Exploring the Effects of Vinegar on Inflammatory Biomarkers and Oxidative Stress in Healthy Adults: A Randomized Controlled Trial

by

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ABSTRACT

This thesis explores the potential anti-inflammatory effects of daily vinegar ingestion in healthy adults over a 4-week period, with a focus on cytokine levels, innate immunity markers, and C-reactive protein (CRP). Participants were healthy, non-smoking adults (n=28, 18-41 years of age) free of chronic disease. Participants were randomized to the active treatment group (VIN; two tablespoons liquid vinegar at mealtime twice daily; n=16) or the control group (CON; one commercial vinegar tablet daily; n=12). A fasting blood sample was collected at the start and completion of the 4-week intervention, and serum was analyzed for 15 cytokines and C-reactive protein (CRP). Fluctuations in the anti-inflammatory cytokines (e.g., IL-4, IL-10, IL-5, and IL-13) did not differ between treatment groups during the trial; however, notable reductions in two pro-inflammatory cytokines were observed at week 4 for VIN participants in comparison to CON participants. IL-1 trended lower for VIN versus CON participants (-2.7±6.0 and 1.0±6.6 pg/ml respectively, p=0.102), and IL-12p40 was reduced significantly during the trial in VIN versus CON participants (-5.7±12.5 and +1.04±9.8 pg/ml; p=0.051). The rise in CRP at week 4 for all participants, while not statistically significant, underscores the complexity of the relationship between inflammatory markers. These findings suggest that daily vinegar ingestion may afford greater resilience to inflammatory conditions (as indicated by increasing CRP concentrations). Recommendations for future research include expanding participant pools, extending intervention periods, and conducting more comprehensive studies to unravel the intricate dynamics of vinegar's impact on inflammatory processes.

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CHAPTER 1

INTRODUCTION

Worldwide, three of five deaths are related to chronic inflammatory diseases (Pahwa, 2022). The empirical evidence shows that low-grade inflammation contributes to cardiovascular disease (CVD), cancer, type 2 diabetes, depression, and many other conditions (Ricciotti, 2020). Diet is a modifiable factor that can impact inflammation risk. Identifying manageable diet strategies to reduce chronic inflammation and the ensuing disease conditions are urgently needed. Acetic acid ingestion resulted in a reduction in lipid accumulation in adipose tissue, reduced hepatic adiposity, and improved glucose tolerance in type 2 diabetic rats (Yamashita et al., 2007); conditions that would reduce inflammatory sequels. Hence, there is much interest in examining the potential benefits of vinegar (the only dietary source of acetic acid) for reducing risk for inflammation.

Acetic acid, also known as vinegar acid or ethanoic acid, is the defining ingredient in vinegar and is largely responsible for the variety of health benefits that result from vinegar ingestion ("Acetic Acid", 2016). Based on the writings of US medical practitioners dating to the late 18th century, many ailments, from dropsy to poison ivy, croup, and stomachache were treated with vinegar, and, well before the production and marketing of hypoglycemic agents, vinegar "teas" were commonly consumed by patients to help manage their diabetes (Johnston and Gaas, 2006). Recently, scientific investigations have verified the antiglycemic property of vinegar (Gheflati et al., Johnston et al., Kondo et al., Liljeberg, etc.); however, it is not yet clear whether the vinegar-induced reductions in blood glucose link to reductions in inflammation. Gheflati

et al demonstrated that apple vinegar consumption improved glycemic indices and oxidative stress in individuals with diabetes and dyslipidemia (Gheflati et al., 2019). Another study found that apple cider vinegar suppressed obesity-induced oxidative stress in high-fat-diet rats through modulation of the antioxidant defense system and reducing atherogenic risk (Halima et al., 2018). Yet, an extended feeding trial in human subjects to directly examine the impact of daily vinegar ingestion on inflammatory mediators has not been conducted.

There are many cellular and biochemical events which occur as a result of tissue injury which is responsible for initiating inflammation. Central to the formation of inflammation are the inflammatory mediators, which include proteins, peptides, glycoproteins, cytokines, arachidonic acid metabolites (prostaglandins and leukotrienes), nitric oxide, and oxygen free radicals. These compounds, described in Table 1 below, are produced by epithelial cells, endothelial cells, and infiltrating inflammatory cells (primarily macrophages and T cells). Inflammatory mediators are a double-edged sword, having the potential to fight off infection, but also to damage the host (Juhn et al., 2008).

Table 1: Inflammatory Mediators (Adapted from Juhn et al., 2008)

Inflammatory mediator	**Source**	**Principal activities**
Cytokines		
Tumor necrosis factor-α	Macrophages Lymphocytes Epithelial cells Endothelial cells	Prostaglandin release, activates neutrophils, eosinophils, macrophages, cytokine release
Interleukin-1	Macrophages Neutrophils Fibroblasts Endothelial cells Epithelial cells	Activates B- and T-cells, epithelial and fibroblast proliferation, cytokine synthesis, histamine release, induces fever, bone resorption

Interleukin-2	T-cells	Activates T-cells
Interleukin-4	T cells Mast cells Basophils	Stimulates T_H2 differentiation and proliferation, anti-inflammatory action on T-cells, B-cells, monocytes
Interleukin-5	T-cells Mast cells Basophils	Stimulates and maintains IgA production by B-cells, eosinophil chemotaxis, stimulates eosinophil production in bone marrow
Interleukin-6	Macrophages Lymphocytes Epithelial cells	Activates B- and T-cells, production of antibodies, induces fever, bone resorption
Interleukin-10	Macrophages	Down regulates inflammatory properties of IL-1, IL-6, TNF-α
Interleukin-12	Macrophages Neutrophils Langerhans cells	Interferon release, activates macrophages, potentiates T-cell proliferation, cytokine production, and cytotoxicity of lymphocytes. Induces T_H1 lymphocyte development
Interleukin-13	T-cells	Similar to IL-4; molecular bridge linking allergic inflammation cells to the non-immune cells in contact with them
Transforming growth factor-ß	Neutrophils Macrophages	Initiation and maturation of inflammatory processes; recruitment, activation, and proliferation of inflammatory tissues and cells
Granulocyte-macrophage colony-stimulating factor	Macrophages	Stimulates production of granulocytes (neutrophils, eosinophils, and basophils)
Chemokines		
Interleukin-8	Macrophages Fibroblasts Epithelial cells	Neutrophil chemotaxis and activation, angiogenesis

	Endothelial cells	
RANTES	Epithelial cells	Monocyte and T-cell chemotaxis
MCP-1	Epithelial cells	Monocyte and T-cell chemotaxis
Histamine	Mast cells Basophils	Increases vascular permeability, vasodilation, neutrophil and eosinophil chemotaxis
Vascular endothelial growth factor	Thrombocytes	Increases vascular permeability, vasodilation, inflammatory cell infiltration
Platelet activating factor	Monocyte Neutrophils Lymphocytes	Chemotaxis and degranulation of neutrophils, increase vascular permeability (1,000x more potent than histamine)
Mast cells	Bone marrow	Release of preformed mediators: histamine and tryptase
	Expressing CD34 molecule	Release of de novo synthesized mediators: leukotrienes and prostaglandins
Arachidonic acid metabolites		
Prostaglandins		
Prostaglandin E_2	Mast cells Neutrophils Monocytes	Vasodilation, mucus production, induces cytoprotection to IL-1
Prostaglandin I_2	Endothelial cells	Vasodilation, inhibits platelet aggregation
Leukotrienes		
LTB_4	Neutrophils	Chemotaxis and degranulation of neutrophils
LTC_4, LTD_4, LTE_4	Mast cells Basophils Eosinophils	Increased vascular permeability, vasoconstriction
Bradykinins	Plasma	Increased vascular permeability, pain

Inflammation occurs when the immune system sends out cells to fight bacteria or heal an injury. One study found that polyphenol-rich vinegar extract regulates intestinal microbiota and immunity and prevents alcohol-induced inflammation in mice (Xia et al., 2021). Shanxi-aged vinegar, a traditional Chinese grain-fermented food that is rich in polyphenols, also shows results that it may have a potential function against inflammation (Du et al., 2021). Another study found that apple cider vinegar attenuates oxidative stress and reduces the risk of obesity in high-fat-fed rats (Halima et al., 2018). As a source of both acetic acid and polyphenols, vinegar may hold great promise in reducing oxidative stress and inflammation.

To date, there has not been a study that directly measures vinegar consumption and inflammation in humans. Many studies have shown the clear link between vinegar ingestion and a decrease in blood glucose levels and other parameters, but there is a need for further research of this other possible relationship between vinegar consumption and inflammation. Further researching this potential link is important because it will help identify a widely available and affordable method of decreasing levels of inflammation, oxidative stress, CRP levels, and in turn, chronic inflammatory disease risk.

Purpose of Study

The purpose of this study is to test the potential ability of red wine vinegar to decrease CRP levels, and in turn, decrease inflammation and oxidative stress in young adults aged 18-41 in the Phoenix metropolitan area. During the 4-week trial, participants will either consume a vinegar drink or low-dose vinegar pill daily.

Research Aim and Hypothesis

H1: Daily red wine vinegar consumption will be associated with decreased CRP levels after 4 weeks compared to the placebo treatment group (low-vinegar pill) in a group of overall healthy young adults aged 18-41.

H2: Daily red wine vinegar consumption will be associated with decreased inflammation and oxidative stress levels (as indicated by a 15-panel cytokine analysis) after 4 weeks compared to the placebo treatment group (low-vinegar pill) in a group of overall healthy young adults aged 18-41.

Definition of Terms

- **Acetic acid/acetate** – the main component of vinegar that is classified as a weak acid with the chemical form CH_3COOH. The average acid content of commercially available vinegar is 5%.
- **Chronic disease** – a persistent or otherwise long-lasting in its effects or a disease that comes with time. The term chronic is often applied when the course of the disease lasts for more than three months.
- **Chronic inflammation** – slow, long-term inflammation lasting for prolonged periods of one year or more and require ongoing medical attention, limit activities of daily living, or both, are identified as chronic.
- **C-reactive protein (CRP)** – level of CRP increases when there's inflammation in the body. Testing this measure checks for inflammation in the body. A CRP level between 1-3 mg/L of blood often signals a low, yet chronic level of inflammation.

- **Glycemic load** – the amount of carbohydrate consumed multiplied by the rate at which the carbohydrate is metabolized and enters the bloodstream.
- **Oxidative stress** – an imbalance between production and accumulation of oxygen reactive species (ROS) in cells and tissues and the ability of a biological system to detoxify these reactive products.
- **Polyphenols** – a compound containing more than one phenolic hydroxyl group. We get polyphenols through certain plant-based foods, they're packed with antioxidants and potential health benefits.
- **Postprandial glycemia** – refers to plasma glucose concentrations after eating. In nondiabetic individuals, fasting plasma glucose concentrations (i.e., following an overnight 8- to 10-h fast) generally range from 70 to 110 mg/dl.

Delimitations and Limitations

Delimitations:

- Generally healthy, nonsmoking adults between the ages of 18-41, from a campus community in Phoenix, Arizona will be recruited for this study. Females must have a dress or pant size greater than 10, and men must have a pant size above 36 inches as this was the most convenient method to assess for high waist circumference prior to their initial visit. Participants should have a BMI greater than or equal to 23. Trial is a short duration, lasting only 4 weeks.

Limitations:

- Adherence to vinegar consumption two times daily, pills may show greater adherence than liquid because of the taste and convenience.
- 24-hour diet recall may not be accurate; therefore, we will implement the double-pass method which will be completed at both visits.
- Small sample size could make it difficult to determine if said outcome is a true finding.

CHAPTER 2

LITERATURE REVIEW

Introduction

Over the millennia, populations across the globe have attributed health benefits to vinegar. However, empirical evidence to link vinegar consumption to health has only recently been examined by researchers. In order to determine suitable methods of determining the true effects of vinegar consumption on overall health, specifically, how it may affect chronic inflammation, a review of studies and experiments done by previous researchers is presented. This review of literature includes details on vinegar as an anti-inflammatory agent, as well as vinegar's relation to oxidative stress, obesity, and chronic inflammation.

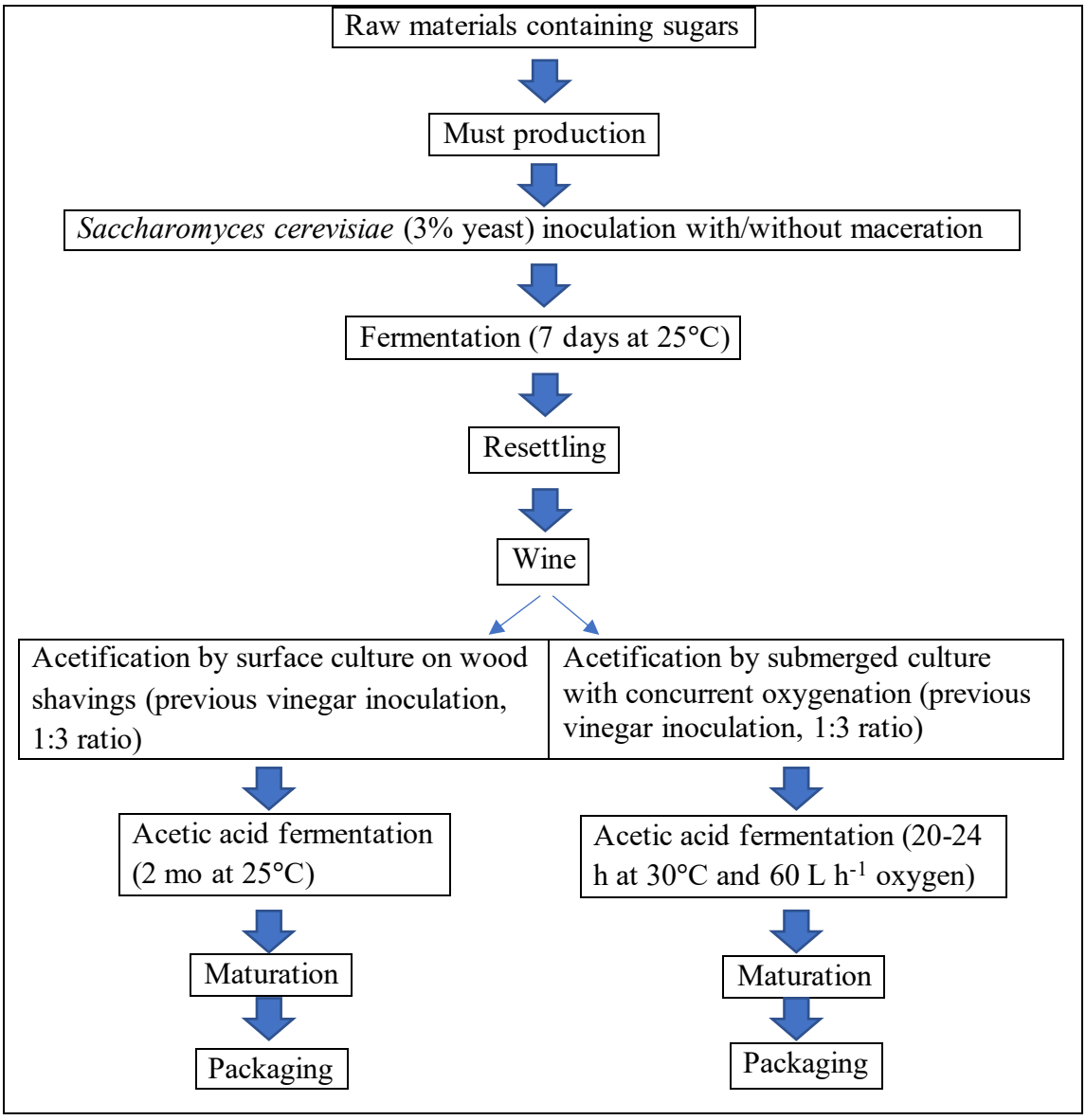
Vinegar

Vinegar Production

Vinegar, from the French *vin aigre*, meaning "sour wine," can be made from almost any fermentable carbohydrate source, including wine, molasses, dates, sorghum, apples, pears, grapes, berries, melons, coconut, honey, beer, maple syrup, potatoes, beets, malt, grains, and whey. The production of vinegar typically involves a first fermentation where simple sugars in raw material are converted to alcohol by yeasts. The resultant alcohol is further oxidized to acetic acid by Acetic Acid Bacteria during the last fermentation (Budak et al., 2014). Commercial vinegar is produced by either fast or slow fermentation processes. For the quick methods, the liquid is oxygenated by agitation and the bacteria culture is submerged permitting rapid fermentation. However, the slow

methods are generally used for the production of the traditional wine vinegars, and the culture of acetic acid bacteria grows on the surface of the liquid and fermentation proceeds slowly over the course of weeks or months. The longer fermentation period allows for the accumulation of a nontoxic slime composed of yeast and acetic acid bacteria, known as the *mother* of vinegar. After opening, *mother* may develop in stored vinegar; it is considered harmless and can be removed by filtering. However, many people advocate retaining the *mother* for numerous, but unsubstantiated, health effects (Johnston and Gaas, 2006).

Figure 1: Production Method for Vinegar (Adapted from Budak et al., 2014)



Types of Vinegar

Specialty vinegars are grouped as herbal or fruit vinegars. Herbal vinegars consist of wine vinegars or white distilled vinegars, which may be seasoned with garlic, basil, tarragon, cinnamon, clove, or nutmeg (Mariana-Atena et al., 2007). Fruit vinegars are wine and white vinegars sweetened with fruit or fruit juice to produce a characteristic sweet-sour taste. Traditional vinegars are produced from regional foods according to

well-established customs. The balsamic vinegar of Modena, Italy, is made from the local white Trebbiano grapes, which are harvested as late as possible, fermented slowly, and concentrated by aging in casks of various woods. Original rice wine vinegars are produced in Asia, coconut and cane vinegars are common in India and the Philippines, and date vinegars are popular in the middle east (Johnston and Gaas, 2006). The table below describes the properties, main country of production, acetic acid content, and the bacterial species of different types of vinegar. These common types of vinegar you most likely know of, include distilled/white vinegar, apple cider vinegar, balsamic vinegar, kombucha, red wine vinegar, and white wine vinegar.

Table 2: Types of Vinegar (Adapted from Budak et al., 2014)

Type	**Properties**	**Main Country of Production**	**Acetic Acid Content**	**Bacterial Species**
Distilled/White Vinegar	Made from distilled alcohol, malt, or corn, often used in foods like ketchup or salad dressings, may be used for cleaning	Worldwide	5-8%	*Acetobacter pomorum, acetobacter obeidiens, Gluconacetobacter entanii*
Apple Cider Vinegar	Gold color, made from pressed apples, sweeter flavor than other vinegars	Worldwide	5-6%	*Acetobacter pasteurianus, aceti and intermedius*
Balsamic Vinegar	Dark brown color, made from	Italy	4-10%	*Acetobacter pasteurianus,*

	Trebbiano grapes, tart			*Gluconacetobacter hansenii, Gluconacetobacter*
Kombucha	Made from fermented black and/or green tea, is a rich source of probiotics, symbiotic culture of bacteria and yeast, contains B vitamins	Japan, United States	5-7%	*Acetobacter xylinum, Gluconacetobacter xylinus, Gluconacetobacter kombuchae*
Red Wine Vinegar	Rich red color, robust flavor, made from red wines like Merlot	Worldwide	4-6%	*Acetobacter pasteurianus, Gluconacetobacter europaeus*
White Wine Vinegar	Typically made from Pinot Grigio, slightly sweet flavor	Italy, Turkey	5-6%	*Gluconacetobacter europaeus, Gluconacetobacter xylinus*

Typical and Historical Medicinal Uses of Vinegar

Based on the writings of US medical practitioners dating to the late 18th century, many ailments, from dropsy to poison ivy, croup, and stomachache were treated with vinegar, and, before the production and marketing of hypoglycemic agents, vinegar "teas" were commonly consumed by diabetics to help manage their chronic ailment (Johnston and Gaas, 2006).

The use of vinegar to fight infections and other acute conditions dates back to Hippocrates (460-377 BC; the father of modern medicine), who recommended a vinegar preparation for cleaning ulcerations and for the treatment of sores. Oxymel, a popular ancient medicine composed of honey and vinegar, was prescribed for persistent coughs by Hippocrates and his contemporaries, and by physicians up to modern day. The medicine was prepared by mixing virgin honey, 4 parts, with white wine vinegar, 1 part, concentrating and clarifying with paper pulp. Undiluted vinegar may be used effectively for cleaning dentures, and, unlike bleach solutions, vinegar residues left on dentures were not associated with mucosal damage (Shay, K., 2000). In the popular media, vinegar is commonly recommended for treating nail fungus, head lice, and warts, yet scientific support for these treatment strategies is lacking (Johnston and Gaas, 2006). Takano-Lee and colleagues demonstrated that, of 7 home remedies tested, vinegar was the least effective for eliminating lice or inhibiting the hatching of eggs (Takano-Lee et al., 2004). Scattered reports suggest that the successive topical application of highly concentrated acetic acid solutions (up to 99%) alleviated warts, presumably due to the mechanical destruction of wart tissue (Steele et al., 1988).

Acetic Acid

Acetic acid, also known as vinegar acid or ethanoic acid, is the active ingredient in vinegar and is largely responsible for the variety of health benefits that result from vinegar ingestion ("Acetic Acid", 2016). The molecular formula for acetic acid is CH_3COOH. It is classified as a carboxylic acid and has a hydroxyl and acetyl group ("Acetic Acid", 2016). In the United States, vinegar products must contain a minimum of

4% acidity. White distilled vinegars are generally 4% to 7% acetic acid whereas cider and wine vinegars are 5% to 6% acetic acid (The Vinegar Institute, 2019). Acetic acid is present in commercially available vinegar, concentrated at values between 4-15% (Ogawa et al., 2000). Acetic acid is produced via *Acetobacter aceti* through the process of fermentation. Acetic acid can also be produced via the human digestion process (Ogawa et al., 2000). When indigestible carbohydrates, typically insoluble fiber such as bran or psyllium, are consumed, they are fermented by the microbiome present in the large intestine and produce short-chain fatty acids, one being acetate, the salt form of acetic acid (Ogawa et al., 2000).

The effects of acetic acid on the body and metabolism are still being explored within the literature; but a recent study has found that acetate improves glucose tolerance (Yamashita et al., 2007). It was found that the acetate treatment resulted in a reduction in lipid accumulation in adipose tissue, reduced hepatic adiposity, and improved glucose tolerance. This study was conducted on a group of obese, type 2 diabetic rats that were orally injected with 5.2 mg/kg acetate (Yamashita et al., 2007). It was found that the acetate treatment resulted in a reduction in lipid accumulation in adipose tissue, reduced hepatic adiposity, and improved glucose tolerance. It was found via Northern blotting analysis that in the liver of the rats, transcription factors of lipogenic genes in the liver were decreased (Yamashita et al., 2007). Upon further analysis, acetate has an inhibitory effect on carbohydrate-responsive element-binding protein (ChREBP) activity in the liver. ChREBP is a transcription factor that facilitates multiple genes that are responsible for converting glucose to fatty acids in the liver, thus explaining the noted decrease in

hepatic adiposity (Yamashita et al., 2007). Another study involving mice fed inulin, a type of easily fermentable fiber, gained less weight, consumed less food, and increased total short chain fatty acid colonic concentrations in comparison to the rats fed poorly fermentable fiber (Yamashita et al., 2007). Acetate was found to be the most abundant SCFA in the inulin-fed rats post intervention. It was also found that acetate is largely responsible for appetite regulation because it encourages the release of the hormones peptide YY and glucagon-like peptide-1 (Yamashita et al., 2007). These hormones are responsible for regulating hunger and satiety, and modulate insulin secretion from the pancreas, respectively (Yamashita et al., 2007).

Safety and Potential Risks of Vinegar Consumption

Vinegar's use as a condiment and food ingredient spans thousands of years, and perhaps its use can be labeled safe by default. Yet there are rare reports in the literature regarding adverse reactions to vinegar ingestion. Inflammation of the oropharynx and second-degree caustic injury of the esophagus and cardia were observed in a 39-year-old woman who drank 1 tablespoon of rice vinegar in the belief it would dislodge a piece of crab shell from her throat (Chung, 2002). Her symptoms resolved spontaneously after several days. Esophageal injury by vinegar is likely very rare but deserves notice. Chronic inflammation of the esophagus is a cancer risk; but, as reported previously, vinegar use was inversely related to risk for cancer of the esophagus (Xibib et al., 2003).

Vinegar ingestion at mealtime is gaining popularity for its antiglycemic effects; however, it is among the most acidic consumable substances. Another study examined tooth wear in healthy adults and found evidence that daily vinegar ingestion may

contribute to erosive tooth wear (Anderson et al., 2021). Given the current popularity of vinegar as a medicinal agent, practitioners should caution patients who utilize this strategy on the possibility of erosive tooth wear.

The unintentional aspiration of vinegar has been associated with laryngospasm and subsequent vasovagal syncope that resolved spontaneously (Wrenn, 2006). Hypokalemia was observed in a 28-year-old woman who had reportedly consumed approximately 250 mL apple cider vinegar daily for 6 years (Lhotta et al., 1998). Although speculative, the hypokalemia was attributed to elevated potassium excretion related to the bicarbonate load from acetate metabolism. These complications attributed to vinegar ingestion are isolated occurrences, but with the increased interest in vinegar as adjunct therapy in diabetes, carefully controlled trials to examine potential adverse effects of regular vinegar ingestion are warranted.

Health Effects of Vinegar

Antiglycemic Properties of Vinegar

The antiglycemic property of vinegar was first demonstrated to extend to individuals with marked insulin resistance or type 2 diabetes in 2004 (Johnston et al., 2004). In this crossover trial, individuals with insulin resistance (n = 11, fasting insulin concentrations greater than 20 mU/mL) or with diagnosed type 2 diabetes (n = 10) consumed a vinegar test drink or placebo immediately before the consumption of a mixed meal (87 g total carbohydrate). In the insulin-resistant subjects, vinegar ingestion reduced postprandial glycemia 64% as compared with placebo values (P = .014). In individuals with type 2 diabetes, vinegar ingestion was less effective at reducing mealtime glycemia

(-17%, P = .149); however, vinegar ingestion was associated with a slight improvement in postprandial insulin sensitivity in these subjects (+19%, P = .07), (Johnston et al., 2004).

The marked antiglycemic effect of vinegar in insulin-resistant subjects is noteworthy and may have important implications. Multicenter trials have demonstrated that treatment with antiglycemic pharmaceuticals (metformin or acarbose) slowed the progression to diabetes in high-risk individuals; moreover, because these drugs improved insulin sensitivity, the probability that individuals with impaired glucose tolerance would revert to a normal, glucose-tolerant state over time was increased (Chiasson et al., 2002). In healthy subjects, Ostman and colleagues demonstrated that acetic acid had a dose-response effect on postprandial glycemia and insulinemia (Ostman et al., 2005). Subjects consumed white bread (50 g carbohydrate) alone or with 3 portions of vinegar containing 1.1, 1.4, or 1.7 g acetic acid. At 30 minutes post-meal, blood-glucose concentrations were significantly reduced by all concentrations of acetic acid as compared with the control value, and a negative linear relationship was calculated between blood glucose concentrations and the acetic acid content of the meal (r = -0.47, P = .001). Bread consumption alone scored the lowest rating of satiety. Feelings of satiety increased when vinegar was ingested with the bread, and a linear relationship was observed between satiety and the acetic acid content of the test meals (r = 0.41, P = .004), (Ostman et al., 2005).

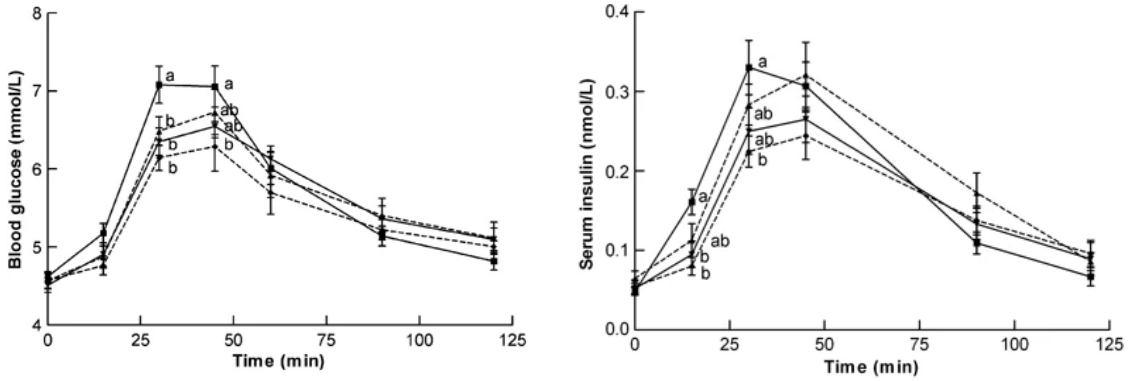
In a separate trial, healthy adult women consumed fewer total calories on days that vinegar was ingested at the morning meal. (Johnston & Buller, 2005). In this trial,

fasting participants consumed a test drink (placebo or vinegar) followed by the test meal composed of a buttered bagel and orange juice (87 g carbohydrate). Blood sampled were collected for 1 hour after the meal. At the end of testing, participants were allowed to follow their normal activities and eating patterns the remainder of the day, but they were instructed to record food and beverage consumption until bedtime. Vinegar ingestion, as compared with placebo, reduced the 60-minute glucose response to the test meal (-54%, $P < .05$) and weakly affected later energy consumption (-200 kilocalories, $P = .111$). Thus, vinegar may affect satiety by reducing the meal-time glycemic load (Johnston & Buller, 2005).

In a later study, paracetamol was added to the bread test meal to permit indirect measurement of the gastric emptying rate. Compared with reference values, post meal serum glucose and paracetamol concentrations were reduced significantly when the test meal was consumed with vinegar (Liljeberg and Bjorck, 1998). Of 20 studies published between 1977 and 1999, 16 demonstrated that low-glycemic index foods promoted post-meal satiety and/or reduced subsequent hunger (Roberts, 2000). A study showed that vinegar supplementation lowers glucose and insulin responses and increases satiety after a bread meal in 12 healthy subjects (Figure 2, Figure 3).

Figure 2: Mean Blood Glucose Responses After Bread & Vinegar (Adapted from Ostman et al., 2005)

Figure 3: Mean Serum Insulin Responses After Bread & Vinegar (Adapted from Ostman et al., 2005)



In a meta-analysis investigating the effects of apple cider vinegar on lipid profiles and glycemic indicators, it was found that overall, 26 studies, including 22 study arms, found that apple cider vinegar consumption significantly decreased serum total cholesterol, fasting plasma glucose, and HbA1C concentrations (Hadi et al., Sohouli et al., Cheng et al.). Additionally, ACV intake appeared to elicit an increase in FPG and HDL-C concentrations in apparently healthy participants (Hadi et al., 2021). Another systematic review and meta-analysis of clinical trials found that vinegar can be effective in reducing postprandial glucose and insulin levels, indicating it could be considered as an adjunctive tool for improving glycemic control (Shishehbor, 2017).

Antitumor Properties of Vinegar

The antitumor properties of vinegar are still being investigated, but current studies have illustrated a significant potential. One study tested the effects of ethyl acetate extract from a Japanese traditional vinegar made from unpolished rice, called "Kurosu" on the proliferation of cancer cells (Nanda et al., 2004). The extract was most effective against the colon adenocarcinoma, showing up to 62% inhibition against cell proliferation. The results of this study suggest that the extract induces cell apoptosis in these cells,

indicating that this treatment may prove effective in reducing or even preventing the spread of colon cancer cells (Nanda et al., 2004).

Another study discovered similar findings involving colon cancer cells but looked at anticarcinogenic potential all of the short chain fatty acids, particularly when they are produced via fermentation of dietary fiber (Fu et al., 2004). It has been hypothesized that the short chain fatty acids (SCFAs) can help prevent carcinogenesis of colon cells because they help maintain colonocyte differentiation. This study focused on a specific phenotype of colonic adenocarcinoma cells called Caco-2 (Fu et al., 2004). The cells were treated with 10 mmol of each SCFAs, and it was found that all of the SCFAs altered the phenotype of Caco-2, prolonging cell doubling time, inhibiting cell motility, and promoted the expression of a differentiation marker. In conclusion, SCFAs produced via fermentation of dietary fiber may be protective against colon cancer (Fu et al., 2004).

New compounds were evaluated in another study for in vitro antitumor activity against tumor cell lines, A549, Bel7402, BGC-823, HCT-8, and A2780 (Li et al., 2017). In vivo testing results indicated that 2 of the 4 (12 and 13) tested compounds had antitumor activity against mouse liver carcinoma. These findings indicate that 20(S)-O-fluorouracil-1'(N)-acetic acid ester derivative of CPTs, 12, could be developed as an antitumor drug candidate for clinical trial (Li et al., 2017).

Vinegar and Dyslipidemia

Dyslipidemia is a condition in which we see abnormally elevated cholesterol or fats (lipids) in the blood. Dyslipidemia increases the chance of clogged arteries, heart attacks, stroke, or other circulatory concerns, especially in smokers. In adults, it is often

related to obesity, an unhealthy diet, and lack of exercise. It is very common as we currently see over 3 million cases in the United States per year (Pappan, 2022).

In a study with 70 participants, who had type 2 diabetes and hyperlipidemia were randomly assigned into an intervention and control group in order to assess the effect of 20 ml apple vinegar per day for 8 weeks (Gheflati et al., 2019). The intervention with apple vinegar could significantly improve FBS ($p = 0.006$) and DPPH ($p < 0.001$) within the intervention group and in comparison with the control group ($p < 0.001$). additionally, the significant increase of MDA in the control group ($p < 0.05$) caused a considerable difference between the two groups (Gheflati et al., 2019). This trial provided some evidence that apple vinegar consumption may cause beneficial effects on glycemic indices and oxidative stress in individuals with diabetes and dyslipidemia (Gheflati et al., 2019). Additionally, another study found that apple cider vinegar can be beneficial for the suppression of obesity-induced oxidative stress in HFD rats through the modulating antioxidant defense system and reduces the risk of obesity-associated diseases, including dyslipidemia, by preventing the atherogenic risk (Halima et al., 2018).

Vinegar and Obesity

Fermented food has been demonstrated to exhibit anti-obesity effects through altering gut microbiota composition and the expression of genes related to metabolic syndrome (Han et al., 2015). Among these fermented foods, vinegar, an acidic food seasoning has recently received substantial attention because it was shown to exhibit multiple bioactivities, including anti-hypercholesterolemia, anti-hyperglycemia, anti-hypertension, anti-microbial, anti-thrombotic and even anti-cancer effects (Mohamad et

al., 2015). Acetic acid, which is the major ingredient in vinegar, has been reported to be a potential agent for preventing metabolic syndrome by reducing obesity in rats (Yamashita, 2015) and even obese human subjects (Kondo et al., 2009).

Vinegar ingestion may decrease the glycemic effect of a meal through satiety this reducing the total amount of food consumed (Budak et al., 2014). In a study reported by Johnston (2006), human subjects consuming two tablespoons of red raspberry vinegar daily with freely access to food and water for four weeks lost weight whereas the control group consuming a similar amount of cranberry juice daily for four weeks had a slight weight gain. In another study, healthy volunteers consumed three levels of vinegar (18, 23, and 28 mmol acetic acid) with a portion of white wheat bread; bread consumption (no vinegar) was used as a control meal. When the hunger and satiety feelings of volunteers were evaluated, it was noted that satiety increased with rising acetic acid level (Budak et al., 2014).

In another study, researchers investigated three treatment conditions (control, consumption of vinegar containing one g acetic acid, or consumption of approximately one oz of peanuts for satiety). In the study, participants ingesting vinegar or peanuts had lower subsequent food consumption accounting for approximately 200 to 275 calories per day. After consumption of the bagel meal, energy consumption for the remainder of the day was weakly affected by vinegar and peanut treatments (a reduction of approximately 200 to 275 kcal, $P = 0.111$). This daily calorie reduction would result in a monthly weight loss of 1 to 1½ pounds (Johnston & Buller, 2005).

Chronic Inflammation

Mediators of Inflammation

The immune system responds to injury or irritation through an innate cascade known as inflammation (Juhn et al., 2008). There are many cellular and biochemical events which occur as a result of tissue injury which is responsible for the outcome of inflammation. Central to the formation of inflammation are the inflammatory mediators, which include proteins, peptides, glycoproteins, cytokines, arachidonic acid metabolites (prostaglandins and leukotrienes), nitric oxide, and oxygen free radicals. These compounds are produced by epithelial cells, endothelial cells, and infiltrating inflammatory cells. Inflammatory mediators are a double-edged sword, having the potential to fight off infection, but also to damage the host (Juhn et al., 2008). Specialized pro-resolving lipid mediators (SPMs) have been demonstrated to be signaling molecules in inflammation (Yang et al., 2021). SPMs are involved in the pathophysiology of different diseases, especially respiratory diseases, including asthma, pneumonia, and chronic obstructive pulmonary disease. All of these diseases are related to the inflammatory response and its persistence (Yang et al., 2021).

Inflammation is a physiologic response against noxious stimuli and microbial invaders. The basic elements of inflammation include host cells, blood vessels, proteins, and lipid mediators, which work together to eliminate the inflammatory stimulus as well as initiate the resolution and repair (Galvao et al., 2018). Mediators of inflammation are regulatory molecules that control the generation, maintenance, and resolution of this response, which is triggered after recognition of infection of injury. The initial recognition of the inflammatory stimuli leads to the production of pro-inflammatory

mediators (Galvao et al., 2018). These mediators are derived from immune cells (i.e., vasoactive amines, lipid mediators, platelet-activating factor, reactive oxygen species, nitric oxide, cytokines, and chemokines) or are acute phase proteins produced by the liver that circulates in the plasma (i.e., the complement, coagulation, and kallikrein-kinin systems). Together, the mediators of inflammation orchestrate all the inflammatory events such as blood vessel dilation, vascular permeability, leukocyte migration to the affected tissue and pain (Galvao et al., 2018).

Inflammation and Obesity

Obesity is a growing public health problem and as of 2016, affects 35% of US adults (Kolb, 2016). It has been estimated that nearly 20% of deaths in US adults between 1986 and 2006 were related to obesity (Masters et al., 2013). The health risks from obesity arise from its association with the increased risk of several diseases including hypertension, type 2 diabetes, cardiovascular disease, osteoarthritis, kidney failure, liver disease, and several types of cancer (Martin-Rodriguez et al., 2015).

Obesity-associated inflammation is first triggered by excess nutrients and is primarily localized in specialized metabolic tissues such as white adipose tissue (Gregor and Hotamisligil, 2011), which acts as a major source of energy and is primarily composed of adipocytes. Adipocytes are endocrine cells that secrete a large range of cytokines, hormones, and growth factors referred to as adipokines, and specialize in the storage of energy as triglycerides in cytoplasmic lipid droplets (Fasshauer and Bluher, 2015). Excess nutrients leads to the activation of metabolic signaling pathways including c-Jun N-terminal kinase (JNK), nuclear factor κ B (NFκB), and protein kinase R (Solinas

and Karin, 2010). Activation of these pathways leads to an induction of low-level inflammatory cytokines resulting in a low-grade inflammatory response (Gregor and Hotamisligil, 2011). This low-grade inflammatory response associated with obesity leads to changes in immune cell infiltration and polarization in white adipose tissue (Han and Levings, 2013).

Obesity caused by long-term energy imbalance is an epidemic disease in developed and developing countries. This chronic disease is one of the major criteria for metabolic syndrome and is also associated with high blood pressure, hyperlipidemia, insulin resistance, and pro-inflammatory status. One study found that apple cider vinegar can be beneficial for the suppression of obesity-induces oxidative stress in high-fat-diet rats through the modulating antioxidant defense system and reduces the risk of obesity-associated diseases by preventing the atherogenic risk (Halima et al., 2018).

Inflammation and Chronic Disease

Chronic inflammatory diseases have been recognized as the most significant cause of death in the world today, with more than 50% of all deaths being attributable to inflammation-related diseases such as ischemic heart disease, stroke, cancer, diabetes mellitus, chronic kidney disease, non-alcoholic fatty liver disease (NAFLD) and autoimmune and neurodegenerative conditions (GBD 2017 Cause of Death Collaborators, 2018). Although intermittent increases in inflammation are crucial for survival during physical injury and infection, recent research has revealed that certain social, environmental and lifestyle factors can promote systemic chronic inflammation

(SCI) that can, in turn, lead to several chronic diseases that collectively represent the leading causes of disability and mortality worldwide (Furman et al., 2019).

Empirical evidence that inflammation plays a role in disease onset or progression is strongest for metabolic syndrome, type 2 diabetes, and CVD. It has been known that patients with autoimmune diseases such as rheumatoid arthritis that are characterized by systemic inflammation have insulin resistance, dyslipidemia, and hypertension, and that they have higher rates of metabolic syndrome, type 2 diabetes, and CVD (particularly ischemic heart disease and stroke) (Straub et al., 2010). Moreover, the inflammatory biomarker high-sensitivity C-reactive protein (CRP) is a predictor of cardiovascular events in men and women (Ridker, 2016). In a recent meta-analysis of data from more than 160,000 people across 54 long-term prospective studies, higher levels of circulating CRP were associated with a relative increase in risk for both coronary heart disease and CVD mortality (Emerging Risk Factors Collaboration et al., 2010).

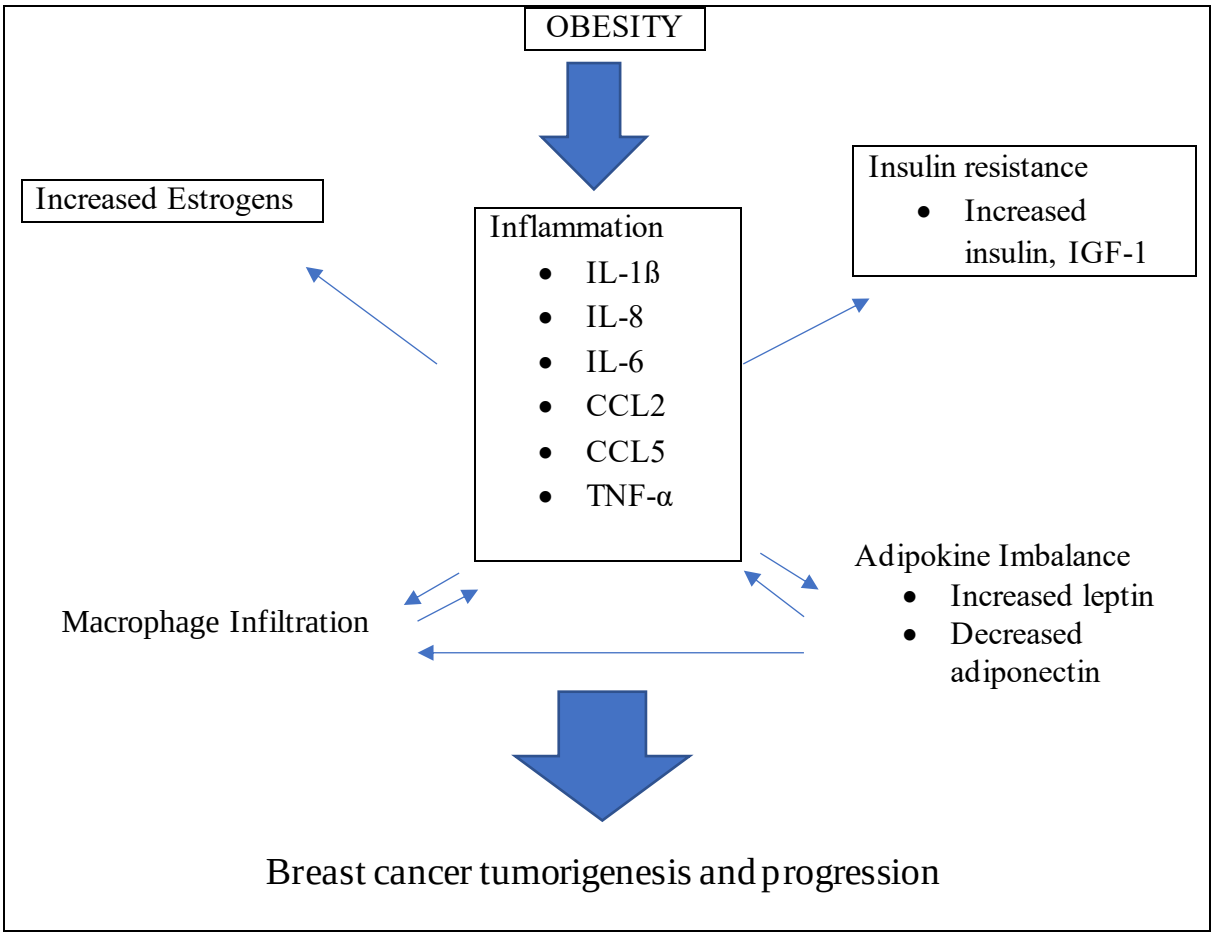
Inflammation and Cancer

Inflammation is the main link between obesity and cancer, and because of this connection, obesity increases the risk of many cancer types and is associated with poor outcomes (Kolb, 2016). It has been estimated that 14% of cancer deaths in men and 20% in women are attributable to obesity (Amer et al., 2006). Chronic inflammation, a phenotype associated with obesity, has been known to be a major factor that contributes to the disease progression of many chronic conditions (Kolb, 2016).

A growing amount of evidence supports a link between obesity-associated inflammation and breast cancer incidence and progression. Adipose tissue in the breast

has been shown to be involved in obesity-associated inflammation, with increased adipocyte hyperplasia and cell death, cytokine production and macrophage infiltration (Sun et al., 2012). Transcriptomic and Gene Set Enrichment analysis of data from luminal breast cancer patients has shown that obesity is associated with the enrichment in genes and pathways associated with inflammation and immune cell trafficking in obese patients compared to non-obese patients (Fuentes-Mattei et al., 2014). These pathways were also enriched in obese MMTV-TGFα/α mice, a model for luminal breast cancer in which obesity promotes tumor progression. A recent report has shown that interaction between adipocytes and breast cancer cells leads to the production of inflammatory cytokines and increase the number of cancer cells with tumor-forming capabilities (Picon-Ruiz et al., 2016). Many studies have demonstrated that elevated levels of inflammatory cytokines including IL-6, TNF-α, and IL-8 are associated with increased breast cancer tumor growth and poor patient outcomes (Kolb, 2016). The figure below demonstrates how obesity leads to breast cancer tumorigenesis and progression.

Figure 4: Obesity and Breast Cancer Progression (Adapted from Kolb, 2016)



Vinegar as an Anti-inflammatory Agent

Vinegar and Chronic Inflammation

Chronic inflammation, also referred to as slow, long-term inflammation lasting for prolonged periods of several months to years, affects the body's ability to repair and overcome damage. Inflammation occurs when your immune system sends out cells to fight bacteria or heal an injury. One study found that polyphenol-rich vinegar extract regulates intestinal microbiota and immunity and prevents alcohol-induced inflammation in mice. This study showed that certain bacteria had a negative correlation with parameters of oxidative stress and inflammation (Xia et al., 2021).

Another study found that ethanol promoted the expression of proinflammatory genes in vitro, whereas coincubation with resveratrol (a potent SIRT agonist) inhibited this effect (Li et al., 2021). Conversely, the addition of sirtinol (a known SIRT inhibitor) augmented the proinflammatory gene expression. Taken together, our findings suggest that melanoidins exert anti-inflammatory and antioxidant functions via abolishing decreases in SIRT1 and SIRT3 expression and cellular NAD^+ levels in ethanol-induced macrophages and may serve as a new therapeutic agent for the prevention of alcohol-induced cell damage (Li et al., 2021). Shanxi-aged vinegar (SAVEP), a traditional Chinese grain-fermented food that is rich in polyphenols, also shows results that it may have a potential function against inflammation (Du et al., 2021). This study further investigated the protective effect of SAVEP on lipopolysaccharide-induced inflammation in RAW 264.7 macrophages and ICR mice. The results showed that compared with those of the model group, SAVEP could remarkably recover inflammation of macrophage RAW 264.7 and ICR mice (Du et al., 2021).

Vinegar and Oxidative Stress

Vinegars are also a dietary source of polyphenols, compounds synthesized by plants to defend against oxidative stress, ingestion of polyphenols in humans enhances in vivo antioxidant protection and reduces cancer risk (Johnston and Gaas, 2006). The expression of genes related to oxidative stress significantly decreases in response to ethanol (Li et al., 2021). One study found that vinegar extract ameliorated ethanol-induced liver injury by reducing the levels of oxidative stress biomarkers (Xia et al., 2021). Additionally, it showed a positive correlation with intestinal immune factors and

antimicrobial peptides, and a negative correlation with parameters of oxidative stress and inflammation (Xia et al., 2021).

Another study found that apple cider vinegar attenuates oxidative stress and reduces the risk of obesity in high-fat-fed rats (Halima et al., 2018). This study tested whether a daily dosage of apple cider vinegar (ACV) would affect cardiovascular risk factors associated with obesity in high-fat diet-induced hyperlipidemic rats. These obese rats showed increased serum total cholesterol triglyceride, low-density lipoprotein-cholesterol (LDL-C), very low-density lipoprotein (VLDL), and atherogenic index after 6 and 9 weeks of being fed an HFD. Importantly, ACV ameliorated all of these parameters significantly. Oxidative stress already developed after 6 weeks of HFD and was significantly reduced by daily doses of ACV (Halima et al., 2018). The findings of this study suggested that HFD alters the oxidant-antioxidant balance, as evidenced by a reduction in the antioxidant enzyme activities and vitamin E level, and enhanced lipid peroxidation. Apple cider vinegar can be beneficial for the suppression of obesity-induced oxidative stress in HFD rats through the modulating antioxidant defense system and reduces the risk of obesity-associated diseases by preventing the atherogenic risk (Halima et al., 2018).

Innate Immunity

Cytokines and Chemokines

Cytokines are involved in virtually every facet of immunity and inflammation, including innate immunity, antigen presentation, bone marrow differentiation, cellular recruitment and activation, and adhesion molecule expression (Figure 5).

Figure 5: Cytokines and Chemokines (Adapted from Borish and Steinke, 2003)

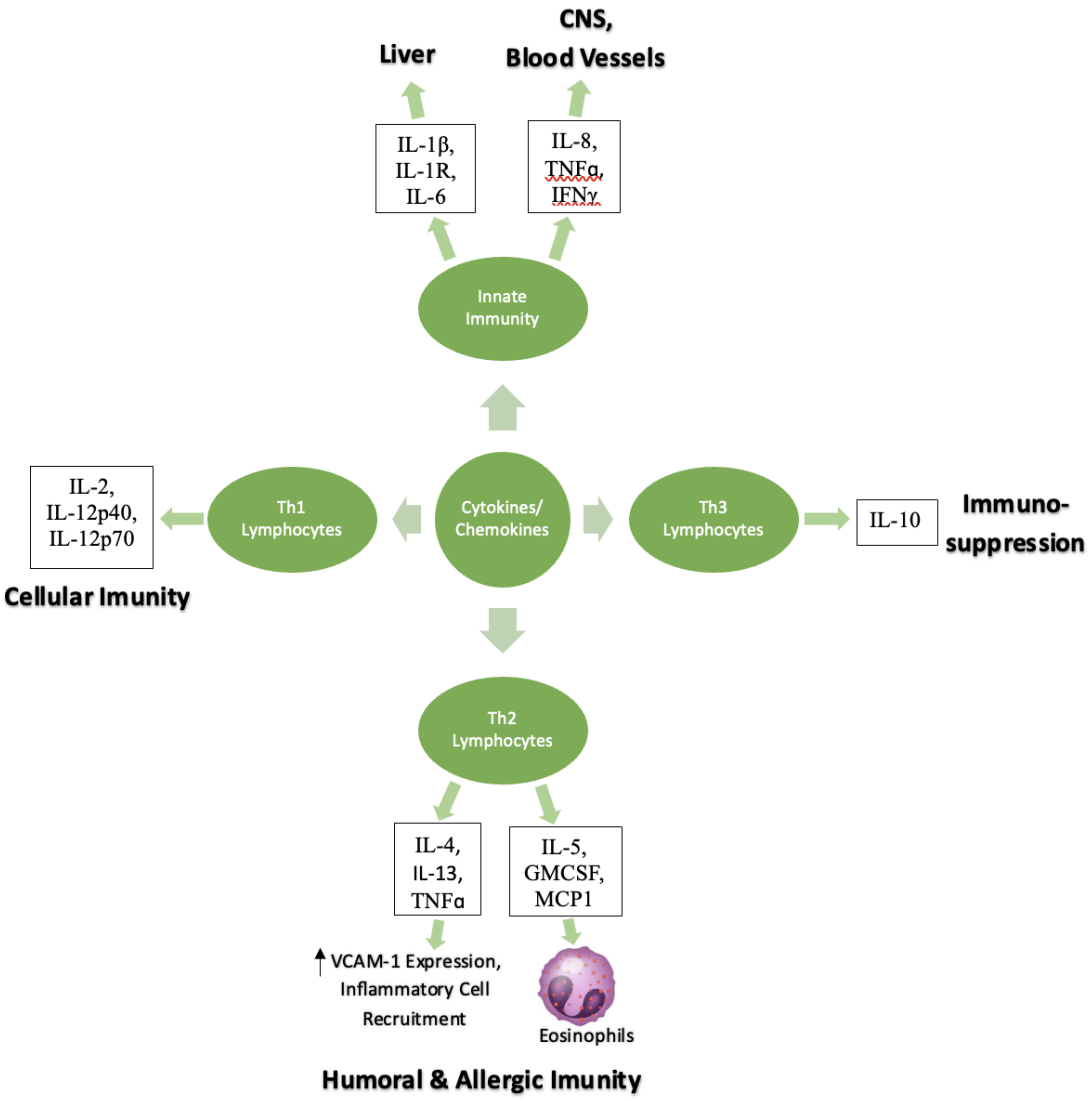


Fig 5. Summary of actions of cytokines and chemokines. Cytokines derived predominantly from mononuclear phagocytic cells are uniquely important in innate immunity and both initiate immune responses and generate symptoms associated with infections and inflammatory disorders. Th1-like lymphocytes are characterized by their production of IFN-γ and primarily contribute to cellular immunity. Th2-like lymphocytes are characterized by their production of IL-4, IL-5, and IL-13 and contribute to humoral and allergic responses. Th3-like lymphocytes have immunosuppressive tendencies and are characterized by their production of IL-10.

Which cytokines are produced in response to an immune insult determines initially whether an immune response develops and subsequently whether that response is cytotoxic, humoral, cell-mediated, or allergic (Borish and Steinke, 2003).

Cytokines and The Immune Responses to Allergens

The pattern of cytokine response to allergens observed in nonallergic individuals is even more complex. Normal individuals are exposed to the same concentrations of allergens as their allergic counterparts living in the same environment. Remaining healthy requires active systems that prevent the development of inflammation. It is frequently stated that the immune response to allergens in nonallergic subjects is characterized by T_H1 lymphocyte responses (Borish and Steinke, 2003). However, functional T_H1 lymphocyte responses stimulate the recruitment and activation of mononuclear phagocytes and are associated with cellular immunity and granuloma formation, features not present in healthy subjects. If present in vivo, these T_H1 lymphocytes must therefore be present in a milieu that prevents cellular inflammation from developing. The absence of inflammation in normal subjects is maintained by influences that promote the development of tolerance (Borish and Steinke, 2003).

Interleukins

IL-2, IL- 5, IL-6, and IL-9 synergize with IL-4 and IL-13 to enhance IgE secretion. IL-4 is responsible for the differentiation of IL-4–producing lymphocytes and recruitment of these T_H2-like cells is promoted by CCL2 (MCP-1). IL-12, IL- 18, and IL-23 inhibit the differentiation of IL-4–producing T cells, and recruitment of these T_H1-like cells is mediated by CCL5 (RANTES). IL-5 is the most important eosinophilopoietin and with

GM-CSF and IL-3 prolongs the survival of mature eosinophils and activates them. These three cytokines, along with TNF and the interferons, are responsible for generating the activated eosinophils that characterize the asthmatic state (Borish and Steinke, 2003).

Many cytokines contribute to the inflammatory state of allergic inflammatory disorders. IL-1, TNF, and IFN-γ increase the expression of endothelial cell adhesion molecules such as ICAM-1 and support the egress of mononuclear cells, neutrophils, and eosinophils into the lungs. Induction of VCAM-1 by IL-4 and IL-13 may promote the selective recruitment of eosinophils, basophils, and lymphocytes. Many cytokines and chemokines may then contribute to the activation of these leukocytes once they reach the airways (Borish and Steinke, 2003).

CHAPTER 3

METHODS

Participants

Participants were recruited after Arizona State University Institutional Review Board (IRB) approval was obtained (Appendix C). Power calculations determined that at least 30 participants should be recruited for this study (Appendix B). Participants were recruited starting January 2023 via social media posts, flyers, emails, and word of mouth from the Arizona State University Downtown Phoenix Campus and the surrounding community. Individuals who were interested in the study filled out a questionnaire to determine eligibility (Appendix D). Participants were healthy, non-smoking adults between the ages of 18-41 years, not taking any new medications within the last 3 months, and free of conditions such as diabetes, heart disease, cancer, gastrointestinal disease, liver or kidney disease, or any other chronic disease. Inclusion criteria include BMI ≥ 25 and ≤ 35. Women were excluded if they were pregnant or lactating. Subjects were willing to adhere to the study design (vinegar drink or pill consumption), and travel to the Wexford building in Downtown Phoenix (850 N. 5th St.) on two separate occasions for 45-minute visits, as well as complete a 24-hour diet recall at each visit.

Subjects were screened for eligibility using an online questionnaire. Females must have a dress or pant size greater than 10, and men must have a pant size above 36 inches, participants should also have a BMI greater than or equal to

23. The trial is a short duration, lasting only 4 weeks. Those who met these criteria were contacted via email to verify eligibility and to schedule the first study visit. These individuals were sent a copy of the consent to read ahead of the first study visit, which was when written consent was obtained. Forty-five eligible participants were randomized into the trial. The number indicated to be sufficient for significant statistical power (.05 alpha and .20 beta) was 30 (Appendix B). Subjects were provided with more information about the study, such as the length of the trial, data collection methods, possible risks and benefits, and contact information for the researchers. At least one researcher was present at each visit to provide further detail about the study and/or answer questions.

Study Design and Procedure

This study was conducted as a 4-week, randomized-controlled, parallel arm trial. After the informed consent was signed, the subjects were stratified by gender, age, and weight, and then randomized into two groups: the intervention group, vinegar drink (VIN), or the control group, vinegar pills (CON). The participants knew which treatment they were given but they did not know which group was the intervention vs. control. Those who received the VIN were instructed to drink 2 tablespoons of red wine vinegar (provided) mixed with at least 8 oz of water before a meal, twice per day for weeks 1-4 of the trial. Those in the CON group were instructed to take two vinegar pills daily (provided) for weeks 1-4 of the trial. Participants were instructed to maintain their current diet and exercise habits during the study. They were provided two diet recall forms to fill out, one at baseline and again at week 4 to ensure typical intake had not changed. Fasting

blood draws, height, weight, and waist circumference measurements were taken before the intervention began and after it ended. Fifty-dollar checks were mailed to participants upon completion of the trial.

Study Variables

The independent variable in this study was acetic acid ingestion. Those in the VIN group were instructed to consume a total of four tablespoons each day, separated into two tbsp twice a day (providing 3,500 mg acetic acid total), mixed with water. They received a 4-week supply of bottles of the red wine vinegar (Pompeian Red Wine Vinegar). Red wine vinegar was chosen because of its palatability compared to other vinegars and affordability. For some, a limitation could be the taste. However, consuming it mixed with water or juice helped dull the sharp flavor. If they were in the CON group, they were provided with one bottle of vinegar pills (Spring Valley Apple Cider Vinegar Pills). They were instructed to swallow two whole pills each day (providing 30 mg acetic acid). The main dependent variable in this study was C-reactive protein (CRP). It was expected that those who are in the VIN group would experience a greater decrease in CRP levels in comparison to those in the CON group. Due to the active dose of acetic acid being 750 mg (according to the experts at Bragg), the VIN received well over the active dose at 3,500 mg acetic acid, and the CON group received well below the active dose at only 30 mg acetic acid per day.

Protocol Procedures

Prior to the initial visit, participants completed a medical history questionnaire (Appendix G). The medical history questionnaire verified that the participants did not

have any known medical conditions or took any medication that would deny them participation in the study. The first visit was approximately 45 minutes long. At this visit, after participants provided consent (Appendix E), they filled out some health screening questionnaires (Appendix F, G, H), had their height, weight, waist circumference, and blood pressure measured, as well as completed a 24-hour diet recall (Appendix I). The diet recall had them list their total food and beverage consumption for the past 24 hours, specifying what they ate, how much, and how it was prepared to the best of their knowledge. Double-pass method for the 24-hour diet recall was completed with the participant to clarify any ambiguities and analyzed by a trained investigator using the Food Processor software (version 7.71; ESHA Research, Salem, OR, USA). Additionally, subjects had to fast for at least 10 hours prior to this visit, with the exception of plain water, as the phlebotomist performed blood draws to test anti-inflammatory markers. Participants were given a 4-week supply of either the red wine vinegar or vinegar pills depending on which group they were randomized into. Instructions regarding proper consumption were given; subjects were told to adhere to the same treatment for the entire 4 weeks. Participants were asked to mark off each day they took their supplement on a compliance calendar. Their second visit was also scheduled at the completion of visit one.

Participants received weekly email reminders that also provided study tips and gave them an opportunity to ask questions if needed. Fasting blood draws, 24-hour diet recalls, questionnaires, and anthropometric measurements were repeated after the four weeks at the final visit. Participants turned in their compliance calendar, as well as their

pill container or vinegar bottles to track adherence to study protocol. Participants were compensated with a $50 check upon completion of the trial (Appendix A).

Laboratory Analyses

Participants arrived at the test center after fasting for 10 hours and had their blood taken via venipuncture performed by a certified phlebotomist. Approximately 10 mL of blood was drawn (2 mL via gray top vacutainer tube, 8.5 mL via red top serum vacutainer tube). Standard Blood Specimen Collection by Venipuncture for Study Protocols and Procedures was used (Arizona State University, 2010). Blood was analyzed for CRP, and adiponectin, as well as fifteen cytokines (See Appendix J). Blood was sent to Eve Technologies for the cytokine analysis using the 15-plex system.

Statistical Analyses

Data are reported as mean values $\pm$ standard deviation. The Statistical Package for Social Sciences (SPSS) software (SPSS Incorporated, Chicago, IL, USA) version 28 was used to complete the statistical analyses of the data. A p-value of $<.05$ was considered significant. Shapiro-Wilk, Kolmogorov-Smirnov, and independent t-tests were conducted to test normality of the data and compare the means of the intervention vs. control group. Repeated measures ANOVA (analysis of variance) was conducted to determine group by time interactions for the outcome variables.

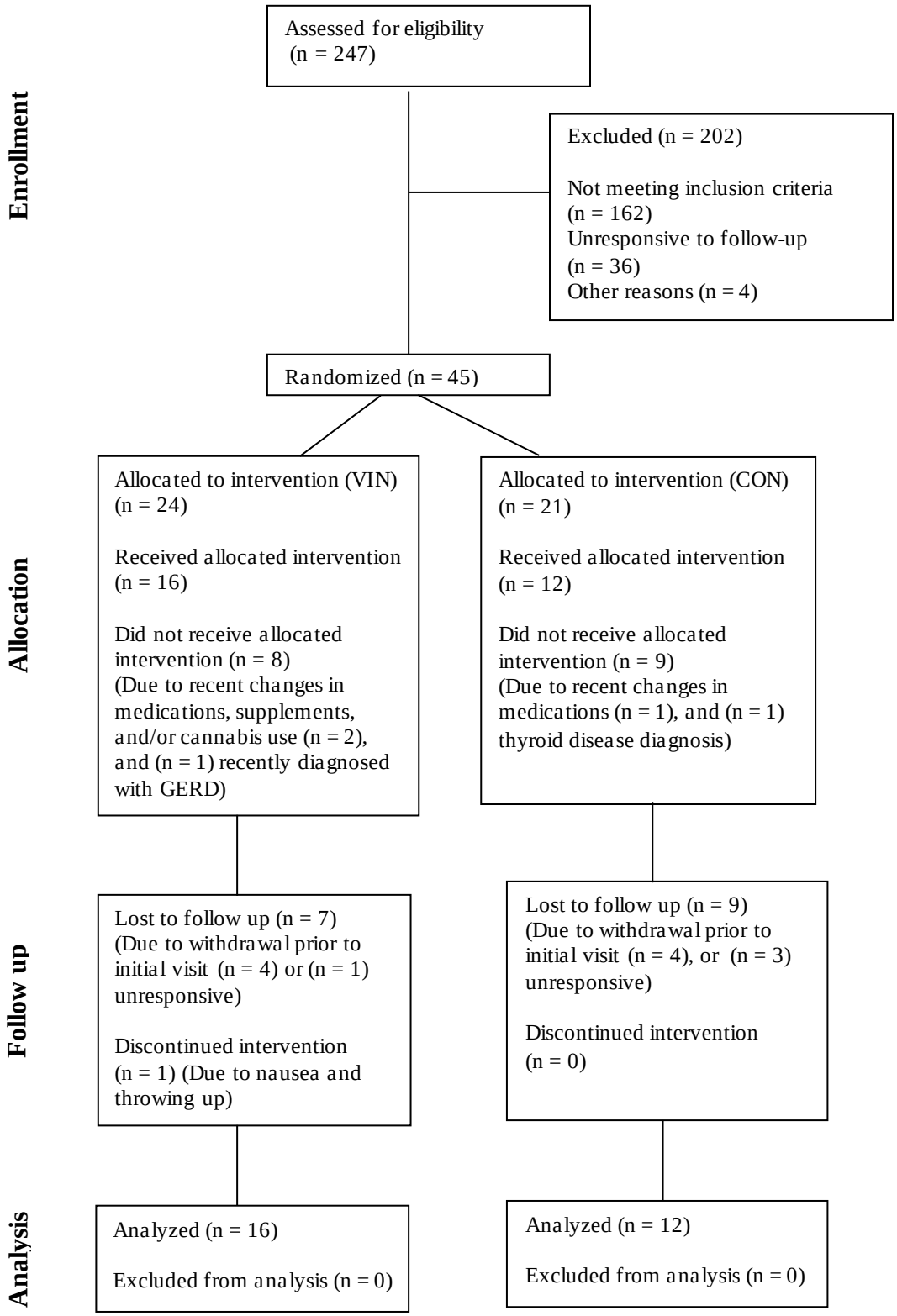
CHAPTER 4

RESULTS

Descriptive Characteristics

A total of 247 participants completed the online survey and were assessed for eligibility. Based on their responses to the survey, 202 participants were excluded from the study based on the established criteria. Specifically, 162 participants did not meet the inclusion criteria, and 36 participants did not respond to follow-up attempts. Additionally, four participants were excluded for other reasons. The remaining 45 participants were emailed to answer any follow-up questions we had regarding the survey and were scheduled for their initial visits. These 45 participants were randomized and assigned to study groups; 24 in the VIN group and 21 in the CON group. For various reasons, only 17 received the allocated liquid vinegar and 12 received the allocated vinegar pills (Table 3). In the VIN group, seven participants did not receive the allocated intervention due to recent changes in medications, supplements, and/or cannabis use, and a recent diagnosis of GERD. An additional participant withdrew during the study due to adverse response to liquid vinegar ingestion. In the CON group, nine participants did not receive the allocated intervention due to recent changes in medications, a recent diagnosis of thyroid disease, or lost to follow-up. For the final analysis, a total of 16 participants from the VIN group and 12 participants from the CON group completed the trial in its entirety and were included in the analysis.

Table 3: Consort Diagram (derived from study participant data)


Enrollment
Assessed for eligibility (n = 247)
Excluded (n = 202)
Not meeting inclusion criteria (n = 162)
Unresponsive to follow-up (n = 36)
Other reasons (n = 4)
Randomized (n = 45)
Allocation
Allocated to intervention (VIN) (n = 24)
Received allocated intervention (n = 16)
Did not receive allocated intervention (n = 8)
(Due to recent changes in medications, supplements, and/or cannabis use (n = 2), and (n = 1) recently diagnosed with GERD)
Allocated to intervention (CON) (n = 21)
Received allocated intervention (n = 12)
Did not receive allocated intervention (n = 9)
(Due to recent changes in medications (n = 1), and (n = 1) thyroid disease diagnosis)
Follow up
Lost to follow up (n = 7)
(Due to withdrawal prior to initial visit (n = 4) or (n = 1) unresponsive)
Discontinued intervention (n = 1) (Due to nausea and throwing up)
Lost to follow up (n = 9)
(Due to withdrawal prior to initial visit (n = 4), or (n = 3) unresponsive)
Discontinued intervention (n = 0)
Analysis
Analyzed (n = 16)
Excluded from analysis (n = 0)
Analyzed (n = 12)
Excluded from analysis (n = 0)

Baseline Characteristics

Initial participant screening included sex, age, BMI, weight, height, ethnicity, and education level using standard protocols to collect the data. Using Mann-Whitney nonparametric tests, there were no significant differences in sex (*P=0.666*), age (*P=0.423*), BMI (*P=0.450*), weight (*P=0.732*), height (*P=0.982*), ethnicity (*P=0.521*), or education level (*P=0.521*) between the VIN and CON groups at baseline (Table 4). There were significantly more females than males included in the study, with 21 females and 7 males. The age range of all participants was between 18-41 years. For the VIN group, the mean age was 25.3±7.3, and for the CON group the mean age was 26.3±6.8. Additionally, for the VIN group, the mean BMI (kg/m^2) was 27.3±3.7, the mean weight (kg) was 79.0±15.2, and the mean height (cm) was 169.9±11.6. For the CON group, the mean BMI (kg/m^2) was 27.9±3.4, the mean weight (kg) was 79.9±13.1, and the mean height (cm) was 169.1±10.1. Ingestion of liquid wine vinegar (VIN group) or vinegar pills (CON group) was tracked using a compliance calendar. The mean compliance percent for the VIN group was 90.3±17.1, and for the CON group 101.1±13.8, (*P=0.029*). Compliance to vinegar supplementation among all reporting participants ranged from 42-100%.

Table 4: Baseline Participant Characteristics (derived from study participant data)

	Vinegar Group	**Control Group**	**p-value**
Sex, n (%)			
Male	4 (25)	3 (25)	0.666
Female	12 (75)	9 (75)	

Age, y	25.3±7.3	26.3±6.8	0.423
BMI, kg/m2	27.3±3.7	27.9±3.4	0.450
Weight, kg	79.0±15.2	79.9±13.1	0.732
Height, cm	169.9±11.6	169.1±10.1	0.982
Ethnicity, n (%) [1]			
Caucasian	9 (56.3)	6 (50.0)	0.521
African American	2 (12.5)	2 (16.7)	
Native American	0 (0.0)	1 (8.3)	
Hispanic	5 (31.3)	3 (25.0)	
Education, n (%) [2]			
High school diploma	6 (37.5)	4 (33.3)	0.521
AA/vocational degree	1 (6.3)	2 (16.7)	
College degree	6 (37.5)	4 (33.3)	
MS degree	3 (18.8)	2 (16.7)	
Adherence, %	90.3±17.1	101.1±13.8	0.029

[1] These chi-square analyses are based on collapsed groups due to sample size. Non-Caucasian groups were collapsed for the analysis.
[2] These chi-square analyses are based on collapsed groups due to sample size. Collapsed to 2 groups for the analysis (HS diploma and AA are one group).

Change Data

Participant data was collected at the initial visit, as well as at the final visit after the 4-week intervention. Data collected included energy intake via 24-hour dietary recalls completed at both visits, as well as physical activity, medications, supplement use, and stress via questionnaires also completed at both visits. Using Mann-Whitney

nonparametric tests, there were no significant differences in energy intake *(P=0.479)*, MET minutes (*P=0.304*), medications (*P=0.365*), or stress (*P=0.907*) between the VIN and CON groups at T1 and T2 (Table 5). T1 and T2 represent Time 1 and Time 2, or Visit 1 and Visit 2, respectively. Supplement use at T1 did not change for T2 (*P=0.823*), therefore, the p-value for this was calculated based on whether supplements were used (yes or no), rather than the change between each visit. For the VIN group, the mean energy intake in kcal for T1 was 2189.2±565.8, and for T2 was 2324.1±697.5. For the CON group, the mean energy intake in kcal for T1 was 2166.5±837.8, and for T2 was 2178.6±796.8. The mean change for energy intake was 134.9±708.1 kcal for the VIN group, and 12.1±760.2 kcal for the CON group. For the VIN group, the mean MET minutes for T1 was 35.6±17.0 MET minutes, and for T2 was 37.8±19.8 MET minutes. For the CON group, the mean MET minutes for T1 was 38.3±20.4 MET minutes, and for T2 was 35.0±18.1 MET minutes. The mean change for MET minutes was 2.2±11.6 MET minutes for the VIN group, and -3.4±18.1 MET minutes for the CON group. For the VIN group, the mean medications for T1 were 0.69±1.08 medications, and for T2 was 0.75±1.07 medications. For the CON group, the mean medications for T1 were 0.50±0.67 medications, and for T2 was 0.50±0.67 medications. The mean change for medications was 0.06±0.25 medications for the VIN group, and 0.00±0.00 medications for the CON group. Regarding supplement use at T1, for the VIN group, 6 (37.5%) said yes and 10 (62.5%) said no. For the CON group, 5 (41.7%) said yes and 7 (58.3%) said no. For the VIN group, the mean stress scores for T1 were 3.9±2.5 arbitrary units, and for T2 were 3.5±2.3 arbitrary units. For the CON group, the mean stress scores for T1 were 2.4±2.3

arbitrary units, and for T2 were 1.7±1.5 arbitrary units. The mean change for stress was -0.38±2.22 arbitrary units for the VIN group, and -0.75±2.93 arbitrary units for the CON group.

Table 5: Participant Change Data (derived from study participant data)

	Vinegar Group	**Control Group**	**p-value**
Energy Intake, kcal			
T1	2189.2±565.8	2166.5±837.8	
T2	2324.1±697.5	2178.6±796.8	
Change	134.9±708.1	12.1±760.2	0.479
Physical Activity, MET minutes			
T1	35.6±17.0	38.3±20.4	
T2	37.8±19.8	35.0±18.1	
Change	2.2±11.6	-3.4±18.1	0.30
Medications			
T1	0.69±1.08	0.50±0.67	
T2	0.75±1.07	0.50±0.67	
Change	0.06±0.25	0.00±0.00	0.365
Supplement Use at T1, n (%)			
Yes	6 (37.5)	5 (41.7)	0.823
No	10 (62.5)	7 (58.3)	
Stress			
T1	3.9±2.5	2.4±2.3	

T2	3.5±2.3	1.7±1.5	
Change	-0.38±2.22	-0.75±2.93	0.907

*T1 and T2 represent Time 1 and Time 2, or Visit 1 and Visit 2, respectively.

Numerous cytokines have both proinflammatory and anti-inflammatory potential, which activity is observed depends on the immune cells present and their state of responsiveness to the cytokine (Borish and Steinke, 2003). The targeted proinflammatory cytokines were IL-1β, IL-2, IL-6, IL-12p70, IL-17A, IFNγ, and tumor necrosis factor (TNF)-α, whereas the anti-inflammatory cytokines were IL-4, IL-5, IL-10, and IL-13. The cytokines and chemokines were grouped by immune function, (see Figure 5 on p. 30). The first group being 'Innate Immunity' which consists of IL-1β, IL-1R, IL-6, IL-8, IL-TNFα, and IL-IFNγ. The second group is 'Th1 and Th3 Lymphocytes' which consists of IL-2, IL-12p40, IL-12p70, and IL-10. The third and final group is 'Th2 Lymphocytes' which consists of IL-4, IL-5, IL-13, GMCSF, and MCP1 (CCL2). All these immune function groups are portrayed in the tables below (Tables 6, 7 and 8) comprising of all the values for vinegar and control groups including p values and effect sizes. C-Reactive Protein (CRP) levels were also analyzed and are portrayed below (Table 9) comprising of all the values for vinegar and control groups including p values and effect sizes.

Table 6: Innate Immunity Cytokines (mean ± standard deviation)

	Vinegar	**Control**	**p***	**Effect Size**
IL-1β pre (pg/ml)	23.2±9.1	7.8±7.7		
IL-1β post (pg/ml)	20.5±37.3	8.8±12.7		

ΔIL-1β (pg/ml)	-2.7±6.0	1.0±6.6	0.102	0.112
IL-1R pre (pg/ml)	9.6±8.1	11.2±10.4		
IL-1R post (pg/ml)	8.6±5.8	11.1±12.2		
ΔIL-1R (pg/ml)	-0.99±6.7	-0.14±10.56	0.855	0.001
IL-6 pre (pg/ml)	4.4±6.2	1.5±0.9		
IL-6 post (pg/ml)	3.7±5.3	1.6±1.2		
ΔIL-6 (pg/ml)	-0.72±2.19	0.09±0.41	0.176	0.078
IL-8 pre (pg/ml)	8.9±2.9	9.2±3.7		
IL-8 post (pg/ml)	9.1±2.7	9.4±3.6		
ΔIL-8 (pg/ml)	0.28±1.8	0.22±1.9	0.938	0.000
TNFα pre (pg/ml)	47.5±36.9	30.2±19.8		
TNFα post (pg/ml)	46.2±31.1	29.5±21.5		
ΔTNFα (pg/ml)	-1.36±13.6	-0.76±7.4	0.765	0.004
IFNγ pre (pg/ml)	6.8±12.4	2.03±2.2		
IFNγ post (pg/ml)	6.4±11.1	2.02±2.8		
ΔIFNγ (pg/ml)	-0.45±2.21	-0.003±1.25	0.420	0.027

*All cytokines are pro-inflammatory.
*p value: for change data, outliers removed and BMI, gender controlled.
Effect size explains the % of variance related to the intervention.

Table 7: Th1 and Th3 Cytokines (mean ± standard deviation)

	Vinegar	**Control**	**p***	**Effect Size**
IL-2 pre (pg/ml)	6.5±13.5	2.2±2.9		
IL-2 post (pg/ml)	7.0±13.3	2.3±4.1		

ΔIL-2 (pg/ml)	0.45±3.5	0.17±1.5	0.862	0.001
IL-12p40 pre (pg/ml)	63.8±48.2	56.5±41.1		
IL-12p40 post (pg/ml)	53.2±42.4	50.6±34.1		
ΔIL-12p40 (pg/ml)	-10.7±16.3	-5.9±24.7	0.552	0.016
IL-12p70 pre (pg/ml)	48.3±97.7	9.3±12.7		
IL-12p70 post (pg/ml)	46.1±98.6	10.4±18.6		
ΔIL-12p70 (pg/ml)	-5.7±12.5	1.04±9.8	0.051	0.162
IL-10 pre (pg/ml)	2.9±1.5	2.5±2.6		
IL-10 post (pg/ml)	2.7±1.3	2.7±3.6		
ΔIL-10 (pg/ml)	-0.13±1.07	0.20±1.22	0.391	0.031

*The cytokines highlighted in green are anti-inflammatory.
*p value: for change data, outliers removed and BMI, gender controlled.
Effect size explains the % of variance related to the intervention.

Table 8: Th2 Cytokines (mean ± standard deviation)

	Vinegar	**Control**	**p***	**Effect Size**
IL-4 pre (pg/ml)	3.2±6.9	0.91±1.2		
IL-4 post (pg/ml)	3.1±6.5	0.84±1.3		
ΔIL-4 (pg/ml)	-0.13±0.99	-0.07±0.365	0.736	0.005
IL-5 pre (pg/ml)	11.4±23.5	4.0±4.6		

IL-5 post (pg/ml)	10.8±22.9	3.6±3.8		
ΔIL-5 (pg/ml)	-0.68±4.02	-0.36±1.74	0.855	0.001
IL-13 pre (pg/ml)	126.6±120.1	61.9±72.7		
IL-13 post (pg/ml)	125.4±112.3	56.7±75.0		
ΔIL-13 (pg/ml)	-1.1±44.4	-5.1±27.3	0.919	0.000
GMCSF pre (pg/ml)	186.7±325.2	40.5±62.7		
GMCSF post (pg/ml)	153.2±269.9	33.3±59.2		
ΔGMCSF (pg/ml)	-33.4±95.5	-7.3±37.4	0.276	0.049
MCP1 pre (pg/ml)	307.6±79.2	330.8±89.1		
MCP1 post (pg/ml)	299.7±80.9	296.3±70.8		
ΔMCP1 (pg/ml)	-7.91±61.9	-34.5±61.1	0.235	0.058

*The cytokines highlighted in green are anti-inflammatory.
*p value: for change data, outliers removed and BMI, gender controlled.
Effect size explains the % of variance related to the intervention.

Table 9: C-Reactive Protein (CRP) Levels (mean ± standard deviation)

	Vinegar	**Control**	**p***	**Effect Size**
CRP pre (pg/ml)	2.2±3.0	2.7±3.1		
CRP post (pg/ml)	3.2±4.9	2.9±3.8		
ΔCRP (pg/ml)	0.94±3.9	0.24±1.04	0.583	0.013

After the analysis, for innate immunity and CRP, there were no significant changes between groups; however, we see that they tend to improve and go in the right

direction (Figures 6 and 9). However, notable reductions in two pro-inflammatory cytokines (IL-1 and IL-12) was observed (Figures 7 and 8).

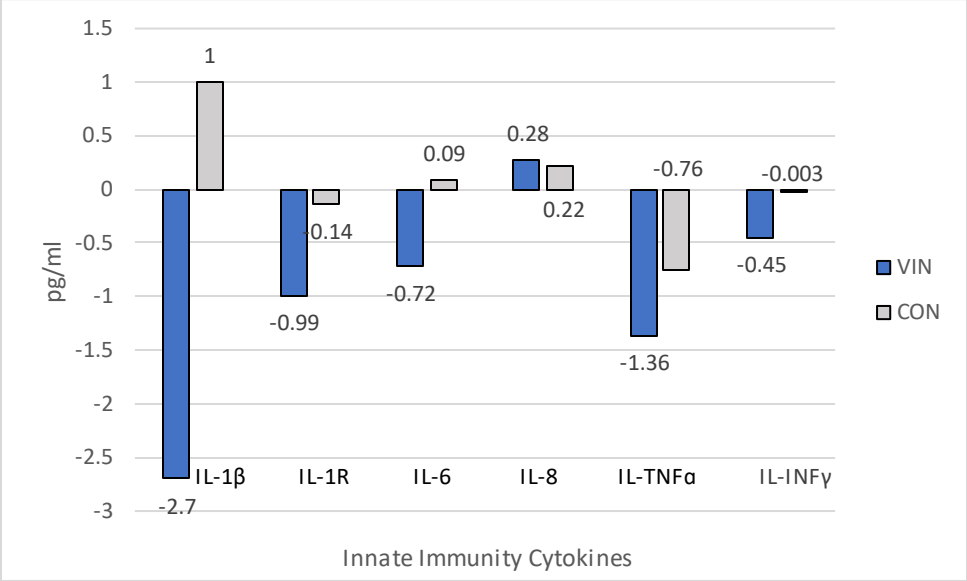


Figure 6: Innate Immunity Cytokines Change Data

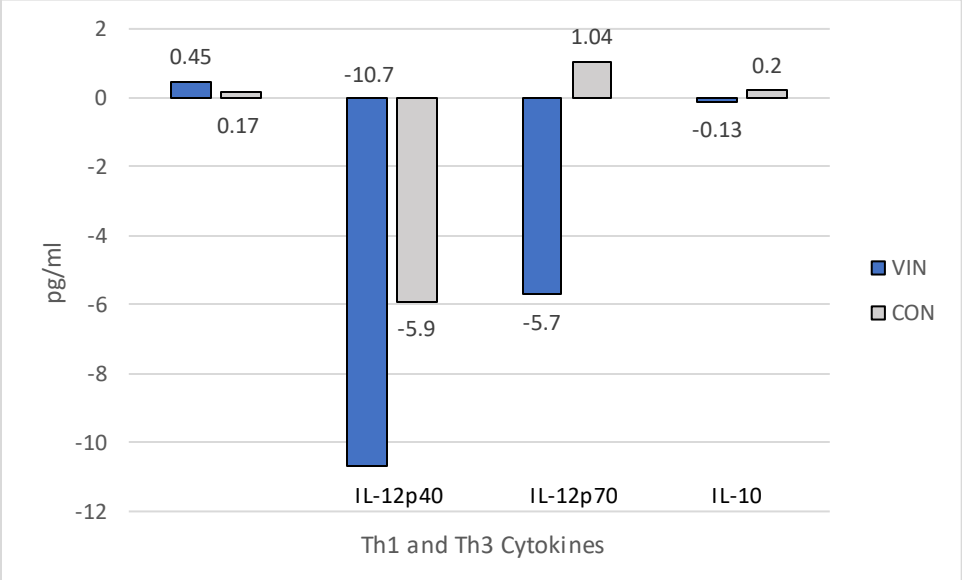


Figure 7: Th1 and Th3 Cytokines Change Data

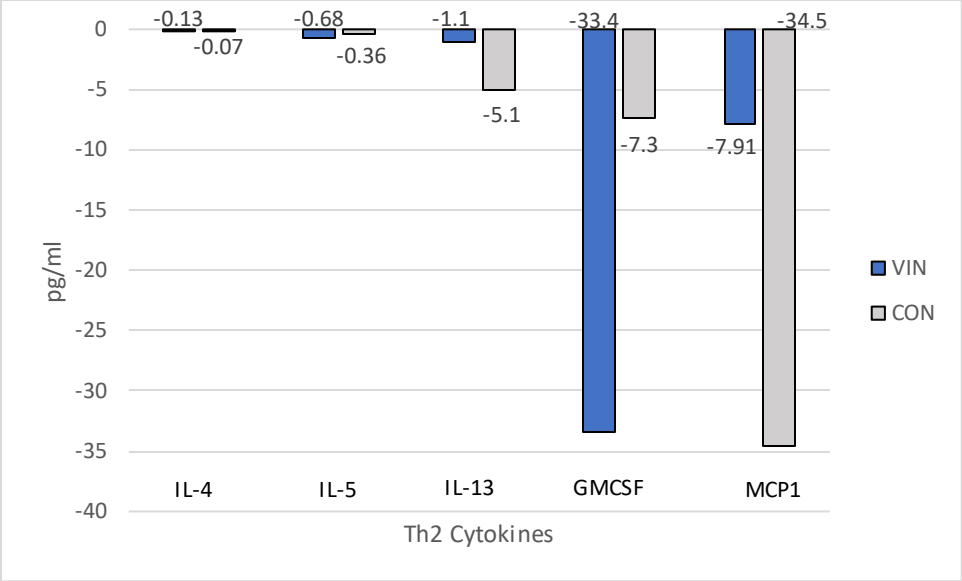


Figure 8: Th2 Cytokines Change Data

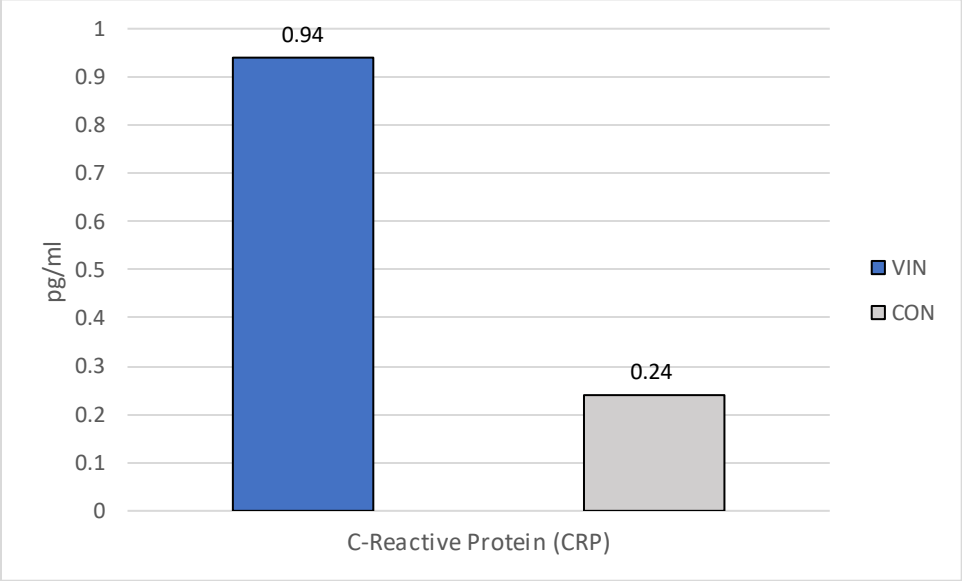


Figure 9: C-Reactive Protein (CRP) Change Data

CHAPTER 5

DISCUSSION

Summary and Discussion

The present study sought to investigate the potential anti-inflammatory effects of vinegar over a 4-week period, focusing on cytokine levels and C-reactive protein (CRP). Notable trends ($p<0.10$) were observed in these parameters, and the study did yield a statistically significant decrease in one of the pro-inflammatory cytokines. One primary limitation contributing to the study not yielding more statistically significant trends is the relatively small sample size, which may have compromised the study's statistical power.

The non-significant findings should be interpreted with caution, considering the inherent variability among participants and the brief, 4-week duration of the intervention. The complex nature of inflammatory responses may require a more extended study period to capture significant changes accurately. Most of the randomized controlled trials (RCTs) examined had durations ranging from 8 to 12 weeks, with the shortest being one month and the longest being three months. It is crucial to highlight the significant and observed trends in the data, suggesting a potential influence of vinegar on cytokine modulation and innate immunity.

Another study sheds light on the significance of the cytokine IL-12 in cellular immunity, notably by promoting lymphocytes to produce IFN-γ. Within this context, the research identifies IL-1β as a novel inducer of IL-12. Collectively, these findings delineate a new mechanism that links adaptive and innate immune responses in the regulation of IL-12 production in dendritic cells, while emphasizing the crucial role of

IL-1β in the development of cellular immunity (Wesa & Galy, 2001). Interestingly, acetate, a component of vinegar, has been associated with lower IL-1 levels, which aligns with the observed modest decrease in IL-1. Although not statistically significant, the decrease in IL-1 can be reported as a noteworthy trend ($p=0.102$) compared to the control group. Importantly, there was a significant decrease in IL-12 ($p=0.051$), a pro-inflammatory cytokine induced by IL-1, suggesting a tandem relationship between these cytokines. IL-1 plays a role in initiating inflammation and IL-12 contributes to the development of cell mediated immunity. The results of this study present a new mechanism linking adaptive and innate immune responses for the regulation of IL-12 production in dendritic cells and underscore the role of IL-1 in the development of cellular immunity (Wesa and Galy, 2001).

The rise in CRP for both groups at week 4 (10-40%), while non-significant, implies a possible increase in inflammation over the course of the trial. A contributing factor to the elevated CRP levels observed in both groups could be linked to the study's participant demographic, predominantly comprised of college students. The study coincided with the end of the semester, a period when students were immersed in final exam preparations. The rise in CRP during the study was significantly correlated to the change in IL-6, the prominent pro-inflammatory mediator ($r=0.397$, $p=0.036$). The observed decrease in IL-1 and the significant reduction in IL-12p70 within the vinegar group, but no tendency to impact on IL-6 or TNFα concentrations, suggests vinegar has a differential impact on the pro-inflammatory cytokines, perhaps related to induction at the cellular level.

Further support for the correlation between acetate and its inflammatory-modulating effects is evident in an additional scholarly work titled "Acetate supplementation attenuates neuroglia activation in a rat model of neuroinflammation." This study explores the mechanisms behind changes in the inflammatory response linked to acetate, including an increase in brain acetyl-CoA, modifications in histone acetylation, and a reduction in interleukin IL-1β expression (Soliman et al., 2012).

There are also various alternative methods aimed at reducing inflammation. These methods often include dietary adjustments, supplements, and herbal remedies. One approach involves incorporating anti-inflammatory foods into one's diet, which can be done by consuming more fruits, vegetables, whole grains, fatty fish like salmon, beans, nuts, seeds, spices and herbs, and olive oil. Like the Mediterranean Diet, these omega-3 fatty acids and antioxidant-rich foods contain compounds known to have anti-inflammatory properties (Brody, 2023). On the other hand, there are also foods that may cause inflammation including ultra-processed, fried, and high in added sugar, salt, or saturated fat. This includes red and processed meats, as well as simple carbs like white breads, cookies, and soda.

Certain dietary and nutritional elements are recognized for their anti-inflammatory and antioxidant effects. These include omega-3 fatty acids, vitamin A, vitamin C, and a range of phytochemicals like polyphenols and carotenoids found abundantly in plant-based foods. Additionally, dietary fiber present in plant-based foods offers various health advantages, including anti-inflammatory properties. This occurs through fermentation by gut microbiota, leading to the production of metabolic

compounds, particularly short-chain fatty acids (SCFA). These active anti-inflammatory compounds play a significant role in maintaining overall homeostasis of inflammation and oxidative stress, both in the pre-infection and acute infection phases. (Iddir et al., 2020).

Moreover, herbs and spices are rich in antioxidants and widely acknowledged for their anti-inflammatory properties. Throughout history, spices have been utilized for their therapeutic, coloring, flavoring, and preservative attributes. Numerous studies have demonstrated that nutraceuticals sourced from spices like clove, coriander, garlic, ginger, onion, pepper, turmeric, and others effectively prevent and treat a range of chronic diseases by targeting inflammatory pathways. This review underscores the link between inflammation and chronic illnesses, highlighting the advantageous role of spices in mitigating these prevalent health concerns (Kunnumakkara et al., 2018).

In conclusion, the current findings contribute valuable insights into the potential of vinegar ingestion to modulate pro-inflammatory cytokines. The observed trends and significant decrease in IL-12, coupled with supporting literature, lay the foundation for further exploration. Recommendations for future research include expanding participant pools, extending intervention periods, and considering the intricate interplay of cytokines in designing comprehensive studies in this domain. The study's limitations, though acknowledged, should not overshadow the promising trends observed, and the groundwork laid invites researchers to delve deeper into the intriguing potential of vinegar to modulate the pro-inflammatory milieu.

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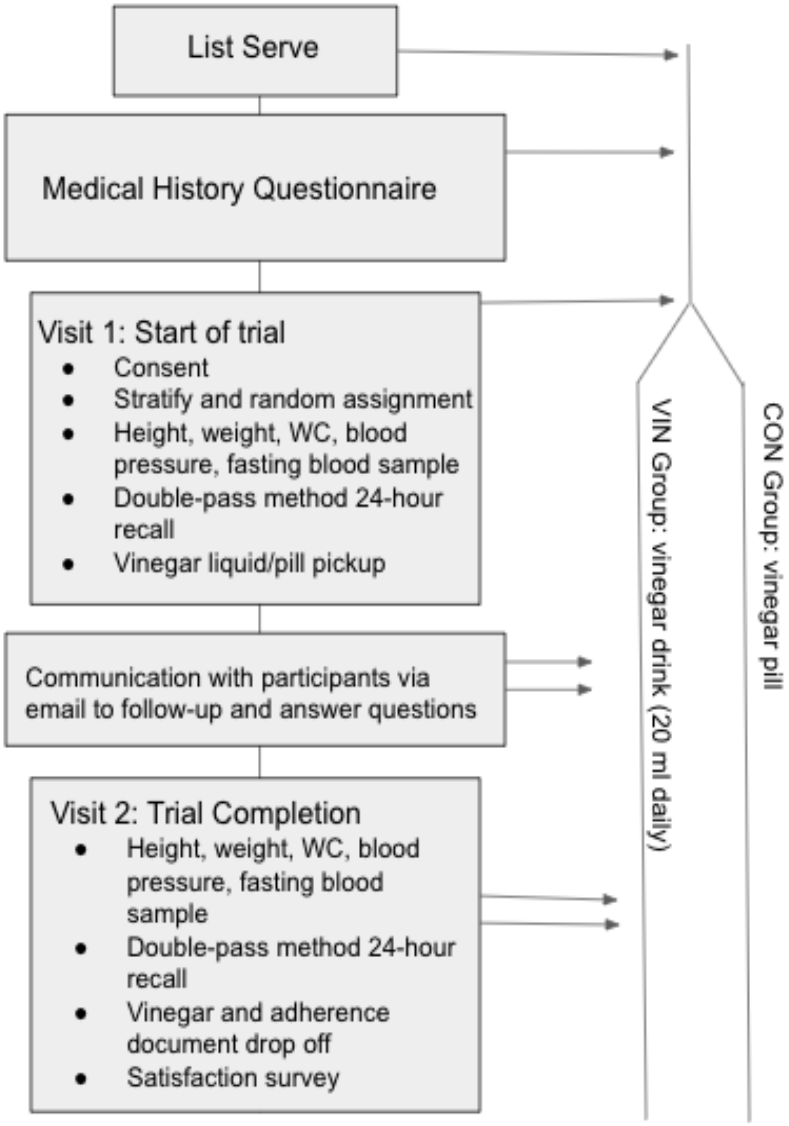
APPENDIX A

STUDY DESIGN FLOW CHART


List Serve
Medical History Questionnaire
Visit 1: Start of trial
Consent
Stratify and random assignment
Height, weight, WC, blood pressure, fasting blood sample
Double-pass method 24-hour recall
Vinegar liquid/pill pickup
Communication with participants via email to follow-up and answer questions
Visit 2: Trial Completion
Height, weight, WC, blood pressure, fasting blood sample
Double-pass method 24-hour recall
Vinegar and adherence document drop off
Satisfaction survey
VIN Group: vinegar drink (20 ml daily)
CON Group: vinegar pill

APPENDIX B

SAMPLE SIZE CALCULATION

Author	year	Change ±SD or correlation coefficient	n per group	Calculated n per group	Age range	Subject health status
Mahmoud, Abeer	2019	1.9	40 per group	71 per group	18-50	Obese adults, BMI ranges between 30 and 39.9 kg/m^2
Kopf, Julianne	2018	.1	16 per group	17 per group	18-70	BMI > 25 kg/m^2, no diagnosed GI diseases
Jovanovic, Gordana	2021	1.2	10 per group	70 per group	18-50	BMI > 30 kg/m^2, with or without obesity related complications
Camhi, Sarah	2009	1.6	35 per group	17 per group	30-64	BMI of at least 34 kg/m^2 for men and at least 32 kg/m^2 for women
Razavi, Roghaye	2020	1	37 per group	9 per group	25-60	BMI of at least 25 kg/m^2
Mohorko, Nina	2019	.1	19 per group	17 per group	30-45	Sedentary obese adults, BMI higher than 30 kg/m^2
Avg.		.98	26	33	23-56	

APPENDIX C

IRB APPROVAL

APPROVAL: EXPEDITED REVIEW

Carol Johnston
CHS: Health Solutions, College of
602/496-2539
CAROL.JOHNSTON@asu.edu

Dear Carol Johnston:

On 12/27/2022 the ASU IRB reviewed the following protocol:

Type of Review:	Initial Study
Title:	Effect of daily vinegar ingestion for four weeks on mood state, inflammatory state, and risk for metabolic syndrome in healthy adults
Investigator:	Carol Johnston
IRB ID:	STUDY00017204
Category of review:	
Funding:	Name: Graduate College (GRAD)
Grant Title:	
Grant ID:	
Documents Reviewed:	• Approval, Category: Technical materials/diagrams; • Calendar, Category: Participant materials (specific directions for them); • CES-D, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • consent, Category: Consent Form; • GCP certificate Johnston, Category: Technical materials/diagrams; • GCP certificate Lish, Category: Technical materials/diagrams; • Health History Questionnaire, Category: Screening forms; • PHQ-9, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • protocol, Category: IRB Protocol;

	• Screener, Category: Screening forms; • Test resuts form, Category: Technical materials/diagrams; • verbal script and ad, Category: Recruitment Materials;

The IRB approved the protocol from 12/27/2022 to 12/26/2023 inclusive. Three weeks before 12/26/2023 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 12/26/2023 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:
Alexandra Lish

APPENDIX D

RECRUITMENT

ASU Nutrition Study: *Vinegar and Health*

THE ASU NUTRITION PROGRAM IS RECRUITING ***HEALTHY ADULTS***
TO EXAMINE HOW VINEGAR IMPACTS HEALTH

Participation is voluntary

If you are a healthy adult 18 to 45 years of age, a non-athlete, and not adhering to a specific diet plan - and if you are willing to incorporate vinegar into your diet daily for 4 weeks – please complete our survey.

Participation includes:

- Two site visits (ASU downtown campus) to meet with study investigators and complete health questionnaires and assessments including height, weight, and blood pressure (<45 minutes)
- At each visit, a fasting blood sample (<1 tbsp) will be collected to measure metabolites linked to health
- Consuming a vinegar supplement daily for 4 weeks (all vinegar supplements provided free of charge)
- You will receive $50 at the completion of the study.
- INTERESTED??? Please visit our recruitment site:
  https://www.surveymonkey.com/r/Vinegar

APPENDIX E

CONSENT FORM

College of Health Solutions
Arizona State University

Informed Consent

Vinegar and Health in Young Adults

INTRODUCTION
The purposes of this form are (1) to provide you with information that may affect your decision as to whether to participate in this research study, and (2) to record your consent if you choose to be involved in this study.

RESEARCHERS
Dr. Carol Johnston (ASU Nutrition professor) and MS students Lexie Lish, Hannah Coven, and Haley Barrong have requested your participation in a research study.

STUDY PURPOSE
The purpose of this study is to investigate the effects of vinegar on health parameters in young adults.

DESCRIPTION OF RESEARCH STUDY
You have indicated to us that you are a non-smoker 18 to 40 years of age and healthy. You are not a competitive athlete, and you do not follow specific diets for weight loss, and if female, you are not currently pregnant or planning a pregnancy. Also, you are willing to ingest vinegar, either in liquid or pill form, daily for four weeks. You will be randomly assigned to the liquid vinegar group (2 tablespoons diluted in water and drunk with meals twice daily) or the vinegar pill group (2 pills daily). Otherwise, your diet and daily activities will remain constant. We ask that you maintain your normal physical activities and not initiate a new exercise protocol. You will come to the test site in a fasted state (no food or drink aside from water for 10 hours) on two occasions (study weeks 1 and 4; each visit ~45 minutes) to provide a blood sample (<1 tablespoon per draw), complete several surveys on health, and undergo measurements (e.g., height, weight, blood pressure, and waist circumference). Your blood will be analyzed for markers related to health and immunity (including these common markers: glucose, cholesterol, and the inflammatory marker, CRP) You will be provided with vinegar (liquid or pills) at the first visit, and you will receive emails weekly during the study to answer any questions you may have. At study completion, you will receive $50.

Your blood results (glucose, cholesterol, CRP) can be made available to you once the study is complete. To receive results, you will need to sign the Research Results Acknowledgement Statement at the start of the study.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this website at any time.

RISKS
There are no known risks to this study other than a change to your daily routine by incorporating vinegar ingestion along with meals twice daily. Vinegar has a distinctive taste, which many find unappealing. *You should not participate in this study if you cannot tolerate the taste of vinegar.* The blood draw may result in faintness, nausea, dizziness, and bruising at the site of the needle insertion. A certified phlebotomist, trained to handle these transient effects will be performing the blood draw using standard, sterile procedures.

BENEFITS
This trial is examining possible health benefits of daily vinegar ingestion. Although you may not benefit directly from this research, the data derived from this research will expand our understanding of how vinegar influences metabolism.

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

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

revealed. To maintain confidentiality of your records, Dr. Johnston will use subject codes on all data collected, maintain a master list separate and secure from all data collected, and limit access to all confidential information to the study investigators.

WITHDRAWAL PRIVILEGE

You may withdraw from the study at any time for any reason without penalty or prejudice toward you. Your decision will not affect you any manner. We ask that you notify us in a timely manner if you decide to withdraw from the study, and we will ask you the reason for withdrawing for our records.

COSTS AND PAYMENTS

You will receive $50 after completing the study at visit 2. There are no payments required for this study. You will receive the vinegar (liquid or pills) free of charge, and parking at the test site will be covered.

COMPENSATION FOR ILLNESS AND INJURY

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

VOLUNTARY CONSENT

Any questions you have concerning the research study or your participation in the study, before or after your consent, will be answered by Dr. Carol Johnston, 550 N. 3rd St., Phoenix, AZ 85004. [carol.johnston@asu.edu]

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

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

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

_____ _____ _____

Subject's Signature Printed Name Date

_____ _____

Contact phone number Email

INVESTIGATOR'S STATEMENT

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

Signature of Investigator_____ Date_____

APPENDIX F

HEALTH SCREENING FORM

Health Screening Form

Date: _____

Study Name: _____

Participant ID: _____

Principle Investigator: _____

***In the last 48 hours, have you had any of the following NEW symptoms:**

Muscle Aches	☐ **YES**	☐ **NO**	**Trouble breathing, shortness of breath**	☐ **YES**	☐ **NO**
Fever-like symptoms (headache, alternating chills and sweating, fatigue)	☐ **YES**	☐ **NO**	**Fever of 100.4 F (37.8C) or above (without the use of fever-reducing medications)**	☐ **YES**	☐ **NO**
New Cough	☐ **YES**	☐ **NO**	**Sore throat**	☐ **YES**	☐ **NO**
Nausea, vomiting or diarrhea	☐ **YES**	☐ **NO**	**Loss of smell or taste, a change in taste**	☐ **YES**	☐ **NO**
Rash (especially new rash that look like pimples or blisters)	☐ **YES**	☐ **NO**	**Swollen lymph nodes**	☐ **YES**	☐ **NO**

***CIRCUMSTANCES:**

Have you had close contact with anyone who was diagnosed with or under investigation for the Coronavirus (Covid-19) in the last 14-days?	☐ **YES**	☐ **NO**
Have you been diagnosed with COVID-19, and if yes, has it been less than 10 days since positive test?	☐ **YES**	☐ **NO**
Have you been exposed to someone with suspected/confirmed monkeypox?	☐ **YES**	☐ **NO**

If the person does NOT have a temperature, has *NO symptoms AND answered *NO to the circumstance questions above: **No monitoring required**

If the person has had close contact with someone who was diagnosed with or under investigation for the coronavirus (Covid-19) or monkeypox in the last 14-days OR has been diagnosed with COVID-19 less than 10 days ago AND NO temperature AND *NO symptoms:

Inform the participant it will be necessary to reschedule & have person contact their primary care provider

If the person has a temperature above 100.4F OR has symptoms listed above OR answered YES to circumstance questions:

Inform the participant it will be necessary to reschedule & have person contact their primary care provider

If the person has a new rash:

Inform the participant we will reschedule until they follow up with primary care provider OR medical oversight team can be consulted (i.e., send photo to Dr. Frank LoVecchio for evaluation on his cell 602-999-9729)

Screener name: _____ **Date:** _____

*Answering "Yes" to some of the above questions does not necessarily exclude participants from studies ***conducting COVID-19 specific research***. If COVID-19 research is being performed, the PI will set up screening guidelines.

APPENDIX G

HEALTH HISTORY QUESTIONNAIRE

HEALTH HISTORY QUESTIONNAIRE ID#_____

1. Gender: M F

2. Age: _____

3. Have you lost or gained more than 10 lbs in the last 12 months? Yes No
If yes, how much lost or gained? _____ How long ago? _____

4. Ethnicity: (please circle one) Native American African-American Caucasian Hispanic Asian Other

5. Education: (please circle) High school diploma AA/vocational degree College degree MS degree PhD degree

6. Do you smoke? No, never _____
Yes _____ # Cigarettes per day = _____
I used to, but I quit _____ months/years (circle) ago

7. Women only: Are you pregnant now or plan a pregnancy in the next 3 months? Yes No

8. Do you take any medications regularly? Yes No *If yes, list type and frequency:*

Medication	Dosage	Frequency

9. Do you currently take supplements (vitamins, minerals, herbs, etc.) ? Yes No *If yes, list type and frequency:*

Supplement	Dosage	Frequency

10. Please check (YES/NO) if you currently have or if you have ever been clinically diagnosed with any of the following diseases or symptoms:

	YES	NO		YES	NO
Coronary Heart Disease			Chest Pain		
High Blood Pressure			Shortness of Breath		
Heart Murmur			Heart Palpitations		
Rheumatic Fever			Any Heart Problems		
Irregular Heart Beat			Coughing of Blood		
Varicose Veins			Feeling Faint or Dizzy		
Stroke			Lung Disease		
Diabetes			Liver Disease		
Low Blood Sugar			Kidney Disease		
Bronchial Asthma			Thyroid Disease		
Hay Fever			Anemia		
Leg or Ankle Swelling			Hormone Imbalances		
Eating Disorder			Depression		

Please turn over →→

11. Have you ever had abdominal surgery? Yes No

12. Do you have any of the conditions listed below? Yes No

acid reflux, ascites, pancreatitis, diverticulitis/diverticulosis, Crohn's disease, and/or irritable bowel syndrome

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

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

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

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

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

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

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

14. Are you healthy and fit? Yes No

Comments: _____

15. How much alcohol do you drink? (average #drinks per week) _____

16. Are you a current smoker (cigarettes or cannabis)? Yes No
Are you a former smoker? Yes No *if yes,* when was the last time you smoked? _____

17. Do you have any food allergies? Yes No
If yes, explain: _____

18. Do you follow a special diet? Yes No
If yes, explain: _____ _____

19. Do you plan to change your diet in the next 8 weeks? Yes No
If yes, explain: _____ _____

20. Do you plan to change your exercise level in the next 8 weeks? Yes No
If yes, explain: _____

21. Are you willing to consume vinegar supplements daily for 4 weeks? Yes No

APPENDIX H

PATIENT HEALTH QUESTIONNAIRE (PHQ-9)

PATIENT HEALTH QUESTIONNAIRE (PHQ-9)

ID #: _____ **DATE:** _____

Over the last *2 weeks*, how often have you been bothered by any of the following problems? *(use "✓" to indicate your answer)*	Not at all	Several days	More than half the days	Nearly every day
1. Little interest or pleasure in doing things	0	1	2	3
2. Feeling down, depressed, or hopeless	0	1	2	3
3. Trouble falling or staying asleep, or sleeping too much	0	1	2	3
4. Feeling tired or having little energy	0	1	2	3
5. Poor appetite or overeating	0	1	2	3
6. Feeling bad about yourself—or that you are a failure or have let yourself or your family down	0	1	2	3
7. Trouble concentrating on things, such as reading the newspaper or watching television	0	1	2	3
8. Moving or speaking so slowly that other people could have noticed. Or the opposite – being so figety or restless that you have been moving around a lot more than usual	0	1	2	3
9. Thoughts that you would be better off dead, or of hurting yourself	0	1	2	3
		add columns	+	+

(Healthcare professional: For interpretation of TOTAL, please refer to accompanying scoring card). TOTAL: _____

10. If you checked off *any problems,* how *difficult* have these problems made it for you to do your work, take care of things at home, or get along with other people?

- Not difficult at all _____
- Somewhat difficult _____
- Very difficult _____
- Extremely difficult _____

A2663B 10-04-2005

PHQ-9 Patient Depression Questionnaire

For initial diagnosis:

1. Patient completes PHQ-9 Quick Depression Assessment.
2. If there are at least 4 ✓s in the shaded section (including Questions #1 and #2), consider a depressive disorder. Add score to determine severity.

Consider Major Depressive Disorder

- if there are at least 5 ✓s in the shaded section (one of which corresponds to Question #1 or #2)

Consider Other Depressive Disorder

- if there are 2-4 ✓s in the shaded section (one of which corresponds to Question #1 or #2)

Note: Since the questionnaire relies on patient self-report, all responses should be verified by the clinician, and a definitive diagnosis is made on clinical grounds taking into account how well the patient understood the questionnaire, as well as other relevant information from the patient.
Diagnoses of Major Depressive Disorder or Other Depressive Disorder also require impairment of social, occupational, or other important areas of functioning (Question #10) and ruling out normal bereavement, a history of a Manic Episode (Bipolar Disorder), and a physical disorder, medication, or other drug as the biological cause of the depressive symptoms.

To monitor severity over time for newly diagnosed patients or patients in current treatment for depression:

1. Patients may complete questionnaires at baseline and at regular intervals (eg, every 2 weeks) at home and bring them in at their next appointment for scoring or they may complete the questionnaire during each scheduled appointment.
2. Add up ✓s by column. For every ✓: Several days = 1 More than half the days = 2 Nearly every day = 3
3. Add together column scores to get a TOTAL score.
4. Refer to the accompanying **PHQ-9 Scoring Box** to interpret the TOTAL score.
5. Results may be included in patient files to assist you in setting up a treatment goal, determining degree of response, as well as guiding treatment intervention.

Scoring: add up all checked boxes on PHQ-9

For every ✓ Not at all = 0; Several days = 1;
More than half the days = 2; Nearly every day = 3

Interpretation of Total Score

Total Score	Depression Severity
1-4	Minimal depression
5-9	Mild depression
10-14	Moderate depression
15-19	Moderately severe depression
20-27	Severe depression

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APPENDIX I

24-HOUR DIET RECALL

24 Hour Dietary Recall Guide

Subject #_____ Date _____

Upon waking, what food and beverages did you consume?

Food/Beverage	Quantity	Portion Size

What was the next thing you ate or drank?

Food/Beverage	Quantity	Portion Size

What did you have to eat and drink for lunch?

Food/Beverage	Quantity	Portion Size

Did you have any snacks or beverages next?

Food/Beverage	Quantity	Portion Size

What did you have to eat and drink for dinner?

Food/Beverage	Quantity	Portion Size

Did you eat or drink anything else throughout the day or night?

Food/Beverage	Quantity	Portion Size

Is there any condiment, topping, seasoning or food you may have missed, such as: sugar, butter, ketchup, salt, cream cheese, etc.?

Think for a minute. Was there any food, beverage or anything else you may have missed that you consumed yesterday?

Was this a typical day in terms of dietary choices and eating patterns? What differs?

APPENDIX J

CYTOKINE REFERENCE CHART

Cytokine Name	Synonym(s)	Description
IL-1β	Catabolin	Potent pro-inflammatory cytokine that is crucial for host-defense responses to infection and injury
IL-1R	IL-1 receptor antagonist	Blocks IL-1 from binding to it's receptor
IL-6	IFN-β2, BSF-2	Induces synthesis of acute phase proteins such as CRP
IL-8	NAF	Neutrophil-activating factor, promotes NK-cell activity (IL-12p40 and IL-12p70)
IL-TNFα	Cachectin	Suppress tumor cell proliferation and induce tumor regression
IL-IFNγ	IFNG	Plays a role in regulating the immune response of its target cell
IL-2	T-cell growth factor	Promotes development of T regulatory cells
IL-12p40	Natural killer cell stimulatory factor 2	Provides a negative feedback loop by competitively binding to the IL-12 receptor
IL-12p70	Natural killer cell stimulatory factor 1	Heterodimeric cytokine consisting of IL-12p35 and IL-12p40, which are encoded by separate genes
IL-10	CSIF	Limits host immune response to pathogens, prevents damage
IL-4	B-cell stimulating factor	Regulated antibody production, inflammation, and the development of T-cell responses

IL-5	Killer helper factor	Promotes growth, differentiation, and activation of eosinophils
IL-13	P600	Regulates eosinophilic inflammation, mucus secretion, and airway hyperresponsiveness
GMCSF	CSF-2	Promotes the differentiation of granulocytes and macrophages
MCP1	CCL2	Vital role, attracts or enhances the expression of other inflammatory factors/cells

*Derived from Cameron, MJ. et al.; Tau, G. et al.; Wynn, TA.; and Singh, S. et al.