallel-arm, randomized, double blinded study that looked at the effect that regular consumption of red wine vinegar (two tablespoons taken two times per day before a meal) had on resting salivary pH and dental erosion compared to a control (low dosage vinegar pill taken two times a day before a meal). Resting saliva pH was measured at home using the pH20H application and pH strips at week 0 and 8 of the trial. Erosion was noted using the Basic Erosive Wear Examination (BEWE) by a registered dental hygienist at week 1 and 8 of trial. Results indicate no mean difference in resting salivary pH in either treatment group after eight weeks (p value, 0.49). However, there was a statistical significant mean difference in dental erosion between the VIN and CON group (p value, 0.05). Statistical significance in dental erosion, typically a gradual process, in just eight weeks is a significant finding and warrants concern about long time use of vinegar and dental health. Further exploration into this relationship is needed.

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CHAPTER 1

INTRODUCTION

This study examines the effects of dietary vinegar consumption on salivary pH and dental erosion. The use of vinegar for medical and health promotion purposes has ancient roots. For example, more than 2000 years ago, Hippocrates, a Greek physician and notable figure in the history of medicine, used vinegar as a wound disinfectant (Johnston & Gaas, 2006). Sung Tse, a great contributor to Chinese medicine, promoted using vinegar and sulfur for hand washing to prevent infection during autopsies in the tenth century (Chan et al., 1994). Today, vinegar is being studied not only for its antibacterial abilities but also for its ability to act as a functional food, or a food that provides a health benefit beyond inherent nutrition (Schmidl &Labuza, 2000). Many scientific studies show that vinegar has a number of medicinal or otherwise health promoting properties. For example, Johnston and colleagues (2004) showed that vinegar taken before a meal reduced postprandial glycemia by 64%, a benefit comparable to conventional pharmaceutical approaches to regulating blood sugar, specifically Metformin. Another study showed that vinegar decreased blood pressure in hypertensive rats by decreasing both aldosterone and renin (Kondo et al., 2001).

Although many studies have examined the health benefits of vinegar, few have considered possible adverse reactions of regular consumption. Johnston and colleagues (2008) studied some adverse effects of daily vinegar ingestion in a randomized, controlled, parallel study and noted that urinary pH was significantly reduced in the vinegar group. This is not altogether surprising. With a pH of 2.2, just above that of battery and hydrochloric acid, vinegar is among the most acidic consumable substances.

However, several unsubstantiated claims exist that vinegar, although acidic has an alkalizing effect on body pH. One area of inquiry, that has not been studied, is the effect that consuming this acidic liquid has on resting salivary pH and dental erosion.

Dental erosion is defined as the irreversible loss of dental hard tissue (enamel and dentin) caused by low intra-oral pH in the absence of bacteria (Loke et al., 2016). Resting salivary pH is between 6.3-7.6 with an average of 6.7 (Baliga et al., 2013). Anytime something acidic is ingested salivary pH drops in response. A drop in pH below 5.5 causes demineralization of the dental enamel, and can eventually lead to dissolution (Zero, 1996). The body in response has a built–in buffering system to bring the saliva back into a neutral state. This is known as the bicarbonate buffering system. The response is generally quick in a healthy individual and takes approximately 30 to 90 seconds (Edward et al., 1999). However, repeated drops of salivary pH caused by acidic substances can lead to dental erosion.

Several studies have considered the intrinsic and extrinsic causative factors that contribute to a drop in salivary pH. Dynesen and colleagues (2008) observed that dental erosion in bulimic patients was significantly higher than the control group and that the duration of the eating disorder greatly influenced the amount of erosion. Erosion in bulimic patients was attributed to frequent episodes of binging and purging that resulted in forcing highly acidic stomach acid into the oral cavity.

Acids of extrinsic origin are also a concern. Soda and fruit juice are both acidic and have been shown to cause dental erosion especially when consumed regularly (Moazzez et al., 2000). Habib and colleagues (2013) showed frequent consumption of acidic fruit juice, one or more daily, in children increased their risk of dental erosion by

2.4 times. The acidity of vinegar is comparable to gastric acid and more erosive than sodas and most fruit juices.

pH is but one factor in determining a liquid's erosive potential. Other factors are chemical, behavioral, and biological in nature. It has also been determined that titratable acidity, which is the liquids ability to buffer itself, also affects erosion (Tenuta et al., 2015). The stronger the buffering capacity of the acid the longer it will take the saliva to reach resting levels after an acidic attack. Also, the content of minerals in the food or drink are determinates in the erosive potential of the substance, with erosive potential decreasing as mineral content increases (Lussi, et al; 2004).

Dental erosion begins with an erosive chemical, but is often exacerbated by behavioral factors. One important factor is tooth brushing, especially after an acidic attack. Jaeggi and Lussi (1999) showed that brushing after an erosive pre-treatment lead to ten times more enamel loss than brushing without an erosive pre-treatment. Also, the method whereby the acidic liquid is consumed (through a straw versus from a can or gulping versus sipping) can affect dental erosion by determining which teeth come in contact with the liquid and the duration of contact (Edwards, et al; 1998).

Saliva is an important biological parameter in determining dental erosion. A decrease in saliva production is the major indicator for determining the risk of dental erosion. Saliva acts as buffer to acidic substances, rinses food and debris from the mouth and aids in remineralization of teeth by providing the minerals calcium, phosphate and fluoride to the demineralized enamel (Hara and Zero, 2014).

In summary, a number of interrelated factors are known to contribute to dental erosion. Some of these factors are behavioral. Others are biological. And others are

chemical. At the same time, vinegar has been used widely for assorted dietary, medicinal and other health purposes. As the scientific literature continues to explore the potential benefits of vinegar, there is growing opportunity to consider the potential risks. The proposed study integrates the research on dental erosion and saliva pH with the research on vinegar consumption.

Purpose of Study

The goal of this eight week study was to determine if taking vinegar daily as a functional food would cause a decrease in resting, unstimulated salivary pH and/or an increase in dental erosion in healthy adults, ages 18-45. There were two different modes of delivery examined. The first was a vinegar drink: two tablespoons of red wine vinegar diluted in eight ounces of water taken two times a day immediately before a meal. The second method was a very low dosage vinegar pill that was ingested two times a day immediately before a meal. The pill served as a control and allowed for blinding of the participants.

Research Aim and Hypothesis

H_1: Daily consumption of vinegar (red wine vinegar) taken 2 times a day before a meal will not be associated with an increase in dental erosion after 8 weeks in healthy adults, aged 18-45 years, compared to the control (vinegar pill) group.

H_2: Daily consumption of vinegar (red wine vinegar) taken 2 times a day before a meal will not be associated with a decrease in resting salivary pH (measured in morning with pH strips) after 8 weeks in healthy adults, aged 18-45 years, compared to the control group.

Definition of Terms

- Enamel –Tooth enamel is the hardest and most mineralized substance in the body. The primary mineral content is calcium and phosphate. It makes up the visible part of the tooth, and is usually white in appearance but can change color due to age, diet, smoking etc.
- Dentin –Dentin is a calcified tissue that lies underneath the enamel of the tooth. It is less mineralized than enamel and is therefore softer. It is yellow in appearance.
- Demineralization – Demineralization of the enamel is the loss of minerals from the enamel matrix, mostly that of calcium and phosphate. Demineralization is influenced by chemical, biological and behavioral factors.
- Dental erosion - Dental erosion is the irreversible loss of tooth structure due to chemical dissolution by acids not of bacterial origin.
- Xerostomia –Xerostomia is the perception of dry mouth, and may or may not be associated with true decrease in saliva flow.
- Incisal – Relating to or involving the cutting edge of an anterior tooth
- Maxillary – term given to teeth in the upper jaw
- Postprandial glycemia - Blood sugar levels after a meal

Delimitations and Limitations

Delimitations:

- Healthy, non-smoking adults between the ages of 19-45 were recruited for this study. Participants were free from chronic diseases and not taking insulin or any medications that could affect body weight. Women were not pregnant or planning to become pregnant in the next 3 months.

Results from this study may not be applicable to other age groups or disease states.

Limitations:

- The length of this study was eight weeks, which is difficult to determine the effects of dental erosion caused by vinegar. For obvious clinical signs to be apparent, a longer time interval would be ideal.
- Participants were asked not to change dietary habits during course of treatment, but adherence to recommendation cannot be controlled.
- Patient strict adherence to treatment plan, vinegar drink vs. vinegar pill, during the eight-week trial cannot be guaranteed.
- Other moderating factors could lead to a decrease in salivary pH and an increase in dental erosion during the length of the trial. Although a survey was designed to address many of these factors, it was impossible to isolate effects of vinegar on salivary pH and dental erosion.
- The Basic Erosive Wear Examination (BEWE) was a subjective screener and was not as accurate at predicting mild to moderate tooth wear.
- Only one registered hygienist performed the BEWE. It would have been preferable to have two or three hygienists performing the same screener and then averaging their scores.

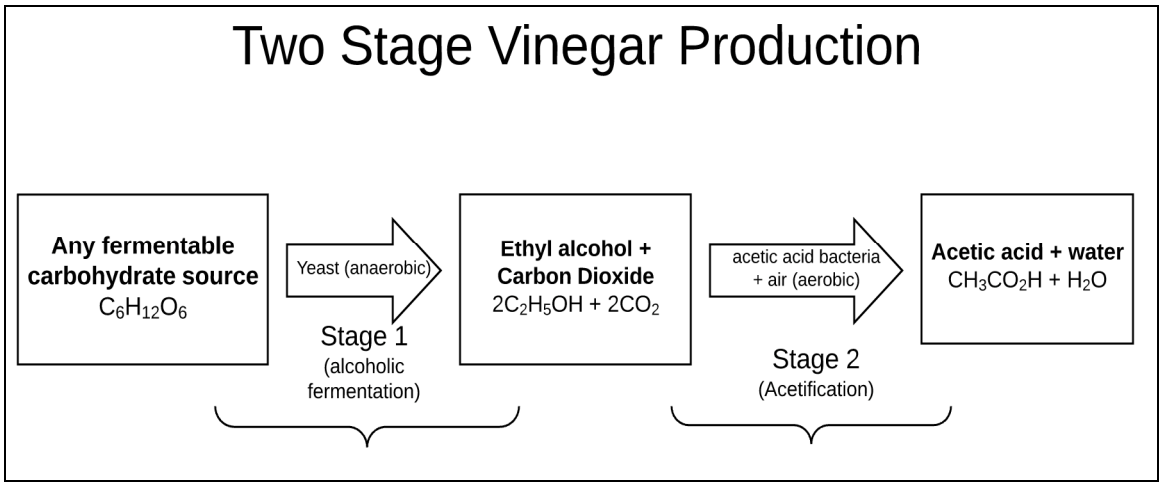
CHAPTER 2

REVIEW OF LITERATURE

Vinegar Production

Vinegar is a consumable liquid containing acetic acid that is produced by a two stage fermenting process (Figure 1). During the first stage of vinegar production, a fermentable carbohydrate source is converted to ethanol typically by the yeast strain, *Saccharomyces cerevisiae*, and in the second stage ethanol is oxidized to acetic acid by bacteria of the genus *Acetobacter* (Ho et al., 2017). This process can occur spontaneously since both types of microorganisms needed for the production, yeast and acetic acid bacteria, are commonly part of the natural micro flora of plants and at each stage of the process conditions are in some way restrictive to prevent microbial competition (Adams, 1998). During the first stage, the high sugar concentration and pH favor ethanol production by yeast, which occurs under anaerobic conditions. At the end of the first stage, when sugar has been consumed, aerobic conditions are re-established at the surface of the liquid, which is necessary for the acetic acid bacteria to further oxidize ethanol to acetic acid. This step drops the pH of the liquid to a pH of 3 or below (Adams, 1998). Thus, a suitable liquid left unattended and exposed to general environmental conditions will over time naturally undergo a fermentation process to produce first alcohol then vinegar. Because of the natural production of vinegar, it is safe to assume that alcohol discovery may have preceded vinegar discovery by days or weeks.

Figure 1:



Although there are several methods of vinegar production, today two primary methods exist. The first method is known as the traditional or "surface method." The transformation of ethanol to acetic acid in this method relies on a static culture of acetic acid bacteria, known as the *mother of vinegar*, at the interface between the air and liquid. (Johnston and Gaas, 2006). In this method, wooden barrels are filled to 2/3 capacity, allowing room for an air chamber. The liquid is left undisturbed and allowed to acetify spontaneously, taking several months. Since this process is operated by the batch, the bacterial film has to reform each time. A newer method, known as the "Orleans method," overcomes the delays associated with the traditional method, which result from the need to re-establish the mother. This is done by adding a funnel with an extension to the base of the barrel. When the liquid in the barrels reaches the appropriate acidity, a proportion of the vinegar is removed and replaced with fresh wine to the bottom of the barrel through the funnel. This allows new liquid to be added while not disturbing the mother (Adams, 1998).

A second method, known as the submerged culture method, is much faster than the traditional methods. In this method, large turbines are used to generate a flow of air bubbles into the wine solution, where the oxidative process occurs at the air-liquid interfaces. The process is quick compared to the traditional method and takes only 24 hours (Mas et al., 2014).

Mas and colleagues (2014) studied the differences in vinegar composition among various techniques and noted that vinegar produced from the traditional method is considered higher quality than the submerged method because of their organoleptic complexity. The submerged method compared to the traditional method produce very few metabolites. As well, many of the volatile compounds present in the original wine in the submerge method are lost in the airflow, leading to an organoleptically limited product. The most important aspect that contributes to organoleptic quality of vinegar is time. This occurs because of the interactions of the liquid with the wood barrels and numerous chemical reactions between acids and alcohols. The result is vinegar with better-integrated aroma and metabolites and a decrease in the acetic acid pungency.

Vinegars also differ from one another by the carbohydrate raw material used in the beginning process. The raw materials used can vary greatly ranging from agricultural surpluses like rice, to high quality substrates like red and white grapes, used in the production of balsamic vinegar. The type of vinegar produced is often dependent on the origin of the production. Several examples of vinegars produced, including the raw material used and it country of origin are listed below (Table 1).

Table 1: Types of Vinegar

Type	Raw Material	Major production country
Rice vinegar	Rice	United States, Taiwan
Balsamic vinegar	Grapes	Italy
Kombucha vinegar	Black or green tea leaves	Japan
Apple cider vinegar	Apples	Worldwide
Distilled white vinegar	Distilled alcohol	United States
Malt vinegar	Barley	England
Sherry vinegar	Sherry wine (fortified)	Spain
White wine vinegar	White wine	Turkey, Italy
Red wine vinegar	Red Wine	Worldwide
Sources: Ho, et al; 2017 and Budak, et al; 2014		

In order for vinegar to be considered vinegar it must contain at minimum 3.75% of acetic acid (Ho et al., 2017). Acetic acid, CH_3COOH, is considered the active ingredient in vinegar. However, the other ingredients vary greatly depending on the raw material and production techniques used. Natera and colleagues (2003) did a study examining the phenolic, organic acids, and volatile compounds in 83 different vinegars as a way to classify vinegars according to raw material and production processes used. The study magnified the fact that volatile acids, organic acids and polyphenols vary greatly between different vinegars. This could be an important concept when using vinegar as a functional food; certain vinegars, due to their constituents, may work better than others to treat specific health ailments.

Acetic Acid Bacteria

Acetic acid bacteria are the main contributors in the production of vinegar. They are gram negative or gram variable, rod or ellipsoidal shaped bacteria that occur in pairs or short chains. They are obligate aerobes that use oxygen as the final electron acceptor during metabolism. They grow best in environments with a pH of 5-6.5 but can grow at lower pH values of 3-4. Their optimal temperature is between 28 to 30 degrees Celsius. (Mamlouk and Gullo, 2013). The two main genera of acetic acid bacteria are: *Acetobacter* and *Gluconobacter*. These two genera can be differentiated by their affinity towards alcohol or glucose. *Acetobacter* oxidizes alcohol over glucose while *Gluconobacter* oxidizes glucose more readily than alcohol (Budak et al., 2014).

Vinegar uses:

Vinegar can be used for a host of different reasons that are both food and non-food related. Anciently, it was used in the production of white lead, which was used in paints and cosmetics found in Egyptian remains (Adams, 1998). More recently, it has been used as an antifungal agent in the production of natural rubber. Due to its high moisture content, fungi can easily grow on natural rubber affecting the quality of the final product. Commercial antifungal agents are toxic and not environmentally friendly. Vinegar, however, can minimize fungi growth while avoiding the other concerns of a commercial product (Ho et al., 2017).

Vinegar has also been used widely in relation to food. The acetic acid present in vinegar is responsible for its distinct flavor. As well, each individual type of vinegar has a particular flavor, which can enhance the taste of food. For example, vinegar is used in salad dressings, marinades, sauces, and condiments like ketchup and mayonnaise.

Vinegar is also used as a way to preserve food as seen in pickling. The acetic acid content in vinegar prevents the growth of most food poisoning and spore-forming bacteria. Although, the popularity of pickling today is primarily due to taste in developed countries, in underdeveloped countries it is a cost-effective way to preserve food (Adams, 1998).

Medicinally, the use of vinegar to treat ailments dates back thousands of years. In 420 BC, Hippocrates used vinegar to treat wounds. He also would treat persistent coughs by mixing honey with vinegar. This mixture is known as Oxymel, and was used by physicians into the 19th century. Sung Tse (10th century), father of forensic medicine, used vinegar mixed with sulfur as hand wash to prevent infection during autopsies (Johnston and Gaas, 2006). In the 18th century, U.S. medical doctors used vinegars as a panacea to treat ailments ranging from poison ivy to croup to stomach ache (Budak at el., 2014).

Antibacterial properties of vinegar

The antibacterial properties of vinegar were recognized early and are still used today as a natural way to kill unwanted bacteria. One area of inquiry is the bacteriostatic and bactericidal effect of vinegar on food borne pathogens. Although foods of animal origin are most commonly implicated as sources of unwanted and harmful bacteria like E Coli and Salmonella, fresh fruit and vegetable consumption have been implicated several times in the last 15 years as culprits for food poisoning outbreaks. There are several reasons for this including increased consumption of fresh fruit and vegetables due to heightened awareness of the positive role they play in health, modern farming practices, demand for prepared and bagged produce, and importation of year round produce from

different countries where standards of hygiene and harvest vary greatly (Heaton and Jones, 2007). The common practice among most, is to wash produce in chlorinated water to rinse off unwanted bacteria and toxic pesticides. However, studies have shown this method to be ineffective in limiting the amount of harmful bacteria (Sengun and Karapinar, 2004). Many chemical sanitizers have proven effective in removing pathogens from fresh produce, but consumers are increasingly interested in consuming food that is free of preservatives and chemicals. Vinegar, therefore, has been studied and found effective in acting as a natural agent in arresting and killing unwanted, harmful bacteria in food. Two different studies done by Sengun and Karapinar (2004) have shown vinegar was effective in significantly reducing *Salmonella typhimurium* in treated produce (carrot, spring onion and rocket lettuce). Entani and collegaues (1998) showed that spirit vinegar with as little as .1% acetic acid inhibited the growth of 17 strains of bacteria including eight strains of E. Coli. Vinegar was shown to be bactericidal against EHEC 0157:H7 and its action was enhanced by the addition of sodium chloride. Also, interesting to note, the bactericidal effect of vinegar was not attributed to the low pH of acetic acid, since E. coli suspended in hydrochloric acid, which has a lower pH than vinegar, did not have any decrease in bacterial count (Etani et al., 1998).

A recent study done by Corteisa and colleagues (2014), demonstrated that acetic acid was effective in killing *Myobacterium tuberculosis* after 30 minutes of exposure to 6% acetic acid. Even the *Myobacterium absceccus* complex, which is heavily resistant to many conventional antibiotic drugs, showed a 6-$\log_{10}$ reduction in colony counts when treated with 10% acetic acid for 30 minutes. Mycobacteria are primarily responsible for causing tuberculosis and often resistant to the current multidrug treatments used, which

makes them biohazards. As well, current disinfectants used to kill Myobacteria can be toxic. The results of this study are promising showing vinegar, which is safe and relatively inexpensive to make is effective in killing even the most resistant strains of Mycobacteria.

Vinegar and blood pressure

Vinegar has been reported to lower blood pressure in hypertensive rats. A study done by Kondo and colleagues (2001) explored the effects of administering dietary vinegar (rice vinegar) and pure acetic acid for 8 weeks on spontaneously hypertensive rats. The results showed a significant difference in blood pressure at week 8 in rats given either treatment of vinegar or acetic acid compared to the control group. Thus establishing the idea that the main contributor in vinegar that promotes a decrease in blood pressure is acetic acid. The study also showed a large decrease in both aldosterone and renin in both experimental treatment groups. The authors hypothesized that a decrease in renin would affect the renin-angiotensin system, which is a typical blood pressure increasing system. A decrease in renin, would therefore cause a decrease in blood pressure.

A more recent study done by Na and colleagues (2015) further explored the biochemical pathways activated by vinegar responsible for decreasing blood pressure. The results of their study demonstrated that the renin-angiotensin system was affected by angiotensin II type 1 receptor (AT1Rs), which is considered the "last decider" in the renin-angiotensin system. Vinegar inhibited AT1R, leading to a decrease in blood pressure. Also, vinegar was shown to activate AMPK (AMP-activated protein kinase), PGC-1α and PPARγ, which cause a decrease in AT1R.

The current literature does not examine the antihypertensive properties of vinegar on humans, but an epidemiological study done in 2006 by Zhao and colleagues found that in Shanxi, China hypertension was lower than in any other region in China. Shanxi is famous for its vinegar production and consumption, ingesting up to 30-50 times more vinegar than other areas in China. Although this does not prove causality, it does reflect an association. This information partnered with the previous studies done on hypertensive rats, reflects that daily vinegar consumption could possibly cause a decrease in blood pressure in humankind.

Vinegar and body fat

Obesity, defined as a BMI greater than 30 in the United States, has increased dramatically in the last few decades. According to the National Center for Health Statistics (NCHS) data brief in 2015, 36.5% of US adults were considered obese (Hales et al., 2017). Obesity is a risk factor for many life-style related diseases like heart disease, type-2 diabetes, and some types of cancer. The fat that is the primary culprit is visceral fat, which is fat stored deep within the abdominal cavity as opposed to subcutaneous fat that is stored just underneath the skin. Although there are medications designed for weight control, a more natural approach using food products is being explored because it is free of undesirable side effects. One food product that has shown promising results is vinegar.

Recent studies done on human subjects have explored the effectiveness of vinegar in reducing fat, both visceral and subcutaneous. Kondo and colleagues (2009) showed that vinegar taken after a meal minimally reduced body weight (1-2 kg), BMI (0.4-0.7 points), visceral and subcutaneous fat, and serum triglyceride levels in obese Japanese

subjects compared to a placebo group in a 12 week, randomized, placebo controlled, double blinded study. These results were seen in both treatment groups (either receiving 15 ml or 30 ml of apple cider vinegar) with more profound results seen in the higher dosage group. The positive effects associated with vinegar ingestion dissipated 4 weeks after treatment ceased, indicating that continuous administration of vinegar is necessary to maintain a reduction in body fat.

The reduction in body fat caused by consuming vinegar has been attributed to the up regulation of adenosine monophosphate-activated protein kinase (AMPK). AMPK is an enzyme that participates in the regulation of energy metabolism. It also participates in fatty acid oxidation. Previous studies done in rats have shown that obese rats given acetic acid had a decrease in body fat caused by activation of AMPK (Yamashita et al., 2007). Park and colleagues (2014) explored if these effects were similar in human subjects. The results of their study showed that participants receiving the treatment of pomegranate vinegar had a significant reduction in visceral fat (10%) compared to the placebo group, which only experienced a 2% decrease. Also, AMPK phosphorylation in adipose tissue increased by a factor of 2.7 compared to the placebo group. This builds on the theory that it is the activation of AMPK that causes a reduction in body fat.

Vinegar and blood glucose

Diabetes is a worldwide issue. It was estimated that there are over 171 million people living with diabetes globally today, and that number is expected to grow to 366 million people by 2030 (Wild et al., 2004). The quality of the diet has been shown to be a factor in the prevention and treatment of diabetes; foods that fall low on the glycemic index are being acknowledged as beneficial. The glycemic index is a number associated

with the carbohydrates in a food source that impact an individual's blood glucose. Numerous factors can affect the glycemic index of a food including: characteristic of raw material (amylopectin vs. amylose), the amount of soluble fiber contained, the amount of whole-intact grain used, and content of protein in the food (Ostman et al., 2005).

Although diets that are low on the glycemic index are recognized as beneficial by the World Health Organization (Ostman et al., 2000), many of the commercially available foods today, like bread and cereal, fall on the high side of the index. However, numerous studies have shown that high glycemic food paired with certain food sources, specifically vinegar, can significantly lower the blood glucose and blood insulin levels compared to eating the food alone. In a crossover study, Johnston and colleagues (2004) demonstrated that vinegar (20g of apple cider) taken before a high carbohydrate meal (white bagel, butter and orange juice – 87 total carbohydrates) increased postprandial insulin sensitivity in insulin resistant participants by 34% compared with the placebo treatment. Ostman and colleagues (2005) confirmed these results. Their study showed that vinegar, containing 18, 23, or 28 mmols of acetic acid, administered before a meal of white-wheat bread reduced postprandial blood glucose and blood insulin levels significantly, and that the response was dose related; a greater response was seen with a larger dose of vinegar. Also, the study examined vinegar and satiety and found that the two were linearly correlated.

The direct mechanism of action behind vinegar ingestion and a reduction in postprandial glycemia and insulin are unknown, but delayed gastric emptying is one hypothesis. However, Brighenti and colleagues (1995) noted using noninvasive ultrasonography, that there was no difference in gastric emptying rates in participants

consuming a bread/vinegar treatment compared to a bread/vinegar/sodium treatment (vinegar is neutralized by the addition of sodium bicarbonate).

Adverse effects of vinegar

Very few studies looked at the relative safety of vinegar when consumed regularly as a functional food. Johnston and colleagues (2008) conducted a study looking at the adverse effects of consuming vinegar medicinally daily. Twenty-seven participants were randomly assigned into three treatment groups each receiving one of the following treatments: a low dose vinegar pill (30 mg of acetic acid), a pickle (approximately 1,400 mg of acetic acid) or a vinegar drink (2,800 mg of acetic acid). A significant reduction in urinary pH was noted at week 12 in the vinegar drink group compared to the other two treatment groups. It was concluded that acetic acid might alter hepatic function, but that further research needed to be conducted especially because the sample size was small. Unsubstantiated claims exist, that although vinegar, specifically apple cider vinegar, is highly acidic it has an overall alkalizing effect on the body; these claims acknowledge that vinegar can cause a decrease in urinary pH as extra acidity is excreted as urine to maintain a proper body pH, but that because of the way the vinegar is metabolized it causes an overall alkaline environment for the body. The effect that vinegar has on resting saliva currently has no research.

One incident in Austria regarding a 28-year-old woman being admitted to a hospital due to muscle cramps and hypokalemia was attributed to the large amount of vinegar she consumed regularly. The woman consumed approximately 250 ml of vinegar daily (200mmol of acetic acid) for six years. Test results showed the women had low levels of potassium and her bone density was significantly reduced placing her in the

osteoporosis category. This seems to be an isolated event, with no further studies showing causality between large amounts of vinegar ingestion and decreased blood and urine levels of potassium and calcium (Lhotta et al., 1998)

Another incident involved a 39-year-old woman who tried to dissolve a crab shell stuck in her throat by drinking one tablespoon of white vinegar. Inflammation of the oropharynx was noted as well as a second-degree caustic injury of the esophagus (Chung, 2002). These results are not substantiated, as various studies have looked at consumption of vinegar in even greater amounts without similar adverse results.

Saliva

Saliva is a watery exocrine secretion comprised of 99.5 % water, proteins in the form of enzymes, antibacterial compounds, mucus, and various electrolytes (sodium, potassium, calcium, magnesium, chloride, bicarbonate and phosphate) (de Almedia et al., 2008). Due to saliva's fluid characteristics and specific components, it is responsible for numerous functions.

Table 2: Functions of Saliva

Lubrication of oral mucosa, including teeth, gingiva (gums) and membrane
Protection against diseases (antibacterial, antifungal, antiviral)
Digestion of food: mechanical and chemical breakdown of food, formation of bolus, swallowing to propel food down to esophagus
Tasting
Buffering/ maintaining pH of saliva within normal limits
Carrier of antibodies, hormones, etc. therefore can be used for diagnostic testing
Remineralization of enamel after demineralization occurs
Source: Wilkins, 1999

Mucins, protein with a high carbohydrate content, located within the saliva are responsible for forming a seromucosal covering that protects the oral tissues from trauma during eating, swallowing and speaking. (de Almedia et al., 2008).

Saliva contains a variety of compounds and enzymes that participate in antibacterial and antimicrobial actions. For example, IgA, found in large quantities compared to other immunologic components like IgG and IgM, serves as an antibody for bacterial antigens and prevents adhesion of bacteria to oral tissues. Another example is lysozyme, an enzyme abundant in saliva, which is responsible for attacking and hydrolyzing the cell wall of some bacteria; Gram negative bacteria are less vulnerable to lysozymes, because they contain an extra protective external lipopolysaccharide layer that Gram positive bacteria does not possess (Marieb, 1999).

The process of breaking down food begins in the mouth during mastication or chewing. During mastication the salivary glands produce more saliva to help in the process of digesting and propelling the food down the GI tract. The saliva mixes with the food to form a bolus. The water present in the saliva softens and moistens the food and the mucins add lubrication to the bolus, which allow it to easily slide down the esophagus without causing any damage to the mucosal GI cells (Pederson et al., 2002) Also, the earliest breakdown of carbohydrates begins in the mouth by the enzyme salivary amylase present in the saliva. Its biological function is to break down starch to maltose, maltotriose and dextrins (de Almedia et al., 2008).

The sense of taste is a stimulant for an increase in saliva production; the amount of saliva produced is dependent on the food ingested. The highest saliva output is obtained with sour food, followed by salt, sweet and bitter (Dawes and Watanabe, 1987).

On the other hand, saliva is essential for taste perception, because taste receptors in the taste buds are only stimulated when food particles are in a solution (Pederson et al., 2002).

Technologies are available to screen for or diagnose several diseases including: HIV, Cushing's disease, Sjogren syndrome, ovarian and breast cancers, and dental diseases (caries and periodontal disease). Hormone imbalances such as, menopause and irregular menstrual cycles, can also be measured in saliva. Also, life insurance companies use saliva screenings to detect nicotine levels to verify smoking status of clients (Navazesh and Kumar, 2008).

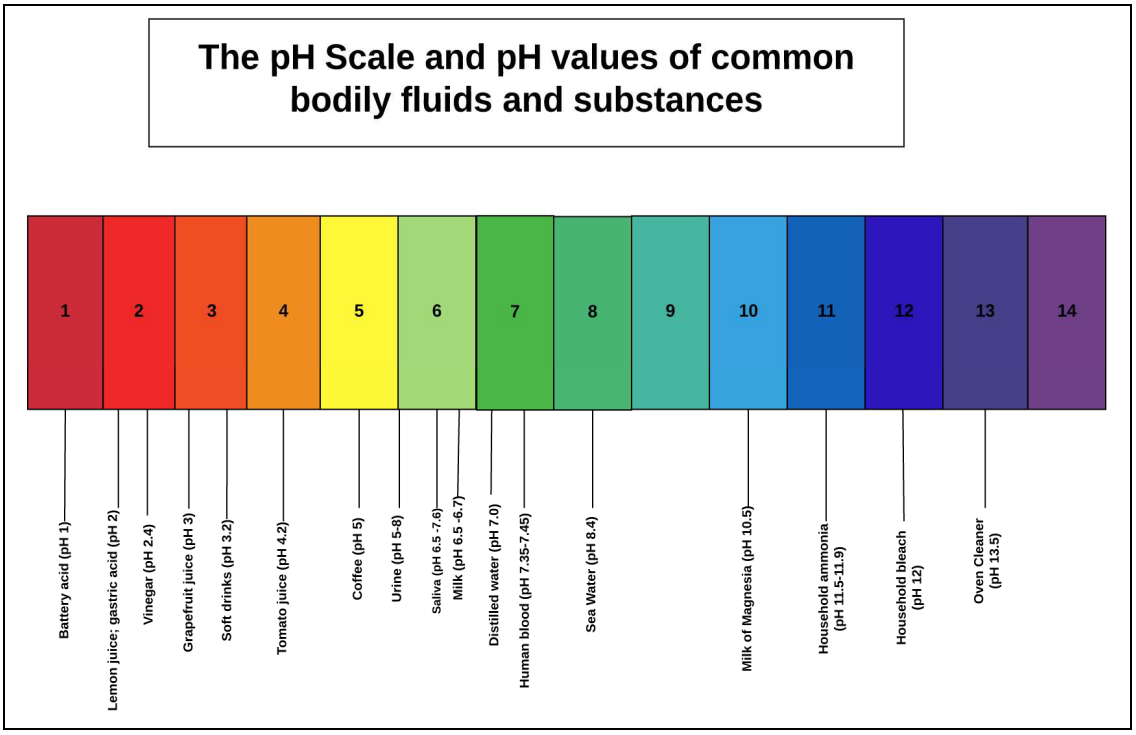
The enamel covering the outer portion of teeth is made of 90% hydroxyapatite, a naturally occurring form of calcium apatite, with a formula $Ca_5(PO_4)_3(OH)$ (Neel et al., 2016). When saliva pH drops below 5.5, the calcium and phosphate bonds forming the enamel matrix begin to break causing a loss of mineral content, primarily calcium, phosphorus and fluoride, from the enamel. The demineralization continues until salivary pH is neutralized; at this point remineralization can begin (Wilkins, 1999). The high concentrations of calcium and phosphate in saliva allow the minerals to be directed to the tooth structure and destruction is arrested (de Almeida et al., 2008).

pH and buffers

Danish chemist, Søren Peder Lauritz Sørensen introduced the idea of a pH (pH meaning potential of hydrogen) scale in 1909. The pH scale is a numeric scale from 0-14 that measures the acidity or basicity of an aqueous solution. The scale is logarithmic, a change from one successive number to the next represents a tenfold change in hydrogen ion concentration, and is defined by the equation: $pH = -\log[H^+]$. A solution with equal

amounts of H^+ and OH^- is neutral and is assigned a pH of 7 on the pH scale. Anytime an ionic or polar substance is dissolved into water it changes the numbers of H^+ and OH^-, causing it to become either acidic or basic. A solution that has an excess of H^+ is acidic (pH of <7) and a solution with an excess of OH^- is basic (pH of >7). A molecule is said to be acidic if it is a proton donor and basic if it is a proton acceptor. The pH of some body fluids and common beverages and foods are below:

Figure 2:



(Source: pH values for chart taken from Marieb, 2001)

The regulation of pH is essential to all living organisms and must be kept within narrow limits. An example of this is normal human blood, which needs to be kept within the range of 7.35 to 7.45. If the blood pH falls outside this range, the results can be disastrous and often fatal (McKee, 2016). pH in humans is kept within these narrow limits by buffers, which are weak acids and their conjugate bases. They function by

resisting changes in pH when a strong acid or base is added to them while remaining unchanged themselves. They accomplish this by releasing hydrogen ions when the pH rises and binding to hydrogen ions when the pH falls (Marieb, 2001).

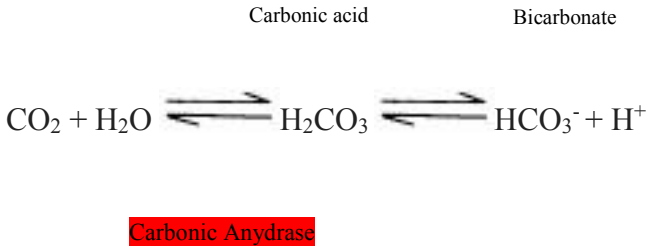
Saliva, like blood, has a range that it must be kept within in order to promote health. The normal range for saliva pH is 6.2-7.6 with an average pH of 6.7 (Baliga et al., 2013). When food or beverages are consumed, the pH in the mouth falls. The degree to which the pH falls is dependent on the pH and titratable acidity of the substance being ingested (Tenuta et al., 2015). When the pH in saliva drops below 5.5 demineralization of the dental enamel begins. Demineralization is the process of removing minerals from dental enamel and leads to its eventual dissolution (Zero, 1996). The body in response counterbalances this drop in oral pH by the neutralizing power of three buffering systems found within the saliva: the bicarbonate, the phosphate, and the protein buffering systems (Bardow et al., 1999).

Whole saliva contains a vast array of different proteins, each responsible for different biological functions (Castle and Castle, 1998). Most of these proteins have capacity to act as buffers when the saliva pH is either acidic or alkaline, because they have a pI within the physiological pH of saliva. Although, they were dismissed in the past as not playing an important role in saliva neutrality, more recent studies show they have an important role in buffering both acidic and alkaline saliva (Bardow et al., 1999).

The phosphate buffering system is most active in unstimulated saliva where there are greater amounts of phosphate concentrations at 5mmol/l compared to only 3mmol/l in stimulated saliva (Bardow et al., 2008). However, because it is found in limited quantities in stimulated saliva, it is not an effective buffer during the ingestion of food or

beverage when saliva production is increased. The bicarbonate buffering system, on the other hand, is found in small quantities in unstimulated saliva but increases dramatically when saliva is stimulated, up to 24 mmol/l. It is therefore considered the major buffering system of saliva (Bardow et al., 1999).

After ingestion of food or drink the pH of the saliva drops. This is due to the pH of the substance and the production of lactic acid by bacteria located inside dental plaque as they ferment the ingested carbohydrates. The saliva glands in response produce an increase of saliva, which causes an increase release of bicarbonate ions from the salivary ducts. The bicarbonate buffering system is as follows:



The enzyme carbonic anhydrase, present in saliva, is responsible for the conversion of CO_2 to carbonic acid and vise versa (Murakami and Sly, 1987). The bicarbonate released during stimulated saliva takes up the extra protons responsible for the drop in pH and drives the equation to the left forming CO_2, which is expelled into the atmosphere. Because the P_{CO2} (partial pressure of CO_2) of the mouth can be as high as 41 mmHg, whereas the P_{CO2} in the atmosphere is .3 mmHg, when saliva is exposed to the atmosphere as in eating or breathing, the equation will rapidly shift to the left. This speeds up the loss of HCO_3^- and protons and causes a change in the oral pH in a more alkaline direction (Bardow et al., 1999).

Important to note, is the ability of the bicarbonate buffering system to buffer acidic attacks on the oral cavity are not fixed and can vary widely from person to person. One of the biggest determinants is the amount of stimulated saliva produced after each acidic attack.

Salivary Flow

Salivary flow is the amount of saliva released by the salivary glands. Salivary flow is an important indicator of oral health. The physiology of salivary flow is dominated by the three major salivary glands: the parotid, submandibular, and sublingual. They are collectively responsible for producing 90 percent of the saliva in the mouth. The remaining 10 percent comes from minor salivary glands, of which there are between 800-1000 located throughout the mouth (Navazesh, 1993). The average amount of saliva produced in a healthy person is between 0.5 and 1.5 Liters per day, the majority being produced while a person is awake (Navazesh and Kumar, 2008).

There are two primary modes of saliva flow: stimulated versus unstimulated. Unstimulated saliva flow occurs when a person is at rest without any outside interference such as food, drink or medication. Unstimulated saliva is secreted continuously in small amounts, which are sufficient to keep the mouth wet (Marieb, 2001). Stimulated saliva accounts for 80-90 percent of total saliva production (Edgar, 1992) and is produced by exogenous stimulation, mechanical, visual or olfactory (deAlmedia, 2008). It is controlled mainly by the parasympathetic nervous system. When stimulated, the salivary glands increase production of serous fluid, an enzyme rich form of saliva. Serous helps aid in the digestion of food (Guggenheimer, 2003). The contribution of saliva produced by each major salivary gland differs depending if it is stimulated or not. For example,

when unstimulated the parotid gland contributes only 20 percent of saliva, whereas upon stimulation it is responsible for more than 50 percent.

Table 3. Stimulated and unstimulated salivary flow rate among different glands

Salivary Glands	% of Saliva in unstimulated SF	% of Saliva in Stimulated SF	Type of Secretion Produced
Parotid	20%	< 50%	Mainly serous
Submandibular	65-70%	>35%	Serous and mucus
Sublingual	7-8%		Mainly mucus
Minor	<10%		Mainly mucus

Source: deAlmedia, 2008

Systematic scientific inquiry into salivary flow has roots that date back at least to the early 1960s. Of central importance is Dawes's research, which has shown repeatedly that saliva, both unstimulated and stimulated follows circadian rhythms (1972, 1973, 1975). A circadian rhythm is an example of an endogenous simulation, built in biological process that oscillates every 24 hours. As part of this rhythm, saliva peaks in the afternoon and wanes in the middle of the night. The circadian rhythm of unstimulated saliva is extremely low during sleep, making patients more prone to decay because the protective mechanisms of saliva are minimal (Schneyer et al., 1956).

Xerostomia/Hyposalivation

The most important factor in determining risk of dental erosion is salivary flow rate, since saliva acts as a buffer to acidic beverages, rinses food and beverages out of the oral cavity, and aids in remineralization by providing calcium, phosphate and fluoride to eroded enamel. Jarvinen and colleagues (1991) showed that a patient with an

unstimulated salivary flow below 0.1 ml/min or less (hyposalivation) was 5 times more likely to experience dental erosion than those with normal salivary rates. During consumption of acidic beverages, the pH at the tip of the tongue drops for approximately 2 minutes and complete clearance of the acidic beverage takes about ten minutes with normal salivary flow rates (Meurman et al., 1987). During compromised salivary flow, the pH in the mouth after an acidic beverage remains low for about 30 minutes (Tenovuo et al., 1977).

Table 4 displays salivary flow rate measurements (mL/min) associated with normal salivation, low salivation and hyposalivation.

Table 4. Summary of salivary flow rates

Salvia Flow Rates	**Normal**	**Low**	**Very Low** (hyposalivation)
Unstimulated	.25-.35 mL/min	.1-.25 mL/min	< .1 mL/min
Stimulated	1-3 mL/min	.7-1 mL/min	< .7 mL/min

Source: Thylstrup, 1994

Numerous factors are attributed to hyposalivation, including medications, age, smoking, radiation and chemotherapy treatments, chronic disease and dehydration.

Since 99 percent of saliva is composed of water, it necessarily follows that dehydration would affect salivary flow. Several studies have measured the correlation between dehydration and salivary flow rate. For example, Ship and Fischer (1997) showed that dehydration is associated with decreased salivary flow rate and that dehydration affects old and young patients similarly. Similarly, Dawes (1987) showed that individual hydration was the number one factor in determining salivary secretion.

Also, it has been shown that dehydration causes the salivary glands to cease production of saliva to conserve water (Holmes, 1964).

As the average life expectancy continues to increase, so does the use of medications (Health Policy Institute, 2002). Xerogenic (causing dry mouth) medications can cause hyposalivation, and the risk increases with the number of medications being taken (Wolff et al., 2008; Guggenheimer and Moore, 2003). There is a variance in prescription drug usage by age groups. Drug use increases from 39% in 19-64 year olds to 74% in individuals over 65 years of age. The number of medications taken also increases with age (Health Policy Institute, 2002). Over 400 medications have been associated with xerostomia (defined as the perception of dry mouth); many are commonly prescribed in the United States. These include, but are not limited to medications in the following categories: antidepressants, antihistamines, antihypertensive drugs, beta-blockers, and diuretics (Smith and Burtner, 1994). Also, the duration of time the medication is taken can influence a subject's perception of xerostomia. Contrary to intuition, the longer certain medications are taken, xerostomia complaints lessen among subjects. For example, a higher percentage of subjects taking tolterodine, a medication prescribed for urinary incontinence, experienced xerostomia at 12 weeks than those taking the drug at months 6 -12 (Carpenter, 2015). Patients becoming more accustomed to feelings of xerostomia could explain this phenomenon, rather than a true increase in saliva production as length of medication usage increases. A study by Nonzee and colleagues (2012), confirmed this hypotheses, showing that the duration of the use of hypertensive drugs from 1 month to 120 months, did not change both stimulated and unstimulated salivary flow rates.

Woolf and colleagues (2008) looked at the relationship between various xerogenic drugs and their effects on salivary flow both stimulated and unstimulated in each individual major salivary gland. Before this, studies only looked at the reduction of saliva from all the glands as a whole in relation to medication. The results showed that different medications affected the glands differently. The parotid gland (PAR) had a significant reduction in saliva flow in only 3 medication categories. The submandibular and sublingual glands (SM/SL) flow rates decreased consistently in all drug categories. These findings suggest that the submandibular and sublingual glands are more susceptible to medications than the parotid.

Chronic disease is another risk factor for hyposalivation. The disease most commonly connected with dry mouth is Sjogren's syndrome (Kassan &Moutsopoulos, 2004). Sjogren's syndrome (SS) is an autoimmune disease affecting the moisture producing glands of the body, including the lacrimal and salivary glands. Sjogren's disease can occur alone, known as primary SS, or in connection with another systemic autoimmune disease (secondary SS); some common autoimmune diseases attached with secondary SS are rheumatoid arthritis and lupus (Al-Hashimi, 2001). The cause is unknown. It is primarily seen in women in later in life, therefore, sex hormones especially estrogen are thought to play a role in development (Voulgarelis & Tzioufas, 2010). Xerostomia in patients with SS is attributed to the destruction of secretory acini cells of the salivary glands, both major and minor, by lymphocytes (Guggenheimer & Moore, 2003). The hallmark symptoms of SS are dry eyes and dry mouth. A study conducted by Al-Hashimi and colleagues (2001) showed that patients with SS

complained of xerostomia 93.5% compared to 2.3% in the control and dry eyes 67.5% in SS patients to 13.6% in control. These are earliest indicators often of the disease.

Another group at risk for dry mouth is cancer patients. Cancer patients receiving treatment of chemotherapy and radiation therapy, specifically head and neck radiation are at risk. Radiotherapy-induced xerostomia is related to the type of radiation, dose and frequency. Since salivary glands are located superficially to most tumors, radiation has to pass through glands often to treat the tumor. Cells with a rapid turnover rate, like cancer cells, are more susceptible to radiation. But cells with a slow turnover rate are still susceptible. For example, salivary gland turnover rate is slow, but production and quality of salvia after radiation changes, thus showing salivary glands are not resistant to radiation therapy (Burlage et al., 2001). Early studies were conducted that showed the effects of radiation on the parotid gland. Shannon and colleagues (1978) showed that a decrease in parotid gland salivary flow is noted 24 hours after the first treatment of radiotherapy at 2 Gray (Gy). Previous to 2001, few studies looked at the effects of radiation on the submandibular and sublingual glands. Burlage and colleagues conducted a study in 2001 that showed patients receiving 60-70 Gy of radiation had a significant reduction in salivary flow at week two in both PAR and SM/SL glands. Both glands showed similar reductions, operating at 20% of the initial flow. However, at 3, 5, and 13 weeks, the SM/SL glands showed less reduction than the PAR gland. This suggests, that the SM/SL glands rebound quicker than the PAR gland to radiotherapy. Six weeks after radiotherapy, there was no significant reduction in salivary flow in any of the major salivary glands. Another study done by Franzen and colleagues (1992) showed that at levels of radiotherapy below 52 Gy, salivary flow recovery began at 2 months after

treatment with continuous improvement until 18 months. Radiotherapy above 64 Gy, however, caused irreversible damage to the parotid gland.

Chemotherapy is a type of treatment used to treat cancer patients. It can include one to numerous types of medication that are designed to attack rapidly growing cancer cells. Unfortunately, other rapidly growing cells are also affected by the medications. Thus, chemo is considered a systemic therapy, because it affects your entire body. Some rapidly growing cells that are often damaged during chemo include, blood cells (white, red and platelets), hair follicle cells, and intestinal cells. Chemotherapy also affects salivary glandular cells. Jenson and colleagues (2006) showed that chemotherapy administered to 45 breast cancer patients for seven cycles decreased both unstimulated whole saliva (UWS) and stimulated whole saliva (SWS) significantly after chemotherapy. SWS returned to baseline levels after six months, while UWS didn't reach baseline until one year after treatment. Xerostomia also increased during chemotherapy treatment, but returned to baseline after one year. It is concluded, that chemotherapy does cause changes in salivary flow but the adverse drug reactions are temporary.

Xerostomia, is a subjective condition in which a patient complains of feelings of oral dryness. It may or may not accompany reduction in salivary flow. Measuring saliva flow is not plausible in many clinical settings, so complaints of dry mouth are often used by clinicians to diagnose low saliva output. Fox and team (1987) identified three questions that could predict possible salivary gland dysfunction. They include:

1. "Do you sip liquids to aid in swallowing dry foods?"
2. "Does your mouth feel dry when eating a meal?"
3. "Do you have difficulty swallowing any foods?"

The study noted that the questions that centered on oral dryness during eating were highly predictive of hyposalivation. Typically maximum salivary output is expected during mealtime when taste, olfactory and mastication all act as stimulators to salvia output.

True hyposalivation can be devastating on the oral cavity, and includes numerous signs and symptoms. Some of the signs and symptoms are:

- Oral candidiasis, which is caused by an overgrowth of Candida species due to the decrease in antimicrobial actions of saliva. It is an opportunistic infection also known as thrush (Samaranayake, 1990).
- Dental erosion – saliva is a buffer against pH drops in the oral cavity; it helps aid in the remineralization of tooth enamel (Hara and Zero, 2014).
- Increase in dental caries – caused by a decrease in the anti-cariogenic properties of saliva. (Guggenheimer and Moore, 2003).
- Mucosal changes including fissured and erythematous tongue, atrophy of filliform papillae, and dry and cracked lips (Guggenheimer and Moore, 2003)

Dental Erosion

Dental Erosion is defined as an irreversible loss of dental hard tissues caused by chemical processes without bacterial involvement. This occurs when the tooth surface loses calcium and phosphate, causing softening of the enamel, which can eventually lead to a loss of dental enamel (Habib et al., 2013). Enamel dissolution causes two different and distinct lesions, a carious lesion or erosion. The two lesions are clearly different. A carious lesion is caused due to lactic acid production formed as a by-product of bacterial degradation of carbohydrates. The carious lesion, therefore, is located underneath the bacterial plaque. Dental erosion is caused by acids of any other origin and typically

appears generalized on exposed surfaces (Sanchez et al., 2003). There are many clinical signs of dental erosion. For instance, the incisal edges on the anterior teeth can become translucent. Also, the teeth may appear more yellowish in tint, caused by the enamel wearing thin and the underlying yellow dentin showing through. A change in shape can occur, like enamel developmental ridges disappearing leading to broad concavities on occlusal surfaces. Amalgam fillings can look clean with no tarnish and may appear as if they are floating above the tooth, because the enamel is eroded around the filling but the metal is unharmed by erosive properties. Loss of enamel can lead to dentin exposure and in extreme cases can extend as far the pulp causing hypersensitivity. (Jarvinen et al., 1990; Habib et al., 2013) .

Dental erosion is a problem of all ages and is evident even in young children. Habib and colleagues (2013) conducted a study looking at the prevalence of dental erosion in American children and found that erosion occurred in 13% of children aged 2-4 and 10% in children that were 12. These were fairly conservative numbers when compared to the 2003 National Children's Dental Health survey. Their results showed 53% of five year olds had dental erosion on their primary maxillary incisors, and 33% of 12-15 year olds had dental erosion on their permanent maxillary incisors (Chadwick et al., 2006).

Like many other diseases, dental erosion is multifactorial in origin and can be attributed to a wide range of factors, which are extrinsic and intrinsic. Dietary factors are thought to be the main extrinsic cause of dental erosion and therefore have been studied extensively. The literature dates back as early as 1907, when WD Miller performed a study looking at the wasting of tooth tissue caused by erosion and abrasion and concluded

that all acids were capable of causing tooth erosion. Since then, numerous studies have been performed to understand the relationship between acidic foods and tooth erosion, including: epidemiological studies, clinical trials (in vitro and in vivo), and case studies.

Epidemiological studies have shown a clear relationship between consumption of acidic beverages and dental erosion. Jarvinen and colleagues (1991) found a strong relationship between patients with dental erosion and consuming citrus fruits 2x a day or more. For soft drinks, a correlation was found if they consumed more that one a day. Apple cider vinegar was two times stronger than soft drinks in its association with dental erosion, although the calculated population attributable risk (PAR) was small because of the small number of people who drank it. Another study, showed that 17% of children consuming fruit juice daily had dental erosion compared to only 8% of children who consumed juice less than once a day (Habib, 2013).

Many in vitro studies have also demonstrated the erosive properties of acidic food and beverages. Rytomaa and colleagues (1988) compared the differences in common acidic beverages by submersing bovine teeth for four hours during constant agitation. In vitro, this study showed that liquids with a pH below 4 caused erosion (cola, orange juice, sport drinks) while liquids with a pH above 4 had no marked erosion (carbonated mineral water, strawberry yogurt, coffee). Meurman and Murtoma (1986) evaluated the possible erosive properties of vitamin C by immersing bovine tooth specimens in 100ml of 8 different concentrations of vitamin C solutions for 100 hours. All vitamin C preparations caused distinct erosion. It was concluded that vitamin C, if left in direct contact with teeth, could be potentially erosive.

Many of the clinical evidences showing correlations between dental erosion and dietary intake of acidic foods are anecdotal. In 1947, Stafne and Lovestedt, observed a significant increase in dental erosion in 50 individuals drinking lemon juice mixed with water daily for therapeutic reasons. Some erosion was noted in just three months. Hicks, 1950, looked at numerous dental clinical cases over a 15-year period involving excessive citrus juice consumption and noted enamel destruction and connective tissue damage in the oral cavity.

An early clinical study by Thomas (1957) evaluated the effect of daily ingestion of different acidic beverages on the anterior teeth microscopically. Seventy participants were assigned into three groups and given three different acidic beverages, orange juice, grapefruit juice and carbonated cola. Each group was subdivided further and issued different amounts of their chosen beverages, 6, 12, 18 or 24 ounces. There were some changes in the enamel in all experimental groups. The earliest signs of microscopic change were noted between 4 and 6 weeks with the largest enamel changes seen in individuals consuming 24 ounces of soda and grapefruit juice. The study indicated that slight modifications in dental enamel topography is possible when consuming 12 ounces or more of soda or grapefruit juice daily for four or more weeks.

Moazzez and colleagues (2000) conducted a clinical study to investigate the relationship between dental erosion, oral pH and consumption of carbonated drinks. The study measured the oral pH during and after drinking a carbonated cola in adolescents with dental erosion compared to adolescents with no erosion. This was done using a small antimony pH electrode at 4 different locations in the mouth. The study also compared the reported drinking habits of both groups and dietary acid intake. All

subjects were healthy and had good oral hygiene practices. Dietary questionnaires were given to measure the amount of acidic beverages (soda and fruit juices) consumed per week. Saliva was collected one hour after any food and 15 minutes at rest to get an accurate representation of unstimulated saliva. A small probe was inserted into the mouth and held into place by a plastic appliance. Each participant was given a carbonated beverage. The oral pH was measured from the ingestion of the soda until the pH returned to normal. The results from the study indicated that although there were differences in pH between the two groups at different locations, that the drop in pH from consuming the carbonated beverage was quickly buffered within minutes in both groups. The questionnaire showed statistical significance between the two groups in the amount of soda, fruit juice and sport drinks consumed on a weekly basis. These findings suggest that the amount of acidic beverages play a larger role in erosion than physiological differences in saliva.

The mode by which the acid is consumed is another aspect to consider when looking at dental erosion. Unusual behaviors that increase the time the acid is in contact with the teeth can cause an increase in dental erosion. For instance, holding drinks in the mouth before swallowing or swishing with the drink increases the time the pH stays dropped in the mouth leading to more dental erosion (Zero & Lussi, 2006). Several studies have compared the differences between drinking acidic beverages with a straw versus a cup or bottle. The results differ depending where the straw is placed in the mouth. If it is placed directed towards the back of the mouth, the liquid bypasses contact with the anterior teeth and was seen as beneficial. However, when the straw is placed on the labial surface of the anterior teeth, it can be very destructive (Mackie & Blinkhorn,

1989). A study performed by Edwards and colleagues (1998) employed the use of vidoefluoroscopy to investigate these drinking behaviors. The study demonstrated that 90% of patients had fluid contact with their incisors when drinking through a cup, while only 29% of patients had contact when using a narrow straw positioned behind the anterior front teeth and 61% of patients had contact of liquid on incisors when a wide straw was used positioned behind anterior teeth.

It is also important to consider the intrinsic sources of acid on the oral cavity, namely gastric acid. Stomach acid is very acidic with a pH of 2.0, which is important for the breaking down and digesting of proteins and inhibiting the growth of many microorganisms (Marieb, 2001), but when the gastric acid leaves the confines of the stomach and moves up the esophagus and into the mouth the acid can de damaging. This destruction of enamel caused by stomach acid can be seen in cases of recurrent vomiting, such as in eating disorders (anorexia and bulimia) and gastroesophageal reflux disease, GERD (Jarvienen et al., 1990).

Several studies have looked at these associations. Pace and colleagues (2008) wrote a systematic review, which looked at GERD and dental lesions. They found a median prevalence of 32.5% of adult patients suffering from GERD had tooth erosion. Another paper published by Altshuler and team (1990) looked at the association between bulimia and erosion and found that dental erosion was a distinct characteristic of bulimia and that there was a linear correlation between length of the disease and intensity of dental erosion noted. Studies have found than even vomiting one time a week can increase dental erosion by 31 times compared to those who vomit less than once weekly (Jarvinen et al., 1991).

Traditionally, it has been understood that a low pH is a valid indicator of the erosive potential of food and drink. But measuring pH gives only a partial picture of the hydrogen ion concentration. Recent studies have shown that measuring the titratable acidity, which is the ability of the liquid to resist a change in pH, is a more realistic predictor of erosive potential (Edward et al., 1999). A recent study published by Andaló and colleagues (2015) compared the salivary pH of eight participants consuming cola, orange juice and a control sucrose solution in a cross over study. Cola had a lower pH than orange juice (2.5 vs. 3.5) but orange juice had a higher titratable acidity (3.17 to .57). The study showed that salivary pH in the participants returned to baseline in 30 seconds after consuming the cola but took 90 seconds after the orange juice. Therefore, it was concluded that a beverage's titratable acidity was a better predictor of dental erosion than a beverage's pH.

Also, interesting to note is the effects that diluting an acidic beverage has on both pH and titratable acidity. The act of diluting drinks may appear as safer. Cairns and collegaues (2002) looked at the effects on pH and titratable acidity of 4 different acidic drinks when they were diluted. The results showed that diluting the 4 different liquids had very little change on their pH. In fact, two of the drinks required a dilution of greater than 1:5,000 before a pH of 7 was reached, while the other two required a dilution of 1:10,000. At a pH of 1:100, all of the drinks appeared as water, but still had pH's that were below 5. On the contrary, dilution caused the titratable acidity of the beverages to fall considerably. It may be concluded that diluting a beverage may not affect the pH, but it can affect the titratable acidity causing a decrease in the erosive properties of the beverage.

Acidic beverages may vary in their degree to cause dental erosion even if they have the same internal pH. Depending on their degree of saturation of certain minerals, like calcium and phosphorus, the erosive properties can differ (Meurman et al., 1987). Certain food/beverages like orange juice supplemented with calcium and phosphate and yogurt showed little erosive effects on enamel after immersion in vivo, even though they had low pH (Larson and Nyvad, 1999).

Although counterintuitive, being overzealous when tooth brushing can lead to damage to the gums and tooth structures which is irreversible. The damage is more severe when the toothbrush used has medium or hard bristles and when the toothpaste used is highly abrasive. Also, the technique used contributes to tooth wear. The scrubbing "back and forth motion" coupled with heavy lateral force over time begins to mechanically wear away dental structures. This is known as toothbrush abrasion. Toothbrush abrasion is accelerated when combined with erosion. When the oral cavity experiences an acidic attack, whether intrinsic or extrinsic, it is more susceptible to abrasion. The initial demineralization of tooth enamel, caused by a drop in pH lower than 5.5, can be reversed and remineralized by saliva. Brushing teeth, however, immediately after an acid attack on the mouth removes the partially demineralized tooth enamel before saliva can repair it. This leads to irreversible loss of tooth structure (Zero, 1996). A study done by Jaeggi and Lussi (1999) looked at the amount of tooth structure lost brushing after an acidic attack compared to brushing without a drop in oral pH. They showed that brushing when the enamel was softened by an erosive liquid resulted in ten times more enamel loss than brushing without an erosive pre-treatment. It was shown by Attin and colleagues (2004), to minimize tooth loss at least 30 minutes should elapse

after an acidic attack before tooth brushing should occur. Acid neutralization prior to 30 minutes can be achieved by rinsing the mouth with water, using an acid-neutralizing solution, one part baking soda to eight parts water or by chewing sugar free gum, which causes in increase in stimulated salvia production (Wilkins, 1999). This increase in stimulated saliva clears the mouth of 95% of residual food debris, increases saliva's buffering capacity to bring pH in the mouth back to a normal state and aids in the remineralization of tooth enamel (Wefel & Hogan, 2003).

Another factor that can exacerbate the effects of erosion is attrition, defined as the physiological wearing away of dental hard tissues through tooth-to-tooth contact. The interaction between these two forces is a minimal concern compared to tooth brushing abrasion and erosion, but should still be considered for this paper. In vitro, enamel/enamel attrition was much greater when combined with the erosive liquid of HCL (pH 1.2) compared to that of water (Kaidonis et al; 1998). This erosive challenge would typically only be a concern in individuals who suffer from GERD or frequent vomiting episodes. When combined with a typical extrinsic source like soda or fruit juice, with higher pH readings, attrition is lower. Although no research could be found on this topic, the consideration of bruxism at night combined with GERD and a low salivary flow rate due to circadian rhythms would be a worthwhile inquiry.

Basic Erosive Wear Examination (BEWE):

There are a myriad of different indices used to measure the severity of dental erosion. In 2008, Bartlett, Ganss and Lussi designed an index called the Basic Erosive Wear Examination (BEWE) as a valid, repeatable and simple screening tool to detect and measure dental erosion that could be easily used by dental practitioners. Prior to the

development of BEWE, the indices used to measure erosion varied greatly in their assessment, choice of teeth and scale, as to make any comparison between the indices difficult. Also, the complexity of many of them made it difficult for implementation into a regular dental practice. The BEWE is a simple and easy to use screening tool that measures the presence and severity of dental erosion. It works by dividing the mouth into sextants. Each tooth is then examined by looking at three different surfaces (facial/buccal, lingual/palatal, occlusal) and assigned a number between 0 – 3 (see Appendix A). The tooth with the highest number in the sextant is recorded. After all sextants are assigned a number, the sum of scores is calculated and patients are classified in risk levels of: none, low, medium and high. Treatment is determined based on the risk level of the patient. A study done by Dixon and colleagues (2012) found that the BEWE is an effective screening tool for detecting severe tooth wear with a sensitivity of 90.0% and specificity of 91.5%, but its accuracy decreases with moderate tooth wear, sensitivity 48.6% and specificity of 96.1%. According to this study, detecting tooth wear with a screening tool like the BEWE is best utilized when detecting severe tooth loss, and its accuracy is positively correlated to tooth wear. These results, however, are similar to other commonly used screening tools for tooth wear like the Tooth Wear Index (TWI). This emphasizes the idea that the BEWE is not a poor screening tool, rather screening tools in general lack the capabilities to accurately predict minimal tooth wear.

CHAPTER 3

METHODS

Participants and Study Design

Recruitment and Enrollement

Before the start of the study IRB approval was obtained. Recruitment began January 2, 2018 and lasted until January 26, 2018. Several methods were employed for recruitment including: ASU Listservs to students, faculty and staff, flyers posted around the ASU Downtown Phoenix Campus, emails sent directly to presidents of ASU health organizations and clubs to distribute to their members, a Craigslist posting, and Facebook advertising. Prospective subjects were directed to a website where they completed an online questionnaire hosted by Survey Monkey. A total of 132 respondents completed the questionnaire of which 106 qualified for a pre-screen visit (visit 1) to further determine eligibility; the twenty-six respondents who were disqualified did not meet assorted inclusion parameters (see Appendix D for CONSORT chart, which includes specific selection criteria). Emails were sent out to all respondents to update them on their eligibility status (eligible or ineligible). Respondents who qualified were asked to schedule an initial visit (visit 1) for further pre-screening. Sixty-three of the 106 respondents scheduled a visit 1 appointment.

Participants

This study was a joint experiment, which partnered with another study looking at the effect that daily vinegar ingestion had on visceral fat reduction. It was a second round of a previously conducted study and replicated the first round's methods. The primary objective of the second round was to increase the number of observations. The

parameters of this study, including participant inclusion and exclusion criteria were therefore determined by the visceral fat study. Participants were included in the study if they were between the ages of 18 to 45 years, healthy, operationalized here as free from untreated medical conditions, and not taking any medication that could influence body weight. Because of the parallel study's focus on visceral body fat, female subjects were selected with a waist circumference greater than or equal to 33 inches and male subjects were selected with a waist circumference greater than or equal to 38 inches. Women were excluded if they were pregnant or planning to become pregnant in the next three months. Participants were excluded if they had any recent abdominal surgeries or any condition that could cause abdominal distention. Participants were willing to adhere to treatments assigned, a vinegar pill or vinegar drink, two times per day for eight weeks. Participants had either an iPhone or Android device so they could download the pH20H application to keep track of their resting salivary pH daily at home for two weeks, specifically week 0 and week 8 on the study. Also, subjects were willing to travel to Arizona Biomedical Collaborative Building in downtown Phoenix (425 N. 5th Street, Phoenix, AZ 85004) on three separate occasions, for a total of three to four hours.

Thirty-four of the sixty-three subjects completing visit 1 met all the inclusion criteria and were enrolled into the study. The other twenty-nine participants were eliminated at the pre-screen appointment (see CONSORT chart for specifics, Appendix D). The thirty-four eligible participants were then stratified by gender, age, height, weight, BMI, and waist circumference and randomized into two groups, intervention (vinegar drink, n=17) and control (vinegar pill, n=17). Six participants withdrew from the trial between visit 1 and visit 2; three participants from the VIN group and three from the

CON group due to: work conflicts (n=3), being opposed to blood draw (n=2), and health issues unrelated to trial (n=1). Twenty-eight participants returned for visit 2 and began trial (VIN = 13, CON = 15). By the end of eight weeks, twenty-two participants finished. A total of six more participants dropped out during the course of eight weeks; three dropouts were in the intervention group and three in the control group. The reasons for dropouts included: health issues unrelated to trial (2), and adverse reaction to vinegar, specifically stomach issues (1). Also, three participants did not show to their scheduled visit 3 appointments. Numerous emails were sent to reschedule their final visit, but these participants were lost to attrition.

Study Design and Procedure

This study was an 8-week, parallel arm, randomized, double-blinded, clinical trial. Subjects were stratified by gender, age and weight and randomly assigned into two groups: Group 1 (control group – vinegar pill) or Group 2 (experimental group – vinegar drink). Those in the control group were instructed to take the provided vinegar pills (one pill, two times per day immediately before a meal) for eight weeks (week 1-8). Those in the experimental group were instructed to make a vinegar drink by diluting two tablespoons of red wine vinegar (provided) with eight ounces of water; they were instructed to drink the liquid two times per day immediately before a meal for eight weeks (week 1-8).

Participants were told not to change current eating and exercising habits during the study. Resting salivary pH was measured every morning for an entire week using pH strips and was recorded using the pH20H app. This was done prior to the start of the study, week 0 and the last week of the study, week 8. Diet recalls were given out at week

1 and week 8 to look for any changes in diet. A survey was given at week 1 and again at week 8 that looked for moderating and mediating factors that could contribute to dental erosion and a drop in salivary pH (discussed in detail later). Anthropometric measurements (height, weight, and waist circumference), fasting blood draws, and DXA scan were performed at week 1 and week 8 for the visceral fat study. Also, a Basic Erosive Wear Examination (BEWE) was done at week 1 and at the end of week 8.

Study Variables

The independent variable in this study was vinegar, specifically acetic acid. Participants were given two different forms of vinegar. The control group received vinegar pills (Apple Cider Vinegar Tablets, NowFoods, Bloomingdale, IL). They were instructed to take one pill, two times per day before a meal for the length of the study. The amount of acetic acid in two pills was very low, 45 mg, therefore it was considered the control group. The experimental group received a bottle of red wine vinegar (Mantova Red Wine Vinegar, Mantova, Broccostella, Italy). They were instructed to mix two Tablespoons of vinegar with eight ounces of water and drink two times per day, before a meal, for the length of the study. They consumed a total of 3.6 grams of vinegar daily. The two dependent variables in this study were resting salivary pH and dental erosion. It was hypothesized that there would be no difference in resting salivary pH or dental erosion between the control and experimental groups.

Protocol Procedures

Prescreening (Visit 1):

Participants were prescreened prior to study to determine eligibility using anthropometric measurements and medical history questionnaires. Participants were

given further information about the study, including length of trial, data collection methods and possible risks and benefits. Also, participants were given instructions (verbally and written) on how to take their resting, unstimulated salivary pH in the morning prior to food and drink. They were given enough pH strips for daily use for two weeks. The pH20H application was downloaded onto their mobile device to track oral pH during the study. A consent form was obtained from participants.

Week 0, before start of trial

Participants collected resting, unstimulated salivary pH every morning for one week. The collection occurred upon waking before food or drink using pH strips; Participants were instructed to swallow two times before expectorating saliva into a spoon. The pH strip was then dipped into the saliva to reveal the saliva's pH. A picture of the pH strip was taken and assigned a pH value using the pH2OH app.

Start of Study (week 1-8)

At the beginning of the visit, participants filled out a diet recall. Since exclusion and inclusion criteria for study was determined by the visceral fat and vinegar study, a survey was given to look at moderating factors (not controlled for in participant recruitment) that could influence dental erosion and saliva pH (Appendix C). The survey was designed to measure the following factors: the earliest signs/symptoms of dental erosion, acidic beverage practices, gastro esophageal reflux disease (GERD), abrasion and attrition risk factors, risk factors for hyposalivation including medication usage, dehydration and perception of xerostomia as a valid indicator of decreased salvia production. In order to compare the survey scores between the two groups, all data contained in the survey was given a numerical value. Signs, symptoms and behaviors that

are correlated to dental erosion were given progressively larger numerical values as the correlation increased (see Appendix E-I for numerical value assignments).

Anthropometric measurements (height, weight and waist circumference) were obtained and fasting blood draws were taken by a phlebotomist or nurse. A certified radiologic technician performed a DXA scan. All women participating in this study needed to test negative on a urinary pregnancy test before the DXA was administered. Anthropometric measurements, fasting blood draw and DXA scan were measurements taken for the visceral fat study. Also, a dental erosion screening (BEWE) was performed by a registered dental hygienist and any erosion was noted. Participants were then randomized into two groups, control group or experimental group and given an eight-week supply of vinegar. Instructions were given regarding proper consumption of vinegar and participants were told to adhere to treatment for entire eight weeks. A compliance calendar was sent home to keep track of days they took their supplement.

Each week during the study, protocol reminders were sent to the participants via email. Also, questions were answered if needed. At the beginning of week 8, an email was sent to remind the participants to start collecting their unstimulated, resting salivary pH every morning for the entirety of week 8. The procedures were similar to week 0.

At the end of week 8, participants returned for last visit. Anthropometric measurements, DXA scan, fasting blood glucose, and BEWE were repeated. A second diet recall was completed and participants turned in their compliance calendar. The survey was re-administered to look at any changes in early erosion signs and symptoms during the course of the trial and to see if other behaviors that could contribute to dental

erosion remained static (see Appendix B). Participants were compensated for their time with a $10 Amazon gift card at the completion of the trial.

Laboratory Analysis

Participants were screened for dental erosion by a registered dental hygienist using the Basic Erosive Wear Examination or BEWE (see Appendix A), which was established by Bartlett and colleagues (2008) as a simple, uniform, and repeatable way to measure and compare dental erosion in patients. The screening tool was conducted by dividing a participant's mouth into sextants. Each tooth in the sextant was examined and given a number, but only the highest number in each sextant was recorded. The recorded numbers from each sextant was then calculated as a summative score to give one total erosive score. Baseline erosion scores were compared to erosion scores at week 8. Salivary pH was determined immediately upon waking in the morning before participants had food or drinks using pH strips, and assigned a value according to the pH2OH application. Although not as accurate as a pH meter, pH strips were chosen because they could be used at home and therefore a daily pH could be established for week 0 and week 8. This allowed a more accurate representation of any changes in the participant's oral pH seen during treatment, instead of measurements taken with a pH meter at visit 2 and visit 3 of study only.

Statistical Analyses

This study included both laboratory and questionnaire based data. Accordingly, the proposed statistical strategy for testing hypotheses began by leveraging the power of random assignment in the laboratory data, specifically relying on comparisons of mean salivary pH and enamel erosion levels across randomly assigned treatment groups. The

Statistical Package for Social Sciences version 25 (SPSS) was used to calculate means and standard deviations between the two groups (experimental versus control). Mann Whitney nonparametric tests were used on all data that was non-normally distributed or comprised of ordinal data. Independent t-tests were employed when data was composed of interval or ratio data and normally distributed. Significance was set at $p \leq 0.05$.

CHAPTER 4

RESULTS

Descriptive statistics were performed to determine mean and standard deviation for baseline characteristics in both groups. Participants in the study ranged in age from 18-41 years. For the VIN group, participants had a mean age of 28.9±9.1 years and the CON group had a mean age of 31.6±6.9 years. Participants ranged in height from 154.8-184.5 cm. The VIN group having an average height of 166.7±8.0 cm and the CON group averaging 169.7±8.0 cm. Weight in both groups was between 56.6 – 104.4 kg, with a mean weight in the VIN group of 76.9±13.6 kg and a mean weight of 83.9±13.5 kg in the CON group. BMI ranged between both groups from 22.1-38.5 kg/m^2. The average BMI was 27.7±4.5 kg/m^2 in the VIN group and 29.1±3.8 kg/m^2 in the CON group. Lastly, the waist circumference measurements ranged from 31.8 – 45.7 inches between the two groups, with a mean waist circumference of 37.5±4.1 inches in the VIN group and 38.3±3.6 inches in the CON group. Normality tests (Shapiro-Wilks) were run on all variables to determine distribution. All variables were normally distributed except for age in the VIN group; transformation of the data using the inverse, log and square root were insufficient in achieving normal distribution. Therefore, to analyze the mean between the two groups (VIN vs. CON) a Mann-Whitney test was used. Since the BEWE was comprised of ordinal data, a Mann-Whitney test was also used. All other data was compared using independent t-tests. There were no significant differences in any of the baseline characteristics between the groups ($\alpha = 0.05$). A chi-squared test was used to analyze gender; the assumptions were violated due to 50% of cells having less than an expected count of 5, so Fisher's Exact test was used instead to obtain a p-value (Table 5).

Table 5: Baseline Characteristics by Group

	VIN (n=14)	CON (n=14)	p-value
Gender (M/F)	2/12	3/11	1.0 [b]
Age (years)	28.9±9.1	31.6±6.9	0.461[a]
Height (cm)	166.7±8.0	169.7±8.0	0.336
Weight (kg)	76.9±13.6	83.9±13.5	0.178
BMI (kg/m^2)	27.7±4.5	29.1±3.8	0.377
Waist circumference (in)	37.5±4.1	38.3±3.6	0.556
pH readings	7.4±0.2[c]	7.5±0.2 [c]	0.358
BEWE* score	4.4±2.5	4.4±3.8	0.642 [a]

[a]Mann-Whitney test ran instead of Independent t-test due to non-normally distributed data OR ordinal data
[b]Chi square assumptions not met, therefore used Fisher's Exact test to obtain p-value
[c]Excluded two participants from saliva pH analysis due to data collection error, VIN = 13 and outlier, CON= 13
*Basic Erosive Wear Examination

After the start of the trial, it became known that one participant in the VIN group was collecting their pH readings incorrectly. The pH readings for that participant, therefore, were completely removed from any statistical analysis. Also, another participant in the CON group was removed from the pH readings; the participant's mean pH reading fell outside three standard deviations from the groups' mean, and was considered an outlier. The two participants were included in all other baseline analyses performed.

A Mann-Whitney nonparametric test was utilized to determine the effect that the assigned treatment had on pH between the two groups, control versus treatment. The test was chosen over a parametric test because the assumption of normal distribution of data was violated. Also, both the BEWE screener and the sensitivity erosion questions embedded in the survey (see Appendix E for questions) administered to the participants were made up of ordinal data and the results were non-normally distributed, so Mann-Whitney tests were used to compare means between the two groups. The results showed that when alpha was set at 0.05, there was no statistically significant mean difference between the two groups in pH Δ, post pH score – pre pH score, (p-value 0.499) and the

sensitivity erosion score Δ, post sensitivity score – pre sensitivity score (p-value 0.358). It is interesting to note, that although there was no statistically significant mean difference in sensitivity erosion Δ between the two groups, that the vinegar group did have an increase in their sensitivity score of 0.70±2.9 while the control group had no change. There was a statistically significant mean difference in the BEWE Δ score between the two groups (p-value 0.05). When a parametric univariate analysis was performed controlling for both compliance and age the mean difference in BEWE Δ scores between the two groups became more statistically significant (p-value = 0.03); this method was not chosen, however, due to test assumptions not being met (see Table 6).

The parameters for participant recruitment, including the number of participants needed to power the study, were set forth by the vinegar and visceral fat reduction study that this study partnered with. A reverse calculation was run after the trial ended to determine if the number of participants recruited was enough to power this individual study (the effects of vinegar on dental erosion and salivary pH). Using the Harvard sample size calculator, it was determined that with 22 participants at a 0.05 significance level with 1.05 difference in means between the two experimental groups (difference in BEWE Δ) there was a 64% probability that the study would detect a difference between treatments. In order to power the study at 80%, 32 participants were needed (see Appendix K).

The mean difference in patient compliance between the two groups was calculated using Mann Whitney nonparametric test, since both treatment groups had non-normally distributed data. There was no statistically significant difference between the

means of the two groups (p value 0.690). The mean patient compliance of the vinegar group was 0.84±0.18 and 0.90±0.10 in the pill group.

Table 6: Changes in Erosion and pH Values (week 1-8)

		VIN (n=10)	CON (n=12)	p-value	Effect Size
pH	Pre	7.468±0.2	7.516±0.2		
	Post	7.418±0.3	7.489±0.2		
	Post pH – Pre pH (Δ)	-0.050±0.2[b]	0.081±0.4[b]	0.499[a]	0.41
BEWE*	Pre	4.43±2.5	4.36±3.8		
	Post	5.20±3.1	3.25±1.8		
	BEWE post – BEWE pre (Δ)	0.80±0.9	-0.25±1.1	0.051[a]	1.04
Sensitivity/ Erosion Survey score	Pre	2.00±2.6	1.83±2.6		
	Post	2.70±2.8	1.83±2.6		
	Post Sen. Score – Pre Sen. Score (Δ)	0.70±2.9	0.000±0.6	0.358[a]	0.33

[a] Indicates not normally distributed OR ordinal data; Mann-Whitney test ran instead of Independent t-test
[b] Excluded two participants from saliva pH analysis. One due to data collection error, VIN = 9; one due to being an outlier, CON = 11
* Basic Erosive Wear Examination

The survey was divided into the following subcategories: Total acidic beverage consumption (total beverage frequency x total beverage consumption), Erosive beverage total (total acidic beverage consumption + beverage drink total + beverage habit total), GERD risk factors, Attrition risk factors, Abrasion risk factors, and Hyposalivation risk factors (dehydration total + medication total + xerostomia total). Each subcategory was then computed as a summative score, except for the Total acidic beverage consumption, which was calculated as a multiplicative function between the average amount of beverages consumed in a week by the typical amount consumed each time. This gave a

gross estimate of the total amount of fluid ounces of acidic beverages consumed weekly. Also, the total summative score of all moderating factors was calculated and recorded as the Total survey score (Table 7).

Mann Whitney tests were run on all moderating factor subcategories, pre, post and change (Δ) to compare the mean difference between the two treatment groups. No statistically significant differences were seen between either treatment groups at baseline among all moderating factors. It is important to note, therefore, that neither group had a greater risk at baseline for dental erosion due to any of the moderating factors being measured. Also, there was no significant mean difference in any of the Δ moderating factor scores between the two treatment groups. Any changes in moderating factors during the course of treatment between both groups were comparable to each other.

Each moderating factor score throughout the course of eight weeks, changed minimally. For example, the Total survey score, a summative score of all the moderating factors, changed only 0.2 points in the vinegar group and -0.5 in the control group; this is an indicator that the moderating factors between both groups held stationary and therefore any changes in erosion seen in the participants over the course of 8 weeks could be attributed to their course of treatment rather than another risk factor.

Table 7: Survey Score Comparison Between Groups

Mediating factors:		**VIN (n=10)**	**CON (n=12)**	**p-value**
Total acidic beverage consumption (Beverage Frequency X Beverage Consumption)	Pre	49.4±43.4	70.1±50.2	0.322
	Post	45±30.9	62.5±42.6	0.391
	Change (Δ)	-4.4±20.5	-7.6±36.7	0.138
Erosive Beverage Total (Total acidic beverage consumption+beverage drink+beverage habit)	Pre	57.3±44.6	76.5±52.6	0.373
	Post	52.6±32.2	69.1±43.9	0.448
	Change (Δ)	-4.7±21.1	-7.7±38.5	0.138
GERD risk factors	Pre	1.0±1.6	1.3±2.8	0.771
	Post	1.0±1.5	1.25±2.0	0.800
	Change (Δ)	0.0±0.5	0.08±1.3	0.749
Attrition risk factors	Pre	0.6±1.3	0.83±1.2	0.280
	Post	0.5±1.1	0.75±1.2	0.400
	Change (Δ)	-0.1±0.3	-0.08±0.3	0.890
Abrasion risk factors	Pre	7.0±0.9	6.6±1.6	0.610
	Post	7.3±0.9	6.7±1.3	0.260
	Change (Δ)	-0.3±1.3	-0.08±0.9	0.830
Hyposalivation risk factors (Total medication usage +dehydration factor + total xerostomia factors)	Pre	4.1±1.9	3.3±2.0	0.260
	Post	3.6±1.2	3.7±2.9	0.810
	Change (Δ)	-0.5±2.0	0.4±1.7	0.210
Total survey score	Pre	35.8±8.0	36.4±10.6	0.920
	Post	36.0±6.7	35.9±11.6	0.790
	Change (Δ)	0.2±6.5	-0.5±7.1	0.350

CHAPTER 5

DISCUSSION

The aim of this study was two fold: first to examine if daily ingestion of red wine vinegar caused a change in resting, unstimulated salivary pH when consumed for eight weeks and second to determine if subjects consuming vinegar daily for eight weeks were at an increase risk for dental erosion.

Prior research has shown that daily consumption of vinegar caused a significant reduction in urinary pH after 12 weeks compared to a control group (Johnston, et al., 2008). Conversely, one published report suggests that acetic acid has an alkalinizing effect in the gut mucosa mediated by attenuation of endogenous prostaglandins (Nobuhara, Takeuchi & Okabe, 1986). There have been other studies that have examined the short-term implications on saliva pH immediately after consuming vinegar and the typical response time it takes the saliva to return to a normal resting state. However, no research has been done yet to explore the relationship between resting saliva pH and regular consumption of vinegar. Does regular vinegar consumption have a more acidic effect on saliva like it can with urine or does it have a more alkalinizing effect as seen in the gut mucosa? The results of this study indicate that daily ingestion of red wine vinegar for eight weeks had no statistically significant effect on resting saliva pH. The mean difference after eight weeks in pH change (Δ) in the VIN group was -0.050 and the CON group +0.080 with no significant mean difference between them (p=value, 0.499).

These results are not surprising. The regulation of pH throughout the body is essential to human life, and physiological buffers operate throughout the body to keep pH within narrow limits. Hence, the body can withstand exposure to acids and bases. The

bicarbonate buffering system is primarily responsible for preventing changes in saliva pH outside its normal limits. This buffering system allows humans to consume a vast array of different substances with varying pH while maintaining the saliva pH between 6.2-7.6 (Baliga, et al., 2013). The results of this study indicated that the pH in both treatment groups remained very stable, around 7.4, at the start and end of the trial.

Numerous studies have examined the relationship between frequent exposures to acidic substances, both internally and externally, and dental erosion. Epidemiological studies have shown a positive correlation between the frequency of acidic beverage consumption or vomiting and dental erosion (Jarvinen, et al., 1991; Altshuler, et al., 1990). These studies only showed associations and lacked the capabilities to show causality. Other in vitro studies, have demonstrated that teeth left in acidic beverages even for a relatively short time exhibit erosion (Rytomaa, et al., 1988); of course, these studies lack the capabilities to account for the bodies natural abilities to buffer when presented with an acidic beverage. One in vivo clinical study done in 1957 by Thomas did try to show causality between frequent acidic beverage ingestion and dental enamel erosion. The study showed that microscopic changes in dental enamel were evident after 4 weeks when consuming at least 12 ounces of soda or grapefruit juice per day (Zero, 1996).

This clinical trial built on these previous studies. After eight weeks of treatment, either a vinegar drink (intervention) or vinegar pill (control), there was a statistical significant mean difference in dental erosion between the two groups ($p= 0.051$). When age and compliance were controlled for the significance was stronger ($p= 0.026$). The results are disquieting given the short duration of the study and taking into account that

erosion is typically a gradual and slow process. However, it is important to note that in a previous study conducted by Jarivnen and colleagues (1991), vinegar ingestion was two times more likely than soft drinks to result in dental erosion. Thus, the amount of erosion change seen in only eight weeks warrants further investigation given the current popularity of the medicinal use of vinegar.

There are some important considerations that need to be addressed in regards to this study. First, the course of treatment was a vinegar drink, which was two tablespoons of red wine vinegar diluted in eight ounces of water. Cairns and colleagues (2002) showed that diluting a beverage does not affect the pH of the beverage, except by extremely large dilutions in the range of 1:10,000, but it does affect the titratable acidity of the beverage. The titratable acidity, according to a study done by Andaló and team (2015) is a better predictor of dental erosion than measuring a substance's pH. The vinegar beverage consumed by the participants during the entirety of this trial had a lower titratable acidity due to the dilution of the liquid making it easier for the body to buffer the liquid and return the saliva pH back to a normal limit quicker. The typical consumption of red wine vinegar is often not in this diluted form, but straight. Therefore, the erosive potential of the non-diluted vinegar mode of delivery could be far greater than the results seen in this clinical trial.

Second, the participants in this trial were instructed to drink the vinegar drink with a meal. The erosive characteristics of acids can be influenced by the degree of saturation of certain minerals like calcium and phosphorus (Meurman, et al., 1987). Because the vinegar treatment was mixed with a meal, the degree of minerals present would naturally increase, which could attenuate the erosive properties of vinegar. This is

important to note, since many people consume vinegar for medicinal purposes without pairing with food, thus potentially making them more prone to dental erosion.

Thirdly, the population sample chosen to participate in this clinical trail was not representative of those likely to be taking vinegar regularly. Participants in this study were healthy adults, operationalized as free of medical conditions and not taking any medication. In general, those taking vinegar daily for medicinal reasons would be using it to treat some health ailment or disease. They would, therefore, be inclined to take one or more medications. Over 400 medications have xerostomia as a side effect, and a decrease in saliva production is the number one risk factor for dental erosion. These facts placed together indicate that a population sample taking vinegar to treat a disease could experience more devastating effects caused by the erosive properties of vinegar than those chosen for this trial.

Fourthly, the parameters established for participant recruitment were set forth by the vinegar and visceral fat study including the number of participants needed to power the study. With only 22 participants finishing the trial, this study was only powered at 64%. To fully power this study at 80%, 32 participants were needed. This is an important fact to consider. Perhaps, statistically significant results would have been achieved in the sensitivity survey score if more participants were involved in the trial. With only 22 participants the sensitivity score did increase in the vinegar group but not at a statistically significant level.

This study tried to isolate the effects of the independent variable (vinegar) on the dependent variables (saliva pH and dental erosion) by developing a survey to measure moderating factors that could contribute to dental erosion and saliva pH changes. The

aim of the survey, which was not validated, was to measure different risk factors for dental erosion including the number and amount of acidic beverages typically consumed in a week, presence of acid reflux, medication use, dehydration and other behaviors that contribute to tooth wear. The results between the two groups showed there was no mean difference in any of these risk factor subcategories. Thus, these other risk factors were likely not significant contributors to the erosion seen. There were a copious amount of studies (Ship & Fischer, 1997; Dawes, 1997; Wolff et al., 2008; Fox, et al., 1987; Jarvinen et al., 1991; Zero & Lussi, 2006) that verified that all areas being measured in the survey were in fact risk factors for dental erosion, but very few studies provided questions that were validated for measuring the specific risk factors. Therefore, despite the logical approach taken, the survey may be incapable of measuring other risk factors that could have contributed to the increase in dental erosion in the vinegar group.

Other limiting factors that could skew the results of this clinical trial include the short duration of the trial for measuring dental erosion. As mentioned above, a study done by Thomas in 1957 showed that microscopic changes were noted in the dental enamel of participants after 4 weeks of consuming an acidic beverage (Zero, 1996); no studies to date measure the typical time it takes to see macroscopic clinical changes in dental enamel. Although statistically significant results were seen after eight weeks, a longer study would provide a more obvious picture of the potential erosive properties of vinegar on teeth at the macro level. Secondly, the measurement tool for erosion, BEWE, was arguably subjective and not as accurate at determining mild to moderate tooth wear compared to severe tooth wear (Dixon, et al., 2012). Although the same registered dental hygienist administered the screening to all patients, the hygienist had not been

trained specifically in the BEWE test. A thorough training would be advisable. Also, a second or third hygienist performing the same test on all participants and averaging the scores between the raters would help reduce error associated with the assignment of subjective numerical values. However, this trial did employ the power of random assignment to treatment groups and blinding of both the participants and evaluator, which does add strength to the statistically significant difference in erosion seen between the two groups. Thirdly, the tool used to measure pH, pH2OH application, was not a validated tool for measuring saliva pH. It was chosen over a pH meter, because the application could be used at home, allowing for a collection of pH for one week before the trial and one week at the end of the trial. The pH meter, although more accurate, would have provided only two data points to compare: one at the beginning of the trial and one at the end. Lastly, compliance of all participants to treatment protocol could not be controlled. A compliance calendar was sent home with each participant and his or her compliance was calculated at the end of the trial. The use of a compliance calendar relies entirely on the participants reporting and therefore the results could be inaccurate.

Taking into account the previous studies that demonstrate that vinegar has many health promoting properties (Kondo, et al., 2001; Na, et al., 2015, Johnston, et al., 2004; Ostman, et al., 2005) and the current results seen in this clinical trial, advising people to quit vinegar consumption taken for medicinal reasons would not be advised. However, the following five recommendations are given to mitigate the erosive potential of vinegar.

1. Drink vinegar diluted with water (Cairns, et al., 2002; Andaló, et al., 2015)
2. Drink vinegar with a meal (Meurman, et al.1987).

3. Drink vinegar through a straw positioned behind the front teeth to decrease the amount of acidic liquid hitting the anterior teeth (Edward, et al., 1998).

4. Do not brush your teeth for 60 minutes after consuming vinegar (Jaeggi & Lussi, 1999).

5. Chew sugar free gum for 20 minutes after consuming vinegar to stimulate the buffering power of stimulated saliva and clear residual vinegar from the mouth (Wefel & Hogan, 2003).

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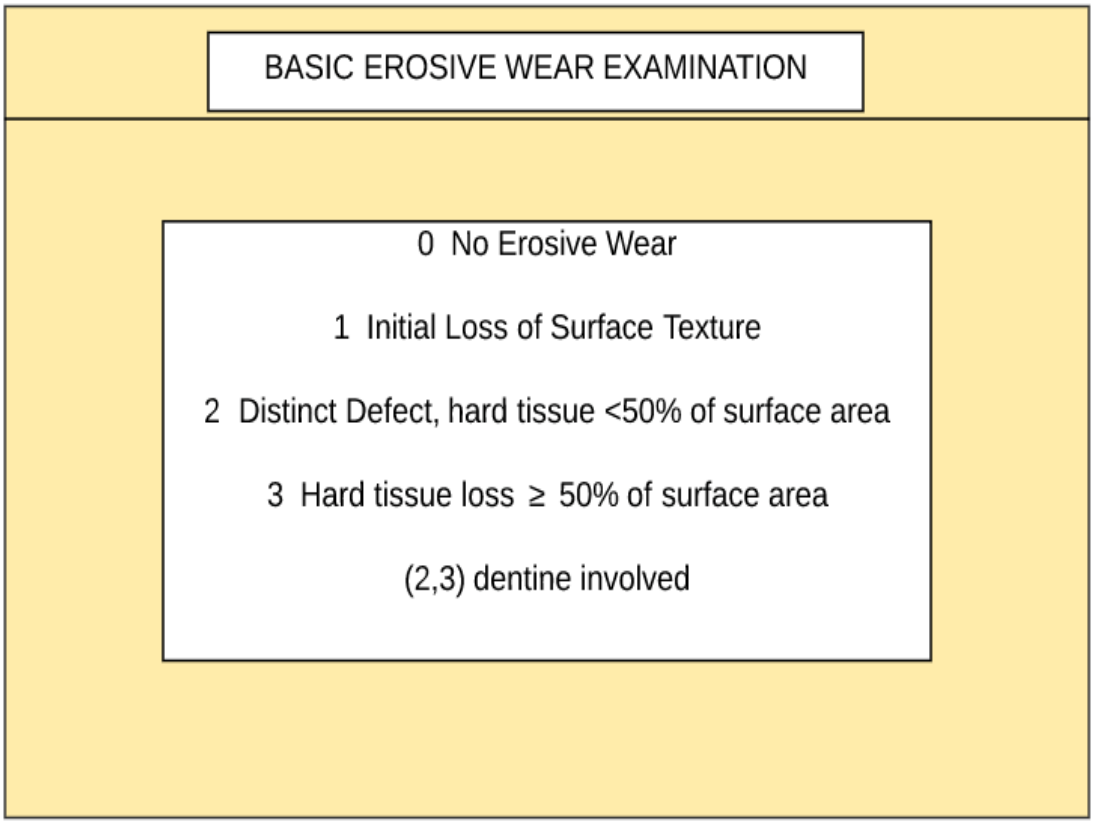
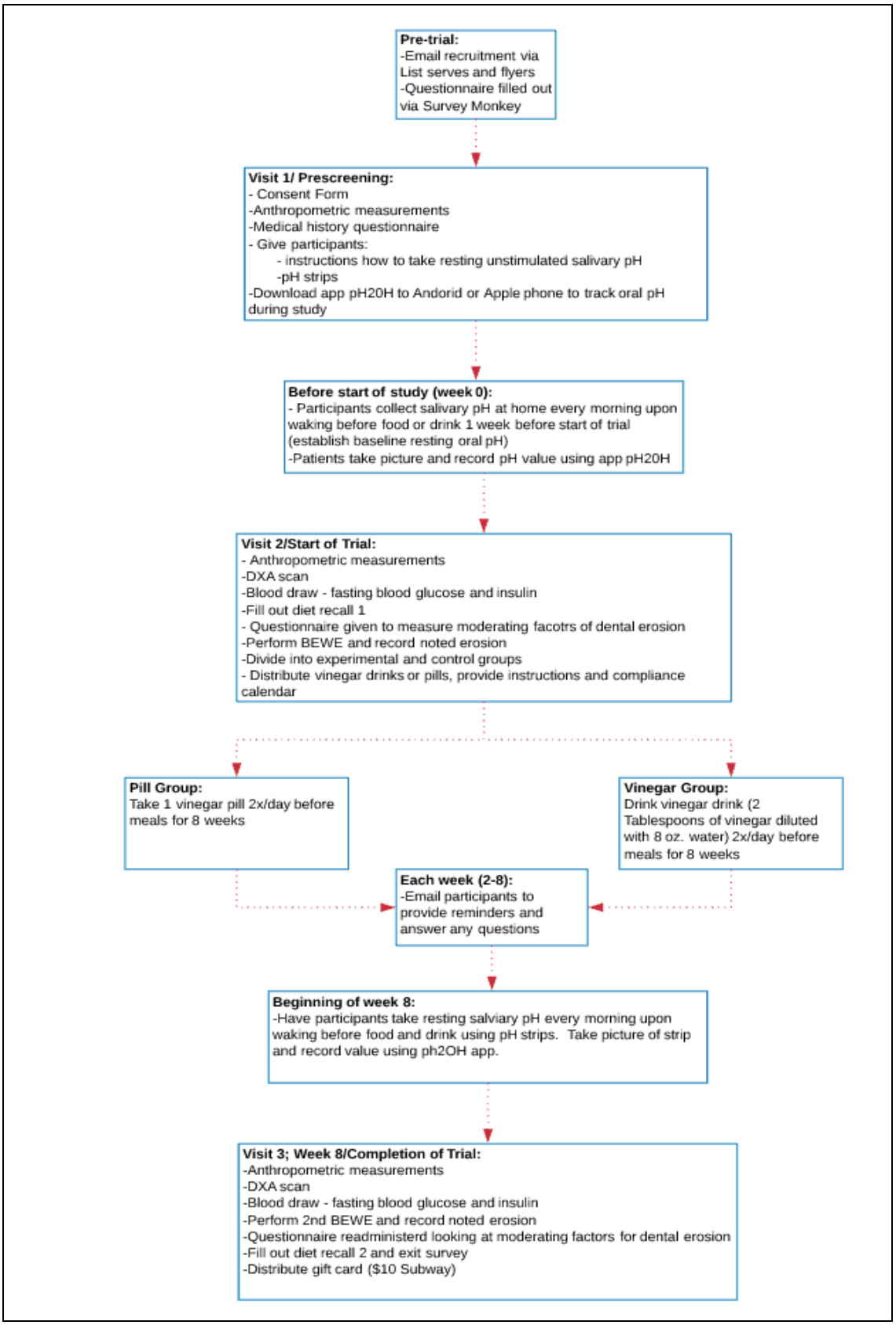
APPENDIX A

BASIC EROSIVE WEAR EXAMINATION (BEWE)


BASIC EROSIVE WEAR EXAMINATION
0 No Erosive Wear
1 Initial Loss of Surface Texture
2 Distinct Defect, hard tissue <50% of surface area
3 Hard tissue loss ≥ 50% of surface area
(2,3) dentine involved

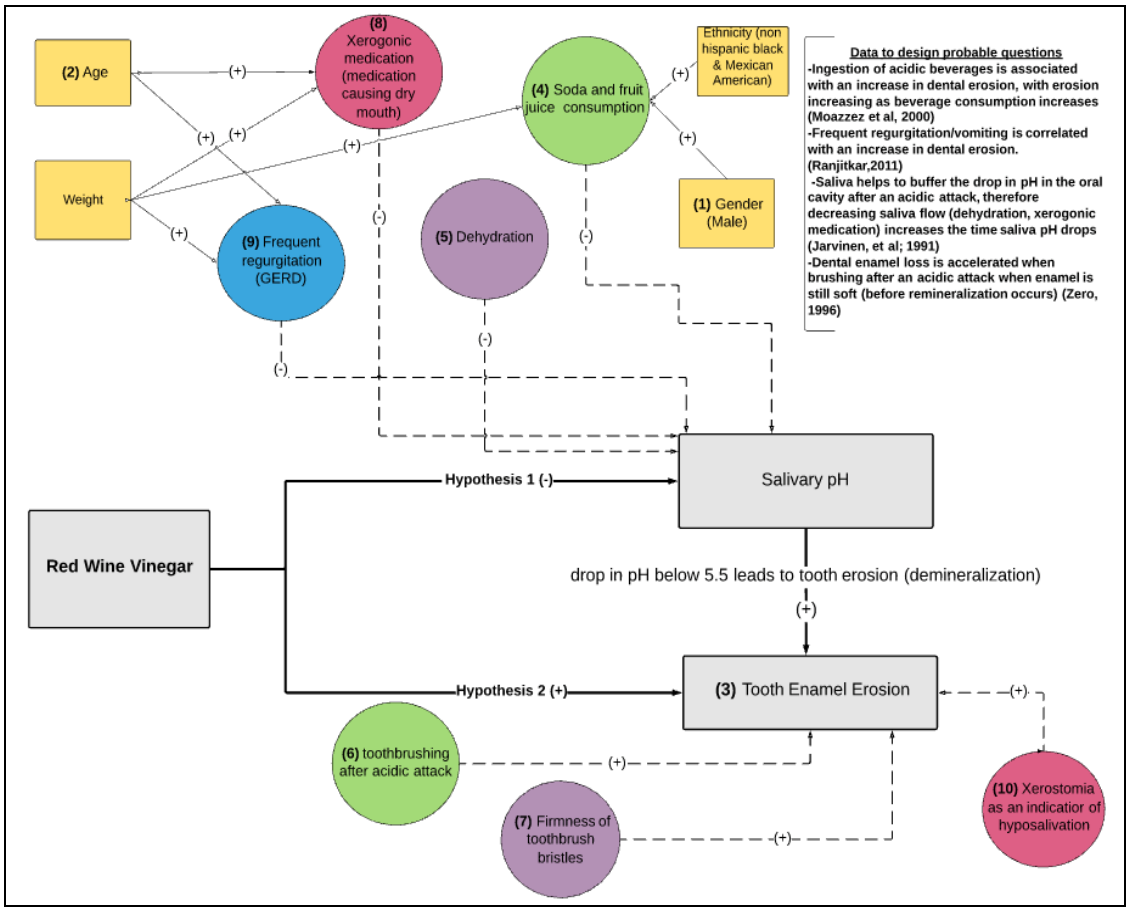
APPENDIX B

STUDY DESIGN FLOW CHART


Pre-trial:
-Email recruitment via List serves and flyers
-Questionnaire filled out via Survey Monkey
Visit 1/ Prescreening:
- Consent Form
-Anthropometric measurements
-Medical history questionnaire
- Give participants:
- instructions how to take resting unstimulated salivary pH
-pH strips
-Download app pH20H to Andorid or Apple phone to track oral pH during study
Before start of study (week 0):
- Participants collect salivary pH at home every morning upon waking before food or drink 1 week before start of trial (establish baseline resting oral pH)
-Patients take picture and record pH value using app pH20H
Visit 2/Start of Trial:
- Anthropometric measurements
-DXA scan
-Blood draw - fasting blood glucose and insulin
-Fill out diet recall 1
- Questionnaire given to measure moderating facotrs of dental erosion
-Perform BEWE and record noted erosion
-Divide into experimental and control groups
- Distribute vinegar drinks or pills, provide instructions and compliance calendar
Pill Group:
Take 1 vinegar pill 2x/day before meals for 8 weeks
Vinegar Group:
Drink vinegar drink (2 Tablespoons of vinegar diluted with 8 oz. water) 2x/day before meals for 8 weeks
Each week (2-8):
-Email participants to provide reminders and answer any questions
Beginning of week 8:
-Have participants take resting salviary pH every morning upon waking before food and drink using pH strips. Take picture of strip and record value using ph2OH app.
Visit 3; Week 8/Completion of Trial:
-Anthropometric measurements
-DXA scan
-Blood draw - fasting blood glucose and insulin
-Perform 2nd BEWE and record noted erosion
-Questionnaire readministerd looking at moderating factors for dental erosion
-Fill out diet recall 2 and exit survey
-Distribute gift card ($10 Subway)

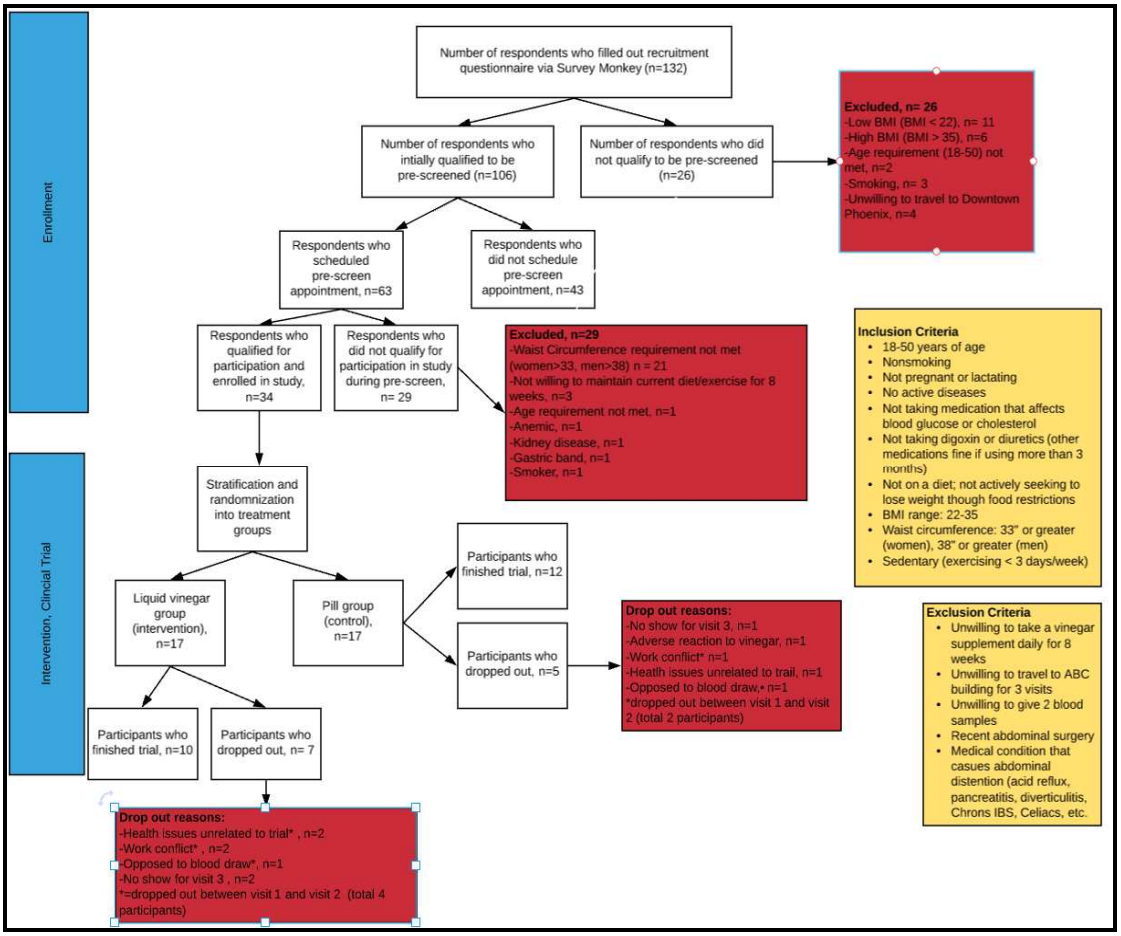
APPENDIX C

CONCEPTUAL FRAMEWORK FOR SURVEY DESIGN


(8) Xerogonic medication (medication causing dry mouth)
(2) Age
(4) Soda and fruit juice consumption
Ethnicity (non hispanic black & Mexican American)
Weight
(1) Gender (Male)
(9) Frequent regurgitation (GERD)
(5) Dehydration
Data to design probable questions
-Ingestion of acidic beverages is associated with an increase in dental erosion, with erosion increasing as beverage consumption increases (Moazzez et al, 2000)
-Frequent regurgitation/vomiting is correlated with an increase in dental erosion. (Ranjitkar,2011)
-Saliva helps to buffer the drop in pH in the oral cavity after an acidic attack, therefore decreasing saliva flow (dehydration, xerogonic medication) increases the time saliva pH drops (Jarvinen, et al; 1991)
-Dental enamel loss is accelerated when brushing after an acidic attack when enamel is still soft (before remineralization occurs) (Zero, 1996)
Salivary pH
Hypothesis 1 (-)
Red Wine Vinegar
drop in pH below 5.5 leads to tooth erosion (demineralization)
Hypothesis 2 (+)
(3) Tooth Enamel Erosion
(6) toothbrushing after acidic attack
(7) Firmness of toothbrush bristles
(10) Xerostomia as an indicatior of hyposalivation

APPENDIX D

CONSORT CHART


Enrollment
Intervention, Clincial Trial
Number of respondents who filled out recruitment questionnaire via Survey Monkey (n=132)
Number of respondents who intially qualified to be pre-screened (n=106)
Number of respondents who did not qualify to be pre-screened (n=26)
Excluded, n= 26
-Low BMI (BMI < 22), n= 11
-High BMI (BMI > 35), n=6
-Age requirement (18-50) not met, n=2
-Smoking, n= 3
-Unwilling to travel to Downtown Phoenix, n=4
Respondents who scheduled pre-screen appointment, n=63
Respondents who did not schedule pre-screen appointment, n=43
Respondents who qualified for participation and enrolled in study, n=34
Respondents who did not qualify for participation in study during pre-screen, n= 29
Excluded, n=29
-Waist Circumference requirement not met (women>33, men>38) n = 21
-Not willing to maintain current diet/exercise for 8 weeks. n=3
-Age requirement not met, n=1
-Anemic, n=1
-Kidney disease, n=1
-Gastric band, n=1
-Smoker, n=1
Inclusion Criteria
18-50 years of age
Nonsmoking
Not pregnant or lactating
No active diseases
Not taking medication that affects blood glucose or cholesterol
Not taking digoxin or diuretics (other medications fine if using more than 3 months)
Not on a diet; not actively seeking to lose weight though food restrictions
BMI range: 22-35
Waist circumference: 33" or greater (women), 38" or greater (men)
Sedentary (exercising < 3 days/week)
Stratification and randomnization into treatment groups
Liquid vinegar group (intervention), n=17
Pill group (control), n=17
Participants who finished trial, n=12
Participants who dropped out, n=5
Drop out reasons:
-No show for visit 3, n=1
-Adverse reaction to vinegar, n=1
-Work conflict* n=1
-Heatlh issues unrelated to trail, n=1
-Opposed to blood draw,* n=1
*dropped out between visit 1 and visit 2 (total 2 participants)
Exclusion Criteria
Unwilling to take a vinegar supplement daily for 8 weeks
Unwilling to travel to ABC building for 3 visits
Unwilling to give 2 blood samples
Recent abdominal surgery
Medical condition that casues abdominal distention (acid reflux, pancreatitis, diverticulitis, Chrons IBS, Celiacs, etc.
Participants who finished trial, n=10
Participants who dropped out, n= 7
Drop out reasons:
-Health issues unrelated to trial* , n=2
-Work conflict* , n=2
-Opposed to blood draw*, n=1
-No show for visit 3 , n=2
*=dropped out between visit 1 and visit 2 (total 4 participants)

APPENDIX E

EARLY SIGNS AND SYMPTOMS OF DENTAL EROSION SURVEY QUESTIONS

NUMERICAL ASSIGNMENT

Question	Numerical value assignment
1. Relative to last year do your teeth appear…	More yellow =1 Less yellow = -1 About the same = 0
2. Do you experience tooth sensitivity in the following situations?	After sugary foods = 0(no), 1 (yes) When consuming hot/cold = 0(no), 1 (yes) During brushing =0(no), 1 (yes) Other = 0(no), 1 (yes)
3. Do you experience sensitivity in a localized or generalized area?	Don't know = 0 Localized =1 Generalized =2
4. How would you rate the sensitivity of your teeth?	Slight = 1 More than slight but less than moderate =2 Moderate = 3 More than moderate but less than extreme =4 Extreme =5
5. Do the edges of your front teeth appear transparent?	No = 0 I don't know = 1 Yes = 2
*Question 3, 4, 5 answered only if responded yes to question 2	

APPENDIX F

GERD SCREENER SURVEY QUESTIONS NUMERICAL ASSIGNMENT

Question	Numerical Value Assignment
1. Have you ever been diagnosed with GERD by a medical doctor?	No = 0 Yes = 1
2. How frequently do you experience heart burn after a meal	Never = 0 Rarely = 1 Sometimes = 2 About half the time = 3 Most of the time = 4 Always = 5
3. How frequently do you have regurgitation after a meal?	Never = 0 Rarely = 1 Sometimes = 2 About half the time = 3 Most of the time = 4 Always = 5
4. How frequently do you have an acid taste in your mouth?	Never = 0 Rarely = 1 Sometimes = 2 About half the time = 3 Most of the time = 4 Always = 5

APPENDIX G

ACIDIC BEVERAGE SURVEY QUESTIONS NUMERICAL ASSIGNMENT

Question	Numerical Value Assignment
1.**Beverage Frequency**: In the past month, how frequently did you consume the following beverages: • Carbonated beverages (soda, energy drinks, seltzer water) • Fruit juice (apple, orange, grapefruit, etc.) • Sport drinks (Gatorade, Powerade, etc.) • Sweetened Juice (Sunny D, lemonade, punch) • Coffee/Tea • Alcoholic beverages (wine, beer, champagne)	0 = Never or less than 1 time per week 1 = 1 time per week 2 = 2-3 times per week 3 = 4-6 times per week 4 = 1 time per day 5 = 2 times per day 6 = 3+ times per day
2. **Beverage consumption**: In the past month, please indicate the amount of each beverage you typically consumed each time: • Carbonated beverages (soda, energy drinks, seltzer water) • Fruit juice (apple, orange, grapefruit, etc.) • Sport drinks (Gatorade, Powerade, etc.) • Sweetened Juice (Sunny D, lemonade, punch) • Coffee/Tea • Alcoholic beverages (wine, beer, champagne)	0 = None 1 = Less than 6 fl. oz (3/4 cup) 2 = 8 fl. oz (1 cup) 3 = 12 fl. oz (1 ½ cup) 4 = 16 fl. oz (2 cups) 5 = More than 20 fl. oz (2 ½ cups)
3. **Beverage drink**: When consuming the following beverages, do you typically use a straw OR drink directly from a cup/bottle/can (only answer for beverages consumed more than 1/week)? • Carbonated beverages (soda, energy drinks, seltzer water) • Fruit juice (apple, orange, grapefruit, etc.) • Sport drinks (Gatorade, Powerade, etc.) • Sweetened Juice (Sunny D, lemonade, punch) • Coffee/Tea • Alcoholic beverages (wine, beer, champagne)	1 = Straw 2 = Cup/bottle/can

4. **Beverage habits:** Do you participate in any of the following drinking habits when consuming the following beverages? • Carbonated beverages (soda, energy drinks, seltzer water) • Fruit juice (apple, orange, grapefruit, etc.) • Sport drinks (Gatorade, Powerade, etc.) • Sweetened Juice (Sunny D, lemonade, punch) • Coffee/Tea • Alcoholic beverages (wine, beer, champagne)	Hold beverage in mouth prior to swallowing = No (0), Yes (1) Swish with beverage prior to swallowing = No (0), Yes (1) Add lemon or lime to beverage = No (0), Yes (1)

APPENDIX H

RISK FACTORS FOR TOOTH WEAR SURVEY QUESTIONS NUMERICAL ASSIGNMENT

Abrasion Questions	Numerical Value Assignment
1. How many times do you brush your teeth in a day?	Free response provided and numerical value taken at face value
2. What times do you typically brush your teeth?	Morning = 0 (no), 1 (yes) Before Bed = 0 (no), 1 (yes) After a meal= 0 (no), 1 (yes) After a sugary snack = 0 (no), 1 (yes)
3. What level hardness are your toothbrush bristles?	I don't know = 0 Extra Soft = 1 Soft = 2 Medium = 3 Hard = 4
Attrition Questions	
4. Has anyone ever told you they could hear you grinding your teeth at night?	No = 0 Yes = 1
5. Have you ever been told by a dentist that you grind your teeth?	No = 0 Yes = 1
6. Has a dentist ever prescribed a night guard for you?	No = 0 Yes = 1
7. Do you typically wake up in the morning with jaw or face pain?	No = 0 Yes = 1

APPENDIX I

RISK FACTORS FOR HYPOSALIVATION SURVEY QUESTIONS NUMERICAL ASSIGNMENT

Dehydration Questions	Numerical Value Assignment
1. What is the typical color of your urine, excluding the first urine of the day (urine color chart provided)	Clear/Pale yellow = 1 Yellow = 2 Dark yellow = 3 Brownish yellow = 4 Brown = 5
Medication Questions	
2. Do you take medication daily, OTC or prescribed (exclude vitamins)?	No = 0 Yes =1
3. How many different medications do you take daily?	Free response provided, and numerical value taken at face value
4. Do you take any of the following medication? • Antidepressants • Antihistamines • Antihypertensive • Antipsychotics • Beta Blockers • Diuretics	No =0 Yes = 1
Xerostomia (dry mouth) Questions	
5. Do you sip on liquids to aid in swallowing dry food?	Never = 0 Rarely = 1 Sometimes = 2 ½ of the time =3 Most of the time = 4 Always = 5
6. Does your mouth feel dry when eating a meal?	Never = 0 Rarely = 1 Sometimes = 2 ½ of the time =3 Most of the time = 4 Always = 5
7. Do you have difficulties swallowing food?	Never = 0 Rarely = 1 Sometimes = 2 ½ of the time =3 Most of the time = 4 Always = 5

*Questions 3 and 4 skipped if answered "no" to question 2

APPENDIX J

IRB APPROVAL

APPROVAL: CONTINUATION

Carol Johnston
SNHP: Nutrition
602/827-2265
CAROL.JOHNSTON@asu.edu

Dear Carol Johnston:

On 12/12/2017 the ASU IRB reviewed the following protocol:

Type of Review:	Modification and Continuing Review
Title:	Effect of Vinegar Consumption on Visceral Fat and Blood Glucose Concentration
Investigator:	Carol Johnston
IRB ID:	STUDY00005418
Category of review:	(2)(a) Blood samples from healthy, non-pregnant adults, (4) Noninvasive procedures, (7)(b) Social science methods, (2)(b) Blood samples from others, (9) Convened IRB determined minimal risk, (7)(a) Behavioral research
Funding:	Name: Graduate College (GRAD)
Grant Title:	None
Grant ID:	None
Documents Reviewed:	• Data release form, Category: Participant materials (specific directions for them); • protocol, Category: IRB Protocol; • dental erosion survey, Category: Screening forms; • exit survey, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • diet recall, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • ad and verbal script, Category: Recruitment Materials; • calendar, Category: Participant materials (specific directions for them); • health history questionnaire, Category: Screening forms; • online screener, Category: Recruitment Materials; • consent, Category: Consent Form;

The IRB approved the protocol from 12/12/2017 to 12/13/2018 inclusive. Three weeks before 12/13/2018 you are to submit a completed Continuing Review application and required attachments to request continuing approval or closure.

If continuing review approval is not granted before the expiration date of 12/13/2018 approval of this protocol expires on that date. When consent is appropriate, you must use final, watermarked versions available under the "Documents" tab in ERA-IRB.

In conducting this protocol you are required to follow the requirements listed in the INVESTIGATOR MANUAL (HRP-103).

Sincerely,

IRB Administrator

cc:
Summer Anderson

APPENDIX K

SAMPLE SIZE CALCULATIONS

Statistical considerations for a parallel trial where the outcome is a measurement

Note: The power calculation uses the non-central t function, pt(x,df,ncen), and it's inverse qt
Power=pt(qt(.025,n-2,0),n-2,-(delta/sigma)/sqrt(4/n)), power is truncated rather than rounded.
If power is specified the other parameters are found by searching.

Request

.05 Significance Level — 2 sided *(default is 0.05, two-sided)*

Standard Deviation of the outcome variable *(if known)*

Enter two of the following three values and the remaining value will be calculated

1. 22 Total number of patients
2. Power (usually 0.8 or 0.9)
3. Minimal detectable difference (specify one of the following):
   a. 1.05 Difference in means
   b. % Location of the mean of one treatement group in terms of a percentile of the other treatment group.

Calculate

Response

Calculation performed at: 8/21/2018, 8:51:17 AM

The provided parameters were: significance level (adjusted for sidedness) = 0.025, standard deviation = undefined, number of patients = 22, power = undefined, difference in means = 1.05, location of mean in one group as a percentile of the other group = undefined.

The variable calculated was the detection probability (power).

A total of 22 patients will enter this two-treatment parallel-design study. The probability is 64 percent that the study will detect a treatment difference at a two-sided 0.05 significance level, if the true difference between treatments is 1.050 times the standard deviation.

Statistical considerations for a parallel trial where the outcome is a measurement

Note: The power calculation uses the non-central t function, pt(x,df,ncen), and it's inverse qt
Power=pt(qt(.025,n-2,0),n-2,-(delta/sigma)/sqrt(4/n)), power is truncated rather than rounded.
If power is specified the other parameters are found by searching.

Request

.05 Significance Level — 2 sided *(default is 0.05, two-sided)*

Standard Deviation of the outcome variable *(if known)*

Enter two of the following three values and the remaining value will be calculated

1. Total number of patients
2. .8 Power (usually 0.8 or 0.9)
3. Minimal detectable difference (specify one of the following):
   a. 1.05 Difference in means
   b. % Location of the mean of one treatement group in terms of a percentile of the other treatment group.

Calculate

Response

Calculation performed at: 8/21/2018, 8:52:07 AM

The provided parameters were: significance level (adjusted for sidedness) = 0.025, standard deviation = undefined, number of patients = undefined, power = 0.8, difference in means = 1.05, location of mean in one group as a percentile of the other group = undefined.

The variable calculated was the total number of patients.

A total of 32 patients will enter this two-treatment parallel-design study. The probability is 81 percent that the study will detect a treatment difference at a two-sided 0.05 significance level, if the true difference between treatments is 1.050 times the standard deviation.