

Reduction of Visceral Fat in Response to Consumption of Red Wine Vinegar

by

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## ABSTRACT

**Objectives:** To investigate the potential of vinegar supplementation as a means for reducing visceral fat in healthy overweight and obese adults, and to evaluate its effects on fasting blood glucose and fasting insulin.

**Subjects and Methods:** Forty-five sedentary overweight and obese adult participants with a waist circumference greater than 32 inches for women and 37 inches for men were randomly assigned to one of two groups, the vinegar group (VIN,  $n=21$ ) or the control group (CON,  $n=24$ ), and instructed to consume either two tablespoons of liquid red wine vinegar (3.6g acetic acid) or a control pill (0.0225g acetic acid) twice daily at the beginning of a meal for 8 weeks. Participants were also instructed to maintain normal diet and physical activity levels. Anthropometric measures, dual-energy x-ray absorptiometry (DXA) scans, blood samples, and 24-hour dietary recalls were collected at baseline and at end of trial. A compliance calendar was provided for daily tracking of vinegar supplementation.

**Results:** Compliance to vinegar supplementation averaged  $92.7 \pm 13.3\%$  among the VIN group and  $89.1 \pm 18.9\%$  among the CON group. There were no statistically significant differences in anthropometric measurements between baseline and week 8: weight ( $P=0.694$ ), body mass index ( $P=0.879$ ), and waist circumference ( $P=0.871$ ). Similarly, DXA scan data did not show significant changes in visceral fat ( $P=0.339$ ) or total fat ( $P=0.294$ ) between baseline and week 8. The VIN group had significant reductions in fasting glucose ( $P=0.003$ ), fasting insulin ( $P < 0.001$ ), and homeostatic model assessment of insulin resistance scores ( $P < 0.001$ ) after treatment.

Conclusions: These data do not support the findings from previous studies that indicated a link between vinegar supplementation and increased fat metabolism, specifically visceral fat reduction.

## DEDICATION

I dedicate this thesis to my mother, Munira Gharib, and in memory of my father, Sami Gharib (1942-2006) who fostered my deep appreciation for food and its pleasurable, cultural, and functional wonders.

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## CHAPTER 1

### INTRODUCTION

The marvels of vinegar have been endorsed throughout history. Evidence of vinegar is speckled along the timeline of ancient civilizations. Babylonians in 5000 BC made date vinegar to preserve food. Urns containing remnants of vinegar were found in Egyptian tombs dating back to 3000 BC. Romans in 1000 BC would dunk their bread in fruit vinegars. Notably, it was in 400 BC in ancient Greece that Hippocrates, deemed the father of modern medicine, would realize its medicinal benefits by prescribing vinegar to treat various illnesses and wounds.<sup>1,2</sup>

The concept that food offers therapeutic benefits in the treatment, prevention and reduction of various diseases would become dubious as the study of pharmacology emerged. However, in this precarious climate of decreased health status in Americans and escalating health care costs, the focus on natural and preventative health practices have taken strong emphasis within public health as obesity and obesity-related conditions became highly prevalent in recent decades.

Obesity-related conditions in particular are of significant concern and pose a great deal of burden on the health care system. Metabolic syndrome is strongly associated with obesity rates.<sup>3-5</sup> This condition is diagnosed when three or more specific risk factors co-exist, increasing risk of stroke, heart disease, and diabetes among others. Those specific risk factors include a waist circumference >40 inches for men and >35 inches for women, high triglyceride levels, low levels of high density lipoproteins (HDL), high blood pressure and high fasting blood sugar.<sup>3-5</sup>

A large body of evidence shows that visceral fat, in particular, has a significantly negative impact on health. Visceral fat is the abdominal fat that surrounds the internal organs and is difficult to lose. On the other hand, subcutaneous fat that builds under the skin has less negative impact on overall health and is relatively easier to lose. Health problems of particular concern include elevated levels of fasting blood glucose and triglycerides, insulin resistance, high blood pressure, and systemic inflammation.<sup>5</sup> Reduction in visceral fat would precede great benefits in overall health as improvements in fasting blood glucose levels, triglyceride levels, insulin sensitivity, blood pressure, and inflammation would be seen.<sup>3-5</sup>

Vinegar consumption has been shown to improve health status as observed in multiple research studies.<sup>6-10</sup> Of substantial relevance is the active ingredient in vinegar, acetic acid. Findings from a rat model revealed a link between the metabolism of acetic acid and fat oxidation.<sup>6</sup> Consumption of vinegar was shown to increase phosphorylation of the adenosine monophosphate-activated protein kinase enzyme, or AMPK, triggering lipolysis in adipose tissue, and fatty acid oxidation in the liver. Despite being fed a high fat diet, increased vinegar consumption in rats resulted in elevated AMPK levels while body weight, fat, and triglycerides decreased.<sup>6</sup>

There is a need for more human studies to identify the potential link between vinegar consumption and fat reduction as observed in several animal trials.<sup>6,11,12</sup> A study that may potentially identify this relationship would provide an optimistic alternative for individuals struggling with associated health consequences or the increased risk of these health consequences.

## **Purpose of Study**

The purpose of this study was to identify a link between vinegar consumption and visceral fat reduction in healthy adults with abdominal obesity. Participants ingested a liquid vinegar drink or a low-dose vinegar control pill two times per day, for an 8-week treatment period.

## **Research Aim and Hypothesis**

**H<sub>1</sub>** Daily red wine vinegar consumption will be associated with visceral fat reduction as measured by Dual-Energy X-Ray Absorptiometry (DXA) after 8 weeks compared to the control (low-dose vinegar pill) in a sample of healthy adults with abdominal obesity.

**H<sub>2</sub>** Daily red wine vinegar consumption will be associated with a decrease in waist circumference after 8 weeks compared to the control (low-dose vinegar pill) in a sample of healthy adults with abdominal obesity.

## **Definition of Terms**

- **Acetic Acid** – The active ingredient in vinegar. It is a weak acid and its chemical structure is  $\text{CH}_3\text{COOH}$ .
- **Abdominal Obesity** – Defined as a large amount of fat in the abdominal region of the body and is an independent risk factor for metabolic syndrome. It is characterized by a waist circumference >40 inches for men and >35 inches for women.
- **Adipokine** – Cell signaling proteins produced by adipose tissue that have a wide range of physiological functions including those to maintain energy homeostasis and promote inflammatory responses.

- **Adiponectin** – An adipokine hormone that functions to help regulate fatty acid metabolism and glucose levels.
- **Body Mass Index (BMI)** – An estimated measure of body fat based on weight relative to height. The formula for BMI is  $[\text{weight (kg)} / \text{height (m)}^2]$ . Adult weight status categories associated with BMI ranges are:
  - Underweight:  $< 18.5 \text{ kg/m}^2$
  - Normal weight:  $18.5 - 24.9 \text{ kg/m}^2$
  - Overweight:  $25.0 - 29.9 \text{ kg/m}^2$
  - Obese:  $\geq 30.0 \text{ kg/m}^2$
- **Dual-Energy X-Ray Absorptiometry (DXA)** – Used to assess bone density and body composition through x-ray technology.
- **Fatty Acids** – Molecules that are a straight, long hydrocarbon chain that terminates with a carboxylic acid group (-COOH) and is a component of more complex lipids. They are classified by their chain length, degree of saturation, specific shape, and essentiality in humans.
- **Free Fatty Acids** – A free fatty acid is a by-product of fat metabolism. They are released from lipid molecules via lipolysis.
- **Leptin** – Known as the “satiety hormone” because it signals the brain to inhibit hunger and promote feelings of fullness. It is primarily produced by adipose cells to maintain energy balance.
- **Metabolic Syndrome** – A specific group of risk factors that are known to increase stroke, diabetes, and heart disease risk. Risk factors include abdominal obesity, elevated levels of triglycerides and fasting blood glucose, low HDL

cholesterol, and high blood pressure. This condition is diagnosed when three or more of these risk factors co-exist, increasing risk of stroke, heart disease, and diabetes among others.

- **Obesity** – A chronic condition defined by excessive amounts of adipose tissue and measured at or above a BMI of 30.0 kg/m<sup>2</sup>.
- **Visceral Fat** – A specific type of fat located in the abdominal cavity of the body and surround the organs; also known as abdominal fat or central fat.

### **Delimitations and Limitations**

Delimitations:

- Recruitment for this study included healthy adults between 18-45 years of age, who are nonsmokers. A minimum waist circumference for men > 37 inches, and waist circumference for women > 32 inches was required. All study participants were healthy, as defined as being free from disease. Female participants could not be pregnant. The results from this study may not apply to age groups or disease states outside these inclusion criteria.

Limitations:

- It was requested that participants maintain normal eating and exercise habits through the 8-week study period. Adherence to this request, however, cannot be guaranteed.
- Participants were given directions for consuming their drink supplements and encouraged to follow them. Adherence to this request, however, cannot be guaranteed.

- Eight weeks may be too short a time span to see a significant change in visceral fat.



## CHAPTER 2

### REVIEW OF THE LITERATURE

#### **Vinegar Sources and Production**

In the simplest definition, vinegar is fermented alcohol. It is the product of the double fermentation of any sugar or starch-containing substrate.<sup>13</sup> Yeasts begin the production process as they feed on the natural sugars found in the carbohydrate source. As it feeds, known as fermentation, yeast breaks down the sugar into carbon dioxide and alcohol (ETOH).<sup>2,14</sup> The acetic acid bacteria (common name), more formally known as *Acetobacter aceti* (*A. aceti* abbreviated), then converts the alcohol to acetic acid, hence it is double fermented.<sup>2,14</sup> For optimal growth of acetic acid bacteria, the temperature range should be maintained at 77-86 degrees Fahrenheit, with a pH within the 5.0-6.5 range.<sup>1</sup> The sour taste and distinctive smell of vinegar emanates from its acetic acid content.<sup>2</sup> Acetic acid is considered a weak acid as it never completely dissociates in water, giving vinegar a very low pH value. It is this low pH value that gives vinegar its antibacterial property. The common commercial vinegar typically contains 4-7% acetic acid. Vinegar with higher concentrations of acetic acid can be unsafe and a health hazard due to risk of skin burns upon contact, and coughing or nausea with inhalation.

Several production methods are used to make vinegar. Commercial production may utilize the traditional, yet slow, process for a higher quality product or speed up the process via submerged culture.<sup>15</sup> The vinegar-making process requires the presence of oxygen, while the fermentation of grapes for wine-making or hops for beer-making requires its absence.

In traditional vinegar-making, a film covers the surface of the solution made up of yeast and acetic acid bacteria. This is known as the mother of vinegar and it attracts vinegar eels. These nematodes are a natural occurrence during the acetification process (conversion of alcohol to acetic acid) and, while harmless, they are filtered from the solution and the vinegar is pasteurized to prevent their reappearance.<sup>2,14</sup> Some vinegar manufacturers promote the mother of vinegar with claims that there are health benefits associated with its consumption beyond that of the vinegar however, these claims have not been scientifically substantiated.

The *A. aceti* bacteria require oxygen to convert alcohol into acetic acid, which is limited to surface exposure to the air in traditional production. In order to speed up the process beyond traditional and naturally organic methods, more oxygen must be provided to the bacteria. The submerged culture method for producing vinegar is characterized by supplying oxygen to the liquid solution during the fermentation process, resulting in an accelerated production of vinegar.<sup>14</sup> The more oxygen that can be provided, the faster the production of the vinegar product. To do this, air bubbles are generated into the solution, increasing the air-liquid surfaces, and thus increasing oxidation.<sup>14</sup> However, an appreciable loss of quality is the consequence of the increase of oxygen flow in order to produce a product quickly and minimize manufacturing costs. To give perspective, a traditional vinegar may take as long as a few months to ferment while industrial vinegar may be produced in as little as one day.<sup>1</sup>

Another variation of vinegar production is an adaptation of the traditional method, known as the Orleans, or continuous, method.<sup>1,2</sup> This vinegar production process allows for air to circulate through holes placed on the sides of the wooden barrels. A funnel is

extended from the barrel base to pour additional liquid directly into the bottom of the barrel solution in order to prevent any alteration to the mother of vinegar sitting on the surface. This method is generally accepted as high quality for its organoleptic complexity.<sup>1,13,14</sup>

The ageing process contributes to the quality of the vinegar product because it takes time for the various elements in the vinegar-making process to permeate, ultimately creating a higher quality product.<sup>15</sup> Elements influencing quality include the source of the vinegar and the complex metabolic and organic chemical reactions that occur during fermentation. Additionally, the biological interactions between the wood materials of the barrels and the bacteria generate a complex network of compounds and aromas, and soften the distinct pungency of the overall product.<sup>1,13,14</sup>

Various organic acids are produced by acetic acid bacteria during the sugar and alcohol oxidation process. While acetic acid is most abundant, other bioactive substances include vitamins, mineral salts, and amino, polyphenolic, and metabolic acids. Red wine vinegar in particular, contains acetic, citric, formic, lactic, malic, succinic and tartaric acids.<sup>1,2,14</sup>

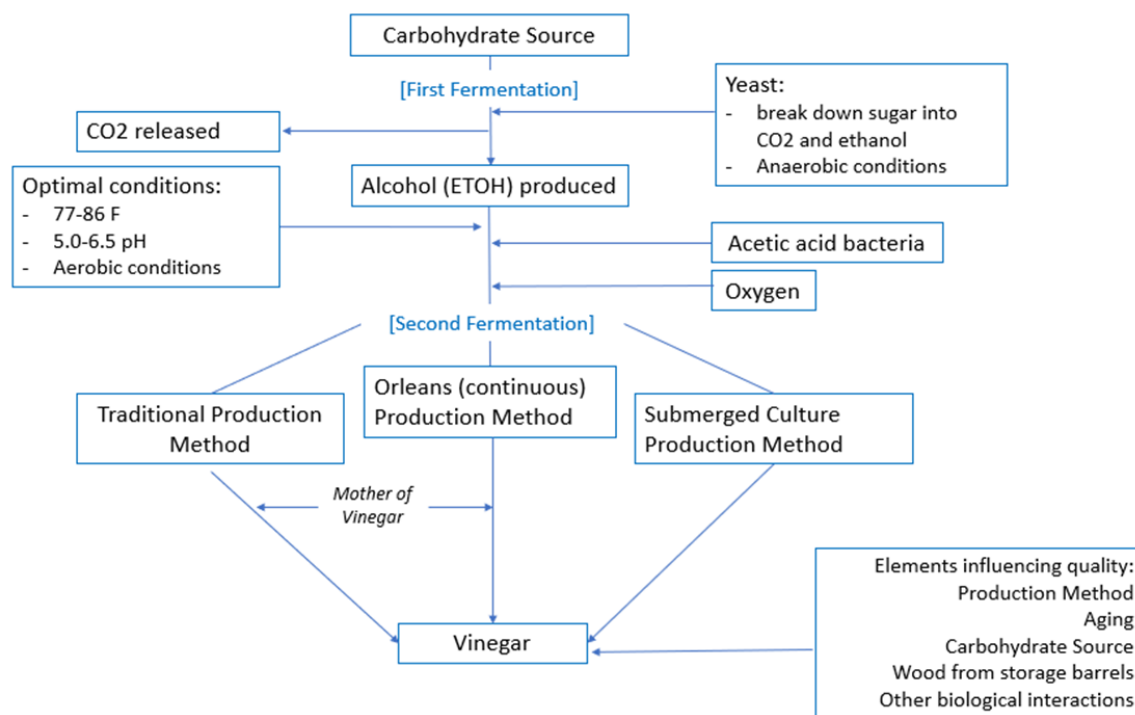


Figure 1. Production of Vinegar Schematic Diagram

Vinegar products sold in the United States must contain at least 4% acetic acid concentration to be classified as vinegar. Common vinegar products will have 4-7% acetic acid concentrations and include red wine, apple cider, raspberry, balsamic, pomegranate, rice, and malt vinegar.<sup>2</sup> In Europe, regional standards are placed on vinegar depending on origin of the vinegar, much like European standards for wine. For example, traditional balsamic vinegar has two classifications (Aceto Balsamico Tradizionale di Modena DOP and Aceto Balsamico Tradizionale di Reggio Emilia DOP) and are regulated products exclusively produced in the Modena province or the greater

Emilia region of Italy and are protected under the *Denominazione di Origine Protetta*, which is translated Protected Designation of Origin (DOP).<sup>15</sup>

## **Vinegar as a Functional Food**

### **History**

The history of vinegar dates back to ancient civilizations from all over the world. In 5000 BC, Babylonians added flavorings to date vinegar such as fruit and honey to season their food.<sup>2</sup> They also discovered its use as a food preservative. In 3000 BC, Egyptians stored vinegar in the tombs of their deceased to be used as gift offerings for their Gods.<sup>2</sup> In 1000 BC, Romans used fruit vinegar as a flavorful condiment to dip their bread in.<sup>15</sup> In 400 BC, the father of modern medicine, Hippocrates from ancient Greece, recognized the medicinal potential of vinegar and used it in treating a variety of ailments.<sup>15</sup>

### **Functional Properties**

Functional food, or nutraceuticals, are defined as a food providing health benefits beyond that of the basic nutritional needs.<sup>16</sup> The therapeutic effects of vinegar are demonstrated in multiple studies.<sup>1</sup> Of significance are those showing how vinegar affects the glycemic response, delays gastric emptying, and functions as an antimicrobial agent.<sup>2,16,17</sup> The mechanisms that are responsible for the health benefits of vinegar are found to be the influence of the main ingredient in vinegar, acetic acid.<sup>18</sup> As such, vinegar has been recognized as a nutraceutical due to its physiologically functional properties.

Polyphenols are known to provide antioxidant benefits. Regular consumption of flavonoids, the major source of polyphenols in the human diet, is associated with reduced

risk of various chronic diseases and cancers.<sup>19</sup> Specifically, flavonoids provide protection against the oxidation of low density lipoproteins (LDLs), reducing oxidative stress and inflammation which, as will be discussed in further detail, may be a factor in the development of visceral fat and fatty liver.<sup>20</sup> Fermented grape and grape juice products, including red wine and red wine vinegar, are excellent sources of polyphenols.<sup>1,20</sup>

### **Recent Popularity**

Vinegar has been increasing in popularity in recent years as a functional food, resulting from the strong emphasis on natural and preventative health practices within public health as the incidence of obesity continues to rise. Lifestyle-related diseases are preventable chronic conditions caused by deleterious lifestyle factors.<sup>21</sup> These conditions include cardiovascular disease, stroke, obesity, and diabetes, among other conditions linked to smoking, and alcohol and substance abuse.<sup>21</sup> Because of its increasing prevalence and the increasing health care costs associated with these diseases, great interest in functional foods has spurred heavy research to combat existing conditions as well as provide scientific evidence to support preventative treatments.

Recent reports in the research literature that vinegar can increase and prolong satiety and independently decrease fat have been touted by mass informational sources. The notion that vinegar is a functional food for chronic metabolic disorders as well as a potential weight loss aid popularized by the media and sparking interest all across the developed world has led to commercially marketed weight loss supplements containing vinegar.<sup>22</sup> While there are studies showing an association between the reduction of fat

and vinegar consumption, the evidence supporting these claims, or the safety of vinegar consumption for that matter, are not sufficient at this time.<sup>4,11,22,23</sup>

### **Acetic Acid**

Acetic acid is part of a family of organic compounds known as carboxylic acids and are identified by the -COOH carboxyl group. The compound is distinguished by the addition of a methyl group (CH<sub>3</sub>). Its molecular formula is CH<sub>3</sub>COOH.<sup>15</sup> Arguably the most important in the carboxylic acid family, acetic acid is the active ingredient in vinegar and is the responsible compound for the various health benefits associated with vinegar consumption.<sup>15</sup> As previously illustrated, acetic acid is fermented by acetic acid bacteria from ethanol and derived from any carbohydrate source.<sup>2,13</sup> Naturally produced vinegar is the product of a chemical reaction in which ethyl alcohol is partially oxidized producing acetaldehyde, and is then converted into acetic acid through an oxidation reaction.<sup>13,15</sup>

Acetic acid is also produced as a byproduct of human metabolism. After a fiber-rich meal, gut bacteria found in the large intestine metabolize insoluble fiber and resistant starch through fermentation, breaking them down and producing short-chain fatty acids (SCFAs).<sup>18</sup> Acetate (the oxidized form of acetic acid), propionate, and butyrate make up about 95% of SCFAs in the body, and they populate the colon and stool at a molar ratio of 60:20:20 respectively.<sup>24</sup> The SCFAs that are produced in the gut can then be rapidly absorbed by colonic cells.<sup>18</sup> Dietary acetic acid however is digested in the small intestines, along with most nutrients including glucose.<sup>18</sup>

It is well documented that vinegar has a hypoglycemic effect however, the biological mechanism remains unclear. The role of acetic acid in human metabolism

continues to be highlighted as the key constituent in vinegar. Based on clinical observations, theories on how acetic acid may effect blood sugar levels include delayed gastric emptying, activation of gluconeogenesis, and the suppression of glucose facilitating enzymes in the epithelial cells of the small intestines.<sup>9,10,18,25</sup>

## **AMPK**

### **Origin and Function**

The evolution of AMPK provides an interesting perspective of its relationship to the insulin signaling pathway. The enzyme is a heterotrimer made up of three subunits, a catalytic alpha and regulatory beta and gamma subunits.<sup>26</sup> The genes that code for these three subunits are found in all eukaryotes including those single celled protozoa.<sup>26</sup> They are more complex in humans as these distinct human genes code for multiple isoforms for each subunit, with at least 12 potential combinations.<sup>26</sup> On the evolutionary timeline, the AMPK signaling pathway occurred well before the insulin signaling pathway. Similar to the action of insulin, fluctuations in glucose levels (such as in starvation and fed states) are at least partially managed by the AMPK pathway.<sup>26</sup> Further, the insulin signaling pathway is only found in multicellular animals.<sup>26</sup> It has therefore become recognized that the AMPK signaling pathway is an important and highly conserved mechanism in the regulation of cellular energy.

The role of AMPK is to detect a fuel deficiency in the cells of the body. It acts as a master switch on certain metabolic pathways including lipogenesis, triglyceride synthesis, hepatic ketogenesis, and carbohydrate regulation in the blood.<sup>27</sup> It regulates metabolism by facilitating the phosphorylation of key proteins that are necessary for the body to adapt to the present feeding conditions, specifically starvation or overfeeding, in



order to maintain energy balance.<sup>28</sup> It may also have long term effects on the expression of genes involved in metabolic regulation.<sup>26</sup> Recently, AMPK has been implicated for its central role in lipid metabolism.<sup>11</sup> Because of the metabolic function of AMPK in the suppression of fatty acid synthesis and promotion of fatty acid oxidation, it is being explored as a promising new drug target in the treatment for obesity and glycemic control for diabetes.

As energy becomes depleted in the mitochondria, body cells become stressed and adenosine monophosphate (AMP) levels increase. This occurs with such conditions as hypoglycemia, hypoxia, or muscle contraction.<sup>26</sup> As cellular energy production decreases, AMPK kick starts various pathways that breakdown other substrates to generate more adenosine triphosphate (ATP), the energy currency for body cells.<sup>26</sup> AMPK becomes activated and phosphorylated, functioning to regulate energy levels, while also protecting transmembrane transporters to maintain membrane potential.<sup>10,26,27,29</sup>

### **AMPK and Insulin Resistance**

It is hypothesized that defects in the activation of AMPK, or a reduction of its activity (resulting from a sedentary lifestyle) exist in those with type 2 diabetes.<sup>27</sup> However, dietary as well as lifestyle changes such as incorporating acetic acid into the diet as well as increasing physical activity levels may improve sensitivity to the activation triggers of AMPK.<sup>10,26,27,29</sup> Clinical treatment in the diabetes population include pharmaceuticals that mimic the biological processes to activate AMPK.<sup>29</sup> Some medications that are commonly prescribed to mediate blood glucose, such as metformin,

interrupts ATP production in the mitochondria and in doing so, these treatments activate AMPK by mimicking a starvation state at the cellular level.<sup>10,27,29</sup>

It is important to note that the type and location of the adipose tissue is more indicative of insulin resistance risk than is body weight per se. Key regulatory adipokine hormones, leptin and adiponectin, act synergistically to maintain energy balance.<sup>30</sup> Leptin inhibits hunger while adiponectin regulates glucose levels and fatty acid metabolism. Together, they have been shown to promote insulin sensitivity and activate AMPK.<sup>27,30</sup> Activation of this enzyme inhibits the synthesis of fatty acids, glycogen, and cholesterol, while stimulating glucose uptake and fatty acid catabolism.<sup>26,27</sup> Further, adipose tissue in the abdominal area surrounding the organs (visceral adipose tissue) are known to possess increased hormonal activity.<sup>31</sup> Higher levels of visceral fat are correlated with decreased levels of adiponectin.<sup>31</sup> Adiponectin at low concentrations is associated with insulin resistance and type 2 diabetes, among other conditions such as cardiovascular disease and some cancers.<sup>29,31</sup>

### **AMPK and Acetic Acid**

Acetic acid has been found to enhance the action of AMPK. This suggests vinegar intake may reduce the accumulation of body fat and increase sensitivity to insulin by promoting AMPK activation. Yamashita and colleagues<sup>11</sup> explored the impact of acetate on lipogenic genes and obesity. In this animal study, male rats with obesity-related type 2 diabetes were split into two groups, an obese treatment group and an obese control group, while another group of rats served as the nondiabetic control.<sup>11</sup> The obese treatment group received an oral administration of acetate at 5.2 mg/kg body weight, while the obese control received 5 mL/kg body weight of distilled water for 6 months.<sup>11</sup>

The results showed the obese treatment group had significantly lower triglycerides and cholesterol levels, and improved glucose tolerance compared to the obese control group.<sup>11</sup> Northern blotting analysis for the obese treatment group showed a reduction in the gene transcription of several enzymes that play a role in lipogenesis in the liver however, there was no statistical significance in those enzymes playing a role in lipolysis.<sup>11</sup> Further, western blotting analysis showed phosphorylated AMPK proteins in the liver was higher in the obese treatment group compared to that of the obese control group.<sup>11</sup>

## **Therapeutic Effects of Vinegar**

### **Antiglycemic Properties of Vinegar**

Of special interest currently are the antiglycemic properties of vinegar. Multiple studies have demonstrated reduced blood glucose levels after meals, as well as improvement to insulin sensitivity. The literature continues to explore the underlying mechanism however, the acetic acid in vinegar is credited for its glycemic lowering effects including the inhibition of glucose absorption and/or promoting cellular uptakes of glucose which consequently reduces glucose levels in the blood.<sup>18</sup>

One of the first studies on the antiglycemic properties of vinegar was conducted in 1987 by Ebihara and Nakajima. In their study, seven healthy participants were divided into two groups; a control group that ingested 300mL of a sucrose solution (53.6g sucrose) and an intervention group that ingested 300mL of sucrose and vinegar solution (50g sucrose with 60mL of strawberry vinegar containing 5% acetic acid).<sup>32</sup> The area under the insulin response curve (insulin AUC) was 20% lower in the vinegar-ingesting group compared to the control group.<sup>32</sup> In this same article, Ebihara and Nakajima also

observed the glucose response to a starch load in 12 rats divided into two groups.<sup>32</sup> One group ingested the control meal containing a corn starch solution (100mg starch per 100g body weight) while the other ingested the test meal containing a corn starch solution plus 2% acetic acid solution.<sup>32</sup> Blood glucose levels were attenuated in the test meal group compared to the control.<sup>32</sup>

A brief overview of carbohydrate digestion begins in the mouth with amylase, an enzyme in saliva. Starch gets broken down into maltose and shorter polysaccharide chains. Digestion continues with pancreatic amylase after passing the stomach as it enters the small intestine where polysaccharides are broken down further as they are hydrolyzed into disaccharides.<sup>18</sup> Production of appropriate glycosidase enzymes (maltase, lactase, sucrase) are upregulated to further break down the disaccharides into monosaccharides.<sup>18</sup> These single unit sugars are then available for absorption into the bloodstream through these monosaccharide-specific enzyme transport systems.<sup>18</sup> While it is still unclear where in this process acetic acid may interfere with the postprandial glucose response, it is thought it may inhibit the absorption of glucose.

In a study that aimed to explore the role of acetic acid in reducing postprandial blood glucose levels and its potential to inhibit the absorption of glucose, Ogawa and colleagues<sup>18</sup> studied the glucose transport and disaccharidase activity in Caco-2 cells. This culture of cells was treated to mimic the epithelial cells of the small intestines for the purposes of examining its physiology. In control cells without acetic acid, a glucose substrate was used to evaluate the glucose transport system.<sup>18</sup> Sucrase enzyme activity increased as expected.<sup>18</sup> Upon administration of acetic acid, disaccharidase enzyme activity and angiotensin-converting enzyme (ACE) activity levels were suppressed,

mitigating the postprandial sugar spike.<sup>18</sup> The results of this study suggest that the mechanism that gives acetic acid its hypoglycemic properties are at least partially related to the suppression of disaccharidase activity.<sup>18</sup>

One study, conducted by Brighenti and colleagues<sup>33</sup>, examined postprandial blood glucose in a more likely, real-life scenario. The glucose response of five healthy subjects was measured over six test-meals which included salads dressed with olive oil only, olive oil with vinegar containing 1g acetic acid, or with olive oil and sodium acetate in the form of neutralized vinegar.<sup>33</sup> White bread containing 50g of carbohydrates were added to three of the six test meals to induce a heavy carbohydrate load.<sup>33</sup> Glucose and blood acetate were considerably lower after vinegar-dressed salads were consumed when compared to salad with olive oil only.<sup>33</sup> While this study was limited due to the small sample size, it is important to note that in a highly likely scenario of a carbohydrate loaded meal, vinegar consumption, even in the small amount provided for salad dressing as demonstrated in this experiment, can have a significant effect in reducing the glycemic response.

Johnston and colleagues<sup>34</sup> explored the effect on postprandial glucose from the time of vinegar intake. Twenty-nine fasting insulin sensitive and insulin resistant participants, and participants with type 2 diabetes were randomly assigned to drink a vinegar (40g water, 20g apple cider vinegar, and 1 teaspoon saccharine) or a placebo beverage.<sup>34</sup> After two minutes, they ate a high carbohydrate meal (meal containing 87g total carbohydrates).<sup>34</sup> Fasting, 30-minute postprandial, and 60-minute postprandial blood samples were collected.<sup>34</sup> One week later, the test was repeated in a cross-over fashion. In insulin resistant participants, the vinegar beverage taken two minutes before

the high carbohydrate meal improved insulin sensitivity at the 60-minute postprandial collection ( $P= 0.01$ ), with improvements also seen in participants with type 2 diabetes ( $P= 0.07$ ).<sup>34</sup> Insulin sensitive participants who consumed the vinegar beverage experienced a significant reduction in postprandial insulin spikes.<sup>34</sup> In another study led by Johnston and colleagues, a 20% reduction in postprandial glycemia was seen with as little as two teaspoons of vinegar when consumed with a complex carbohydrate-rich meal compared to a placebo treatment.<sup>35</sup>

A recent systematic review and meta-analysis was conducted on clinical control trials reporting the effect of vinegar intake on the postprandial glucose response.<sup>25</sup> Across 11 clinical trials, 204 subjects were included with a range of 5-12 participants in each study.<sup>25</sup> Participants' disease states included healthy adults, individuals with type 1 and 2 diabetes (type 1 diabetes involved in one study only), and those with insulin resistance and impaired glucose tolerance.<sup>25</sup> Findings verified a statistical significance in the reduction of postprandial glucose and insulin responses as a result of vinegar consumption in healthy participants as well as those with a glucose disorder.<sup>25</sup> It is noteworthy to point out that in a subgroup meta-analysis of participants with impaired glucose tolerance, insulin resistance, or diabetes, a stronger effect size in glucose AUC was observed.<sup>25</sup>

### **Antiobesity Properties of Vinegar**

Vinegar as a functional food to help manage blood glucose and insulin sensitivity in those with pre-diabetes and type 2 diabetes may also help control body fat content. Several vinegar experiments and clinical trials have reported subsequent weight and fat

loss after vinegar treatment, as well as increased and prolonged satiety.<sup>4,9,10,22</sup> However, the current scientific evidence on the effects of vinegar in reducing fat are weak.

Findings from several studies have proposed mechanisms by which vinegar promotes antiobesity effects.<sup>1,4,18</sup> Acetic acid, as the key component in vinegar, as well as acetate (conjugate base to acetic acid) are implicated in the activation of these proposed mechanisms. These substrates have been shown to enhance the uptake of glucose and its utilization, and concurrently promote fat metabolism.<sup>8,11</sup>

Ostman and colleagues evaluated the dose response effect of vinegar supplementation (white vinegar with 6% acetic acid; 18g, 23g, or 28g) on blood glucose and insulin, and satiety after 12 healthy participants consumed a bread meal containing 50g of carbohydrates for breakfast.<sup>9</sup> It was reported that as acetic acid content increased, satiety levels increased (linear relationship between satiety AUC and acetic acid content, ( $P= 0.004$ )).<sup>9</sup> Likewise, the vinegar supplementation prolonged satiety levels. Postprandial satiety increased greater than two-fold when participants consumed their bread meal with the highest dose of vinegar (28g) compared to that of the control meal containing only bread with no vinegar.<sup>9</sup> This effect of vinegar on satiety may be an indirect mechanism for reducing body fat.

Ok and colleagues<sup>6</sup> used a rat model to examine how pomegranate vinegar (PV) consumption would affect adiposity in obese rats. Fifty rats were equally split into five experimental diet groups: a high fat diet group (41.2% energy from fat), a low dose acetic acid group (high fat diet with 1.6% acetic acid per rat), a high dose acetic acid group (high fat diet with 3.2% acetic acid per rat), a low dose PV group (high fat diet with 1.62% PV per rat), and a high dose PV group (high fat diet with 3.2% acetic acid per

rat).<sup>6</sup> After 16 weeks, weight gain as a result of the high fat diet was suppressed in the high dose acetic acid and low dose PV rats.<sup>6</sup> These same diet groups also experienced a decrease in white adipose tissue.<sup>6</sup> Triglyceride levels declined in all dose acetic acid and vinegar groups while only low dose PV rats experienced a reduction in hepatic triglyceride levels compared to the control group.<sup>6</sup> Investigators reported that the low dose PV group experienced about the same, or slightly better, results on body weight, plasma triglycerides, and hepatic utilization of fat than the high dose acetic acid group.<sup>6</sup> It was also noted that phytochemicals may be the reason for this property in vinegar compared to the acetic acid treatment alone.<sup>6</sup>

Kondo and colleagues<sup>4</sup> were among the first to study the impact of daily vinegar intake on body fat in humans. In this randomized control trial, 155 obese (defined in Japan as BMI of 25-30 kg/m<sup>2</sup>) Japanese participants were placed into one of three test beverage groups: a placebo ( $n= 58$ ; 500mL beverage containing 0mL apple vinegar/ 0mg AcOH/ 1250mg lactate), low dose ( $n= 59$ ; 500mL beverage containing 15mL apple vinegar/ 750mg AcOH), or high dose ( $n= 58$ ; 500mL beverage containing 30mL apple vinegar/ 1,500mg AcOH) group.<sup>4</sup> Lactate was used to mimic the taste of vinegar in the placebo drink. Beverages were consumed daily for 12 weeks by drinking half (250mL/ meal) after the breakfast meal and the rest after the dinner meal.<sup>4</sup> Anthropometric measurements as well as blood samples and blood pressure were taken and recorded on weeks 0, 4, 8, and 12 of the treatment period, and week 16 (post-treatment).<sup>4</sup> Measurements from computed tomography (CT) scans were used to quantify total fat area (TFA), and visceral and subcutaneous fat areas (VFA, SFA), which were recorded at week 0 and week 12 of the treatment period.<sup>4</sup> Diet and physical activity levels were



maintained over the 12 weeks with no significant differences between groups.<sup>4</sup>

Significant reductions in body weight, BMI, and body fat ratio (BFR) were seen as early as week 4 in the low dose and high dose groups, and reductions were dose dependent in that reductions in the high dose group was greater than that of the low dose group.<sup>4</sup> VFA and TFA decreased significantly in both vinegar-consuming groups.<sup>4</sup> Further, triglyceride levels had a significant decrease in the low dose group at the fourth, eighth, and twelfth weeks, and a significant decrease in total cholesterol was seen at the twelfth week.<sup>4</sup>

Darzi and colleagues<sup>22</sup> were the first to investigate the palatability and tolerability of vinegar on appetite control and food intake using validated appetite-related visual analogue scale (VAS) questionnaires to assess appetite and nausea. Palatability is the pleasure experienced by the food and beverages an individual consumes, and thus they are satisfying to the palate and enjoyable to the individual. Appetite is controlled by both extrinsic and intrinsic factors that trigger food palatability, and signal satiety (the physical feeling of fullness) and satiation (the hormonally-driven response to stop eating) cues.<sup>36</sup> The tolerability of a food is the ability to consume and digest a food without side effects such as nausea. Two related randomized crossover studies were conducted on healthy adults.<sup>22</sup> The first examined the postprandial effects of vinegar consumption when the palatability of the vinegar had been altered but the acetic acid content remained the same.<sup>22</sup> A breakfast meal (jam sandwiches; 8g olive oil spread, 16g strawberry jam, and two 38g slices of bread) was provided with one of the following vinegar beverages (white wine vinegar, 6% acetic acid): unpalatable (first drink: 25g vinegar, 25g sugar-free squash, 100g water; second drink: 50g sugar-free squash, 100g water), palatable (divided

into two drinks: 25g vinegar, 75g sugar-free squash, 250g water), or control beverage (divided into two drinks: 75g sugar-free squash, 275g water).<sup>22</sup> The second study examined the effects of the orosensory stimulation of vinegar, sans acetate, on appetite and glycemic response by employing the modified sham feeding (MSF) technique.<sup>22</sup> A milkshake (21g PRO, 60g CHO, 12.6g FAT) was consumed with a subsequent MSF phase that included a vinegar (30g white wine vinegar, 6% acetic acid with 150g water) or a control (180g water) beverage which was equally divided into 10 cups.<sup>22</sup> The participants were instructed to drink and hold the beverage from one cup in their mouths for 25 seconds before expectoration, then to repeat with the next cup.<sup>22</sup> An ad libitum meal was provided after 180 minutes for both studies. VAS results from the first study found significant differences between the palatability of all beverages, with the unpalatable drink rated as least pleasant, the palatable drink rated more pleasant, and finally the control non-vinegar drink rated as the most pleasant to taste.<sup>22</sup> A dose dependent relationship was shown between increased vinegar content and nausea.<sup>22</sup> Feelings of nausea significantly increased after consuming both vinegar drinks (palatable, unpalatable) compared to the control. The unpalatable beverage induced greater feelings of nausea compared to the palatable beverage however, this increase was insignificant. Coinciding with nausea ratings was increased satiety, reduced hunger, and an influence on prospective food intake. Ad libitum energy intake 3-hours and 24-hours post treatment was significantly lower in the vinegar beverage groups (mean energy intake in order from least to most: unpalatable, palatable, control). As would be expected, VAS results on appetite showed that energy intake significantly increased in participants who rated the taste of their drink more pleasant and their breakfast more palatable.<sup>22</sup> In the

second (orosensory) study, VAS survey results showed the vinegar beverage (held in mouth then expectorated) to be significantly less pleasant than the control beverage however, there were no differences in feelings of nausea between the vinegar and control treatments.<sup>22</sup> Additionally, post-treatment appetite assessment showed no difference between beverages.<sup>22</sup> Ultimately, consumption of vinegar had a significant influence on decreasing appetite<sup>22</sup>, supporting findings from other studies that have noted this effect. Orosensory stimulation (without ingestion) from a vinegar-containing beverage, as investigated in the second study, had no effect on appetite. Symptoms of nausea significantly increased as the vinegar content in the beverage increased (and thus palatability decreased), indicating poor tolerance to vinegar ingestion but not to the pungent effects of vinegar on the senses. Based on these findings, investigators reported that nausea following the ingestion of vinegar may be the underlying cause of what appears to be the effect vinegar has to decrease appetite, and increase satiety resulting in an overall reduction in subsequent energy intake.<sup>22</sup>

### **Risks of Consumption**

Very few acute incidences have been documented related to the consumption of vinegar and, of those, they present as isolated cases.<sup>37,38</sup> No other cases with similar acute adverse reactions have been reported.

### **Dental Erosion**

Dental erosion, the permanent loss of dental hard tissue caused by chronic chemical (not bacterial) exposure, has had an increase in prevalence due to the increased intake of dietary acids.<sup>39,40</sup> A growing body of evidence indicates that consuming acidic drinks such as in soft drinks, fruit juices, teas, alcoholic drinks, as well as some acidic

foods such as yogurts and fruits can cause erosion to the teeth.<sup>39</sup> Vinegar, which can be found in salad dressings, marinades, alcoholic cocktails, and currently trending fermented drinks, is among these highly erosive agents. This is exacerbated by the increased popularity of vinegar as a functional food and weight loss product in recent years.

An *in vitro* study tested the erosive potential of 60 agents consisting of dietary substances and medications for changes in the hardness of tooth enamel with an initial two minute exposure and a subsequent two minute exposure.<sup>39</sup> The chemical properties of these agents were characterized by pH value, titratable acidity, buffering capacity as well as its Ca, Pi, and F concentrations.<sup>39</sup> A significant reduction in the surface hardness of the enamel specimens of extracted teeth was found in all but coffee, teas, unflavored mineral waters, some alcoholic drinks, medications, and yogurt.<sup>39</sup> It was shown that the agents that had the highest potential of erosion were those with the lowest pH.<sup>39</sup> With a very low pH level that can range from 2.0 to 3.5 depending on dilution and acetic acid concentration, consumption of vinegar has been implicated in contributing to dental erosion.<sup>39</sup>

Another *in vitro* study used human enamel samples to test the erosion potential of 30 different vinegar varieties. The enamel samples were incubated in a vinegar liquid for 4 or 8 hours.<sup>41</sup> Mineral loss was demonstrated, with the highest mineral losses to the enamel sample incubated in the bio vinegar (pH of 3.1) and raspberry vinegar (pH of 2.7).<sup>41</sup>

Finally, a pilot *in vivo* human study examining resting saliva pH and vinegar ingestion, was conducted during an 8-week double-blind clinical trial (unpublished).<sup>42</sup> Investigators studied the daily intake of red wine vinegar and its effect on resting,

unstimulated salivary pH as well as dental erosion risk.<sup>42</sup> Participants were randomly assigned to a vinegar treatment group or a control group.<sup>42</sup> The vinegar group consumed a liquid vinegar drink containing two tablespoons of vinegar with 8-ounces of water two times per day just before a meal.<sup>42</sup> Using a smart phone application, resting salivary pH was measured for seven days at baseline and for the final seven days (week 8) of the treatment period.<sup>42</sup> Erosion was measured by a registered dental hygienist using the Basic Erosive Wear Examination (BEWE) protocol.<sup>42</sup> No significance was found on saliva pH ( $P=0.499$ ) after 8 weeks of vinegar treatment.<sup>42</sup> However, changes in BEWE mean scores between the vinegar and control groups after 8 weeks was found to be significant ( $P= 0.051$ ).<sup>42</sup>

### **Obesity Epidemic**

Obesity is characterized as the excessive accumulation of adipose tissue in the body and identified when actual body weight is more than or equal to 20% above the ideal body weight for height and measured using the body mass index (BMI). Gynoid and android obesity are two phenotypes of interest in current research, and they are classified based on the distribution of fat in the body. Accumulation of excess fat in the abdominal area is known as android obesity, while gynoid obesity is the accumulation of excess fat in the lower trunk (hip and thighs) of the body.<sup>21</sup> Much of the research on obesity in recent decades have sought to distinguish between these two obesity phenotypes in relation to metabolic health. It has been found that increased visceral fat significantly increases risk of metabolic disorders as well as cardiovascular conditions.<sup>21,31,43</sup>

It is thought that obesity is the result of the metabolic inability to fully oxidize the fat consumed in the typical diet. Excess fat accumulation occurs when fat intake chronically exceeds fat oxidation, which ultimately results in obesity.<sup>44</sup> Studies have found that the utilization of fat in the body is limited compared to the utilization of carbohydrates and proteins.<sup>44,45</sup> Flatt and colleagues<sup>45</sup> investigated how the addition of fat to a typical meal with mixed macronutrient content would effect substrate oxidation. The addition of dietary fat above the standard intake of a mixed diet was shown to have no effect on either fat nor carbohydrate oxidation.<sup>45</sup> This is undesirable because if increased fat intake does not increase fat oxidation, then it will be stored in adipose tissue adding to the total fat content in the body.

Acheson and colleagues<sup>46</sup> studied the impact of a high carbohydrate meal on macronutrient utilization. They found that a meal high in carbohydrates hampered the oxidation rate of fat, and there was no indication that the excess carbohydrates were converted and stored in adipose tissue.<sup>46</sup> In addition, glycogen stores increased up to 33% above the maximum amount of total glycogen reserves that was previously believed the body can hold in storage.<sup>46</sup> It was determined that carbohydrate oxidation adjusted (increasing in rate) to the excess carbohydrates consumed, after glycogen stores reached their maximum.<sup>46</sup> This supports previous findings concluding that excess intake of carbohydrates leads to the upregulation of its oxidation; and in contrast, excess dietary fat does not increase its own substrate oxidation but rather, it is downregulated leading to increased storage of fat.<sup>45</sup> Ultimately during periods of overfeeding, substrate oxidation will adjust to reach a balance between carbohydrate intake and utilization for energy, and

when the energy needs of the body have been met and glycogen stores have reached full capacity, all dietary fat consumed will be stored.<sup>45,46</sup>

A similar investigation was conducted by Horton and colleagues, who also examined the difference in fuel utilization but extended the period of overfeeding to 14 days.<sup>47</sup> Sixteen lean and obese participants consumed excess energy 50% above baseline intake amounts during two separate overfeeding phases; an all-carbohydrate overfeeding phase and an all-fat overfeeding phase.<sup>47</sup> Energy expenditure and substrate oxidation were measured using whole room indirect calorimeter, a gold standard for assessing fuel utilization.<sup>47</sup> It was demonstrated that fat overfeeding did not promote any significant changes in fat oxidation or total energy expenditure (TEE) however, the oxidation of carbohydrates and TEE increased almost two-fold with the 14-day carbohydrate overfeeding period.<sup>47</sup> They found that fat overfeeding led to the storage of 90-95% of the excess dietary fat over the 14-day trial period.<sup>47</sup> The investigators noted that the positive fat balance was the result of the coordinated effect of decreased fat oxidation following an increase to carbohydrate oxidation.<sup>47</sup> These findings are consistent with other overfeeding studies that examine fuel utilization and storage. It is important to determine dietary strategies to stimulate fat oxidation in an effort to control body weight and reduce the risk of obesity.

### **Metabolic Syndrome**

Metabolic syndrome, diagnosed when three or more specific risk factors co-exist, increases risk of stroke, heart disease and diabetes among others.<sup>3,4</sup> Risk factors include a large waist circumference, high triglyceride levels, low HDL levels, high blood pressure, and high fasting blood sugar levels. Substantial evidence is showing that increased levels

of visceral fat, characterized by waist circumferences greater than 40 inches for men and greater than 35 inches for women, is an independent risk factor for metabolic syndrome and may lead to a significantly negative impact on health.<sup>3-5</sup> Therefore, a reduction in visceral fat would precede great benefits in overall health as improvements in fasting blood glucose levels, triglyceride levels, insulin sensitivity, blood pressure, and inflammation would be seen.<sup>3-5</sup>

### **Visceral Fat**

Visceral fat is characterized by adipose tissue that accumulates in the abdominal area and surrounds the vital organs. Subcutaneous fat, on the other hand, may be distributed anywhere underneath the skin. The distinct differences between visceral and subcutaneous fat depots elucidate their metabolic contributions and disturbances.<sup>48</sup> As the body ages, visceral fat accumulation tends to increase.<sup>49</sup> Additionally, this adipose phenotype may be responsible for disturbing the normal metabolic processes in the body.

### **Health Complications of Visceral Fat**

Visceral adiposity is an independent risk factor of metabolic syndrome and elevated levels increase the risk of metabolic disturbances.<sup>31</sup> Other associated risks include cardiovascular and neoplastic diseases, as well as respiratory and inflammatory issues.<sup>31</sup>

Venous blood drainage from subcutaneous adipocytes is transported into systemic circulation, while drainage from visceral adipocytes migrate to the liver via portal vein.<sup>49</sup> It is theorized that free fatty acids (FFAs) and adipokines released by visceral adipose tissue are transported into portal circulation directly to hepatocytes and potentially other downstream organs. This would ultimately lead to an accumulation of fat in liver tissue



and trigger inflammatory responses causing short term as well as long term complications.<sup>49-51</sup>

Korenblat and colleagues found a direct association between intrahepatic triglyceride (IHTG) content and impaired insulin function in the liver and skeletal muscle, as well as total fat content.<sup>52</sup> Nielsen and colleagues used isotope dilution via catheter to the hepatic vein in 44 obese men and women and 24 lean men and women.<sup>53</sup> They determined that hepatic fat content is largely originated from intra-abdominal fat cells.<sup>53</sup> A 20% greater concentration of plasma FFAs were seen in the obese group.<sup>53</sup> Further, the FFAs from visceral lipolysis that are transported to hepatocytes accounted for up to 50% of the liver fat.<sup>53</sup> As visceral fat increased among the obese group, the hepatic FFA content increased as well ( $P=0.002$  in obese men and women).<sup>53</sup>

### **Inflammation and Visceral Fat**

Chronic inflammation and oxidative stress in the body are side effects of obesity. This obesity-related condition has been implicated as an important link to insulin resistance and cardiovascular disease.<sup>54</sup> The Framingham Heart Study investigated circulating inflammatory markers to assess whether visceral fat has a stronger association to inflammation than subcutaneous fat.<sup>54</sup> While both fat depots were found to have similar associations with elevated inflammatory markers, only visceral adipose tissue (VAT) was associated with multiple elevated markers of inflammation after adjusting for typical clinical measures associated with obesity.<sup>54</sup>

An adipocyte is hypertrophic when it is expanded and reaches its maximum lipid storage capacity, and new subcutaneous adipocytes are recruited when excess lipids necessitate storage.<sup>55</sup> When this mechanism of recruiting new adipocytes is impaired,

chronic hypertrophy of adipocytes can promote lipolysis and cell death in adipose tissue.<sup>55</sup> Macrophage infiltration of adipose tissue occurs as an inflammatory response to remove the dying or dead cells.<sup>55</sup> This inflammatory response has also been implicated in the renin-angiotensin system (RAS) dysfunction which results in hypertension (another risk factor for metabolic syndrome).<sup>56</sup> Hypertrophic adipose cells are more resistant to insulin action and ultimately lead to the accumulation of ectopic fat in the visceral organs, liver, and muscle.<sup>51,55</sup>

### **Insulin Resistance**

Peroxisome proliferator-activated receptor (PPAR $\gamma$ ) functions as an activator of a gene network involved in the regulation of adipose cell differentiation, lipid storage and metabolism, as well as insulin sensitivity throughout the body.<sup>55</sup> Impairment of PPAR $\gamma$  caused by gene mutation increases risk of insulin resistance and type 2 diabetes.<sup>55</sup>

Another inflammatory consequence of hypertrophic obesity is retinol-binding protein 4 (RBP4).<sup>43</sup> It is correlated with visceral adipose tissue and its secretion is triggered when glucose uptake is impaired.<sup>55</sup>

### **Fatty Liver**

Non-alcoholic hepatic steatosis (NAFLD) is a fatty liver disease that results from causes not related to excessive alcohol consumption and it is highly prevalent in the obese and in individuals with type 2 diabetes.<sup>57</sup> There is a strong and direct correlation between visceral fat and intrahepatic fat.<sup>55</sup> It can be argued that visceral fat, an important risk factor in metabolic syndrome, may be a secondary condition to the more indicative risk factor of intrahepatic fat.<sup>50,55</sup> Fabbrini and colleagues<sup>50</sup> aimed to distinguish intrahepatic triglyceride (IHTG) content as a primary marker of metabolic disorders and

not VAT. It was noted that the link between increased VAT and metabolic disorders is primarily due to the correlation between VAT and IHTG, and increased IHTG should be recognized as an independent marker for obesity-related metabolic diseases.<sup>50</sup>

### **Measurement Methods**

Lee and Gallagher (2008) conducted a review of assessment methods commonly used in determining human body composition, including visceral fat measurements.<sup>58</sup> It was noted that there is no error-free method or one that can provide an accurate measure of all body areas.<sup>58</sup> Among the methods reviewed that assess visceral fat levels, the dual X-ray absorptiometry (DXA) was found to be a generally noninvasive and safe method with good accuracy and reproducibility that is acceptable for any age range.<sup>58</sup> Other commonly used methods for measuring body composition, including bioelectrical impedance analysis (BIA), ultrasound, and computed tomography (CT) scans are not as safe, or produce poor accuracy estimations.<sup>58</sup>

BIA measures the resistance of an electrical current that passes through the body's water stores and estimates fat mass versus fat free mass as well as body weight.<sup>58</sup> The benefits to this method include its ease of use, safety, and affordability compared to other methods.<sup>58</sup> BIA may be inaccurate in the elderly due to the natural loss of total body water that occurs with age, and may overestimate fat free mass in the obese population or underestimate fat free mass in normal weight populations.<sup>58</sup>

Computed tomography (CT) scans measures visceral fat through its cross-sectional imaging capability. A CT scan can create cross sectional sliced images of the body organs and tissues from rotating x-ray beams. CT scanning is currently the gold standard for high accuracy in assessing adiposity, including visceral fat mass. However,

this method is not without risks. These scanners emit ionizing radiation which is known to break chemical bonds in bodily tissues, damage DNA by freeing charged ions, and increase risk of cancer.<sup>59</sup>

Measuring waist circumference is an easy, convenient, and low-cost screening tool that can provide an accurate estimate for assessing visceral fat.<sup>60</sup> The World Health Organization (WHO) has determined that increased risk of comorbidities associated with obesity for the Caucasian population is indicated at a waist circumference of 38 inches or more (>94 cm) for men and 32 inches or more (>80 cm) for women.<sup>61</sup> Gradmark and colleagues<sup>60</sup> tested the validity of DXA, ultra sound, and anthropometry for assessing visceral fat compared to CT scanning, the gold standard method. Anthropometric measures included BMI and waist circumference.<sup>60</sup> The results from the DXA measurements were comparable to or less strongly correlated with CT measurements of adiposity in all comparisons in relation to waist circumference or BMI.<sup>60</sup>

Based on the data from the third National Health and Nutrition Examination Survey (NHANES III) conducted by the National Center for Health Statistics, Zhu and colleagues concluded that lowering the threshold for waist circumference as an indicator of obesity-related health risks in the Caucasian male population to 35 inches and keeping with the WHO cut off of 32 inches for women may provide better health outcomes.<sup>62</sup> Berber and colleagues found that the optimal waist circumference cut offs were about the same in the Mexican populations.<sup>63</sup> They also noted that their findings were similar to the established thresholds recommended for the Asian populations.

## CHAPTER 3

### METHODS

#### **Participants and Study Design**

This study was a 2-arm parallel group, randomized, controlled trial. The treatment period was 8 weeks, with one initial pre-screening visit before the start of the trial. Participants met the following criteria: healthy man or woman between the ages of 18-45, sedentary as defined by exercising < 3 times/week, not taking insulin or medications that may have affected their weight, and were willing to adhere to the study protocol of maintaining dietary intakes and consuming a vinegar drink or pill. The waist circumference requirement for female participants was >32 inches and for male participants was >37 inches. Individuals were excluded if they were pregnant or trying to get pregnant; had recently undergone any surgery to the abdomen or had a condition that may cause distention or swelling in the abdomen, such as pancreatitis, diverticulitis/diverticulosis, Crohn's disease, irritable bowel syndrome, or ascites; and/or were taking medications, such as digoxin or diuretics, which can affect body weight. Data are reported for participants who completed the 8-week study ( $n=45$ ). All participants provided written consent. This study was approved by the Arizona State University Institutional Review Board (IRB).

#### **Study Variables**

Acetic acid consumption was the independent variable in this study. The participants in the VIN group were given instructions to drink the total of four tablespoons of vinegar each day (which provided a total of 3.6g acetic acid), mixed with water. An 8-week supply of the red wine vinegar was pre-packaged in their original

manufactured bottles (Mantova Red Wine Vinegar, Mantova, *Broccostella*, Italy) and provided to the VIN group participants at the beginning of the trial. While vinegar has many sources, red wine vinegar was specifically chosen because of its palatability, affordability, and common grocery store accessibility compared to other vinegar options. A limitation to this supplement may be the taste however, mixing with water (as instructed to study participants) helps dull the sour flavor. For those participants in the CON group, they were given one bottle of vinegar pills (Apple Cider Vinegar Tablets, NowFoods, Bloomingdale, IL). Instructions were given to swallow two whole pills each day (which provided a total of 0.0225mg acetic acid). The visceral fat mass was the main dependent variable in this study. It was hypothesized that participants in the VIN group would experience a greater loss of visceral fat and greater reduction in waist circumference in comparison to participants in the CON group.

### **Protocol Procedures**

Data collection occurred at the start and upon completion of the trial period. Three visits were required of the participants; before the trial began, at the beginning of the trial period (week 1 of intervention), and lastly after completion of the trial. Upon the first visit (before the intervention period) a consent form was signed by all candidates before continuing with pre-screening protocols to determine qualification per study criteria. Pre-screening protocols of anthropometric measurements included height, weight, and waist circumference. Subjects were stratified by gender, age, and weight, then randomly placed into two groups, an intervention group who were instructed to drink red wine vinegar (VIN), and a control group who were instructed to consume vinegar pills (CON).

Upon the second visit, participants were instructed to fast for 8 hours prior to their visit, with the exception of water. Height, weight, and waist circumference were re-measured. A certified phlebotomist performed blood draws to test levels of fasting blood glucose and insulin. Assessment of body fat was measured using the standard protocol of anthropometric measurements as well as from DXA scan data. A certified radiologic technician performed the DXA scans. Participants also completed a medical history questionnaire as well as the first of two 24-hour dietary recalls. The second dietary recall form was to be filled out upon completion of the trial, which would then be used to confirm dietary maintenance throughout the trial. VIN group participants received an 8-week supply of red wine vinegar, and the CON group subjects received an 8-week supply of vinegar pills. Participants received instructions for proper consumption of their vinegar supplement, and adherence to the assigned supplement for the full 8-week period was emphasized. A compliance calendar was also provided to the participants to check mark each time they consumed their respective vinegar supplements. This meant that 100% compliance would have two check marks per day for 8 weeks.

Participants in the VIN group were directed to mix two tablespoons of the provided red wine vinegar with 8 ounces of water before a meal, two times per day for the entire 8-week trial period. CON group participants were directed to take one of the provided vinegar pills before a meal, twice daily for the entire 8-week trial period. All participants were asked to continue current dietary and exercise behaviors throughout the study. To ensure eating habits did not change, a 24-hour diet recall form was completed at baseline, as well as upon completion of the trial (third visit). Again, anthropometric measurements including height, weight, and waist circumference as well as DXA and

blood draws per protocol were taken upon completion of the 8-week intervention period (third visit). The compliance calendar was turned in to ensure adherence to the protocol. Participants also completed an exit survey to collect subjective data, such as any physical or symptomatic issues experienced with vinegar consumption as well as overall opinions on how the study was conducted. Gifts cards valued at ten dollars were offered as compensation upon completion of the 8-week trial period (third visit).

### **Laboratory Analyses**

Participants arrived at the test laboratory after an 8-hour fast and had their bloodwork taken via venipuncture by a certified phlebotomist. Ten milliliters of blood was drawn (2mL via gray top vacutainer tube, 8.5mL via red top serum vacutainer tube). Blood drawing protocols via *Standard Blood Specimen Collection by Venipuncture for Study Protocols and Procedures* was used (Arizona State University, 2010). Assessment of fasting insulin levels were measured using a radioimmunoassay (Human Insulin-Specific RIA) kit. Assessment of fasting blood glucose levels were measured using a Cobas analyzer (Cobas c111 Analyzer, Cobas, Indianapolis, IN). Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) scores were estimated using the standard HOMA calculation to estimate insulin sensitivity:

$$\frac{\text{fasting glucose (mmol/L)} \times \text{fasting insulin } (\mu\text{U/L})}{22.5}$$

Each participant had a DXA (Lunar iDXA, General Electric, Fairfield, CT) scan performed by a certified radiologic technician for assessing total body fat and distribution including visceral fat mass. Female participants provided a urine sample (at least 2 ounces) to confirm absence of pregnancy (Medline Urinalysis Reagent Strips, Medline,



Mundelein, IL) due to the potential risk of fetal harm from exposure to minimal amounts of radiation from DXA scanning.

### **Statistical Analyses**

Data are reported as mean values  $\pm$  standard deviation. Shapiro-Wilk and independent t-tests were used to test normality of the data. Data were not normally distributed, and the Mann-Whitney nonparametric test was used to compare means between the experimental and control groups at baseline. One way (ANOVA), univariate (ANCOVA), and multivariate (MANCOVA) general linear models (GLM) for repeated measures were used to determine significant treatment effects. Chi square statistic was used to assess exit survey responses between groups and Cramer's V for effect size. A *P*-value of  $<0.05$  was considered significant. The Statistical Package for Social Sciences (SPSS) software (SPSS Incorporated, Chicago, IL, USA) version 23 was used to complete the statistical analyses of the data.

## CHAPTER 4

### RESULTS

Recruitment of study participants occurred in two rounds. In total for both recruitment periods, 297 respondents completed the survey. Based on their responses, 62 did not meet the qualifications of the study. The remaining 235 respondents were emailed to schedule a pre-screen visit and 103 respondents made it to their scheduled pre-screen visit. The first pre-screening visit allowed investigators to verify survey responses after obtaining a written consent. Forty eight were excluded after the first pre-screening visit: 37 were excluded because they did not meet the minimum requirement for the waist circumference measurement; three were not willing to maintain their current diet and exercise levels for the following 8 weeks; one did not meet the age range requirement; one was anemic; one had kidney disease; one had a gastric band; one was a smoker; one was excluded due to dietary restrictions; one had transportation and scheduling conflicts; and one was already taking vinegar supplements. After pre-screening, 64 participants were eligible to continue the 8-week trial.

Participants were paired by age, gender, and BMI. They were then assigned to one of two groups by coin flip: the treatment group (VIN;  $n=32$ ) or control group (CON;  $n=32$ ). Participants tracked their daily intake of liquid vinegar (VIN group) or vinegar pills (CON group) on a compliance calendar. The trial and data collection began from their second visit. Of 64 participants who proceeded to start the trial, 45 completed the 8-week trial (VIN,  $n=21$ ; CON,  $n=24$ ). Nine participants dropped as a result of attrition, four due to health issues unrelated to this trial, three due to work conflicts, two due to an adverse reaction to the liquid vinegar, and one due to pregnancy. One participant could

not provide a blood sample due to difficulties obtaining the sample and another refused, which explains the smaller sample size in the blood data analysis. Four men and 41 women completed the entire 8-week trial.

Initial participant screening included age, height, weight, BMI, and waist circumference using standard protocols to collect these data. Using Mann-Whitney nonparametric tests, there were no significant differences in age ( $P=0.882$ ), weight ( $P=0.335$ ), BMI ( $P=0.851$ ), or waist circumference ( $P=0.906$ ) between the VIN and CON groups at baseline (Table 1). One participant from the CON group was a multivariate outlier (specifically BMI, weight, and waist), as they were three standard deviations away from the mean. Baseline data from this participant was excluded from baseline analysis. Height was significantly higher in the CON group ( $P=0.033$ ; Table 1) with a mean height of  $167.9 \pm 7.4$  cm compared to the mean height among VIN group participants of  $163.3 \pm 7.0$  cm. The age range of all participants was between 18-45 years. For the VIN group, the mean weight was  $74.3 \pm 11.5$  kg and mean BMI  $27.8 \pm 4.0$  kg/m<sup>2</sup>. The CON group had a mean weight of  $80.7 \pm 16.9$  kg and mean BMI of  $28.5 \pm 4.9$  kg/m<sup>2</sup>. Mean waist circumference was  $36.5 \pm 3.3$  inches among the VIN group compared to  $36.9 \pm 4.0$  inches among the CON group. Ingestion of liquid vinegar (VIN group) or vinegar pills (CON group) was tracked using a compliance calendar. The mean compliance percent among the VIN and CON group was  $92.7 \pm 13.3\%$  ( $n=18$ ) and  $89.1 \pm 18.9\%$  ( $n=20$ ) respectively. Seven participants lost their compliance calendar, 4 of those participants were in the CON group and 3 from the VIN group. Compliance to vinegar supplementation among all reporting participants ranged from 19.6-100%.

Table 1: Baseline Characteristics by Group<sup>a</sup>

	VIN ( <i>n</i> =21)	CON ( <i>n</i> =24)	<i>P</i> value
Age (years)	29.6 ±8.1	30.1 ±7.4	0.882
Height (cm)	163.3 ±7.0	167.9 ±7.4	0.033
Weight (kg)	74.3 ±11.5	80.7 ±16.9 ( <i>n</i> =23)	0.335
BMI (kg/m <sup>2</sup> )	27.8 ±4.0	28.5 ±4.9 ( <i>n</i> =23)	0.851
Waist circumference (in)	36.5 ±3.3	36.9 ±4.0 ( <i>n</i> =23)	0.906
% Compliance	92.7 ±13.3 ( <i>n</i> =18)	89.1 ±18.9 ( <i>n</i> =20)	0.764

<sup>a</sup>Data are mean±SD. *P* value for baseline differences between groups (Mann-Whitney nonparametric test). Data considered significant at *P*<0.05. One outlier removed from baseline data (outlier ≥3 SD from mean).

Eight-week variances between the VIN and CON groups are shown in Table 2. Pre and post data were analyzed for time and interaction effects. General linear model univariate analysis was used to determine significance for the 8-week change between groups. Age, gender, baseline BMI, and change in physical activity (measured in metabolic equivalents or METs) were controlled confounding variables in all analyses (Table 2) with the exception of mean METs. No statistically significant differences were seen between groups in anthropometric measurements of weight (*P*=0.652), BMI (*P*=0.855), and waist circumference (*P*=0.694) between weeks 1 and 8. Similarly, DXA scan data did not show significant changes between groups in visceral fat (*P*=0.368) or total fat (*P*=0.918) between weeks 1 and 8. There was a significant increase in both groups in physical activity levels (*P*=0.032 with a large effect size of 0.102) between weeks 1 and 8. There was no difference, however, between the groups (*P*=0.916) indicating that both groups increased their physical activity levels equally.

Table 2. Anthropometry: Baseline data, week 8 data, and the 8-week change data, for the vinegar (VIN,  $n=21$ ) and control (CON,  $n=24$ ) groups <sup>a</sup>

	VIN			CON			<i>P</i> value (effect size)
	baseline	week 8	change	baseline	week 8	change	
Age, y	29.6± 8.1			30.1± 7.4			
Gender, M/F	20/1			21/3			
METS <sup>b</sup>	28.0± 16.8	36.7± 34.3	8.8± 24.9	34.2± 28.3	42.2± 29.5	8.0± 25.6	0.916 (0.032)
Body Mass Index, kg/m <sup>2</sup>	27.8± 4.0	27.9± 3.8	0.03± 0.69	28.5± 4.9	28.5± 5.0	0.08± 0.49	0.855 (0.077)
Weight, kg	74.3± 11.5	74.3± 10.9	0.01± 1.79	80.7± 16.9	80.9± 17.2	0.25± 1.32	0.652 (0.157)
Waist circumference <sup>b</sup> , cm	92.7± 8.4	91.4± 9.4	-1.1± 3.3	93.7± 10.2	92.5± 10.9	-1.3 ± 2.7	0.694 (0.085)
Visceral fat, cm <sup>3</sup>	672.0± 469.5	688.4± 471.9	16.4± 94.1	569.4± 547.1	562.0± 536.0	-7.4± 85.2	0.368 (0.266)
Body fat, %	40.4± 7.2	40.2± 7.3	-0.17± 0.93	37.9± 7.4	37.8± 7.8	-0.08± 1.57	0.918 (0.067)

<sup>a</sup>Data are mean±SD; baseline values do not differ between groups ( $P>0.05$ ; independent t-test). *P* value for 8-week change between groups (GLM univariate analysis; age, gender, baseline body mass, and change in METS were controlled in all analyses with the exception of METS). Effect size is Cohen's *d* (0.2=small; 0.5=medium; 0.8=large). <sup>b</sup>Significant time effect.

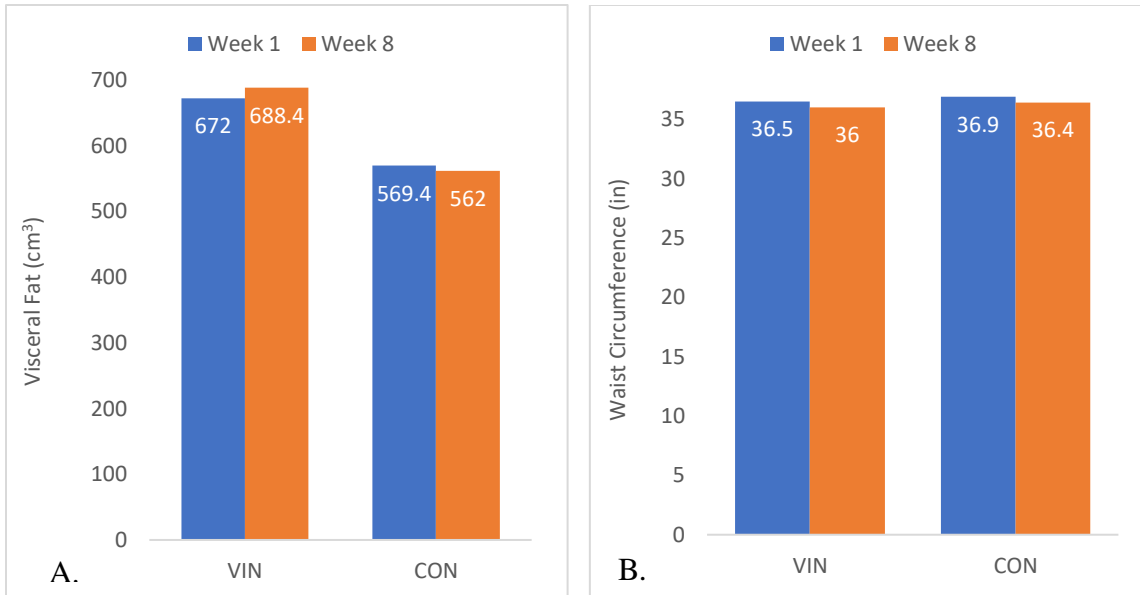


Figure 2. A: Comparison of mean visceral fat (cm<sup>3</sup>) between groups at week 1 and week 8 ( $P=0.368$ ,  $d=0.266$ ). B: Comparison of mean waist circumference (in) between groups at week 1 and week 8 ( $P=0.871$ ,  $d=0.871$ ). Data considered significant at  $P<0.05$ . VIN:  $n=21$ , CON:  $n=24$ .

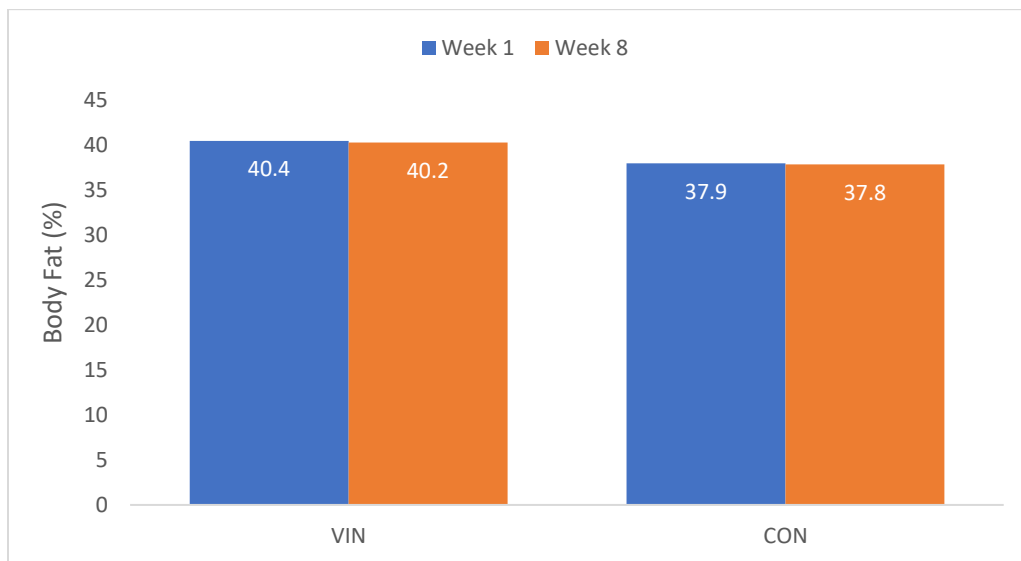


Figure 3: Comparison of mean body fat (%) between groups at week 1 and week 8 ( $P=0.294$ ,  $d=0.067$ ). Data considered significant at  $P<0.05$ . VIN:  $n=21$ , CON:  $n=24$ .

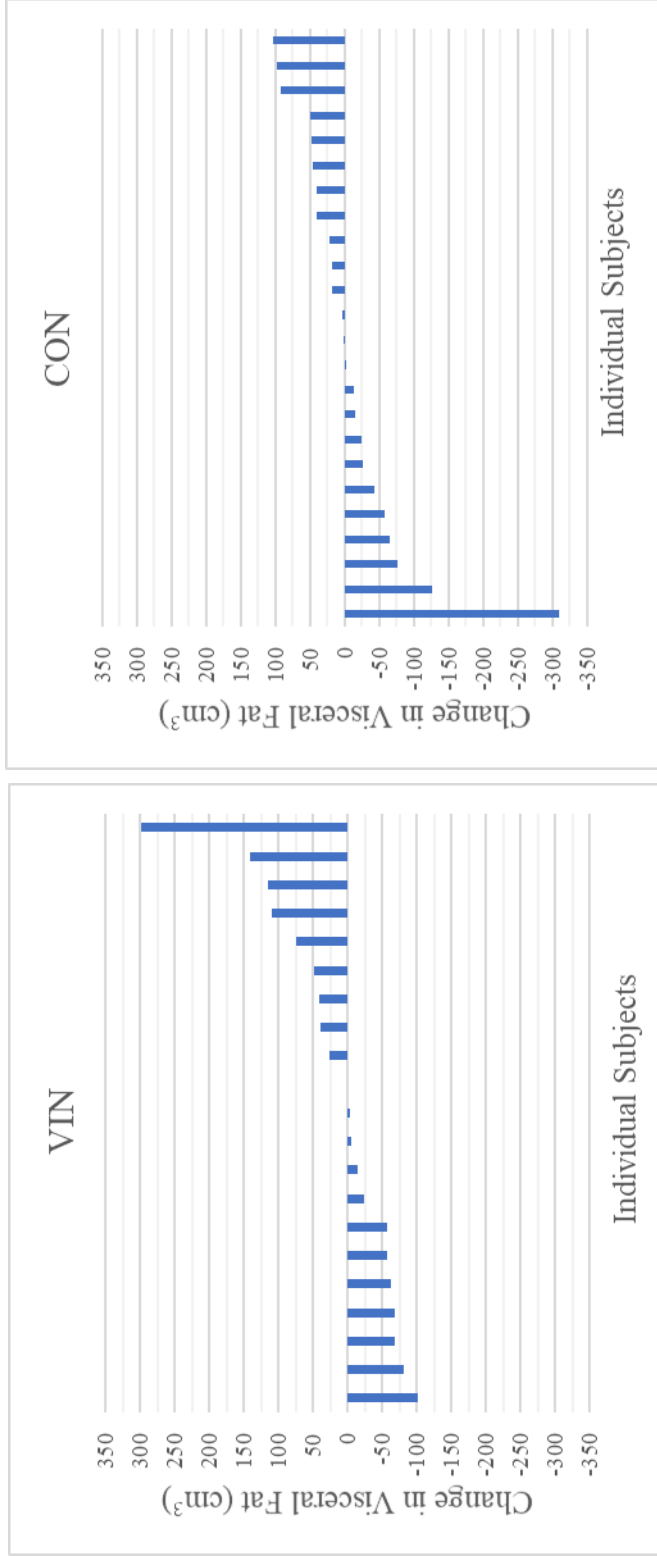


Figure 4: Comparison of individual changes in visceral fat between groups at week 8. Data represents all participants who completed the trial ( $n=45$ ). VIN:  $n=21$ , CON:  $n=24$ .

Blood samples were obtained in a fasted state at the beginning and end of the trial. Samples from two participants, one in the VIN group and one in the CON group, were not collected which explains the smaller sample size in each group. One participant refused to have blood drawn, and the other due to difficulties obtaining the sample. There were no outliers found in the blood sample analysis.

Changes in blood indices between the VIN and CON groups at weeks 1 and 8 are shown in Table 3. General linear model multivariate analysis was used to determine significance for the 8-week change between groups, controlling for age, gender, and baseline data for BMI, insulin, glucose, HOMA, and change in physical activity (METs) in all analyses (Table 3). The 8-week change in all blood indices showed a statistically significant difference between the vinegar and control groups; fasting blood glucose ( $P=0.003$ ,  $d=0.487$ ), fasting insulin ( $P<0.001$ ,  $d=0.618$ ), and HOMA-IR scores ( $P<0.001$ ,  $d= 0.607$ ). Further, Cohen's effect size suggested a moderate to high practical significance in all blood indices.



Table 3. Blood Indices: Baseline data, week 8 data, and the 8-week change data, for the vinegar (VIN,  $n=20$ ) and control (CON,  $n=23$ ) groups <sup>a</sup>

	VIN			CON			<i>P</i> value (effect size)
	baseline	week 8	change	baseline	week 8	change	
Fasting glucose, mmol/L	5.1±0.3	5.1±0.3	0.03±0.25	5.2±0.5	5.2±0.4	0.02±0.39	0.003 (0.487)
Fasting insulin, μU/L	15.1±8.4	14.2±7.0	-0.9±5.1	13.1±5.9	14.3±6.5	1.2±4.4	<0.001 (0.618)
HOMA-IR	3.6±2.1	3.3±1.7	-0.3±1.2	3.1±1.5	3.4±1.7	0.3±1.2	<0.001 (0.607)

<sup>a</sup>Data are mean±SD; baseline values do not differ between groups ( $P>0.05$ ; independent t-test). *P* value for 8-week change between groups (GLM multivariate analysis; age, gender, and baseline data for body mass, insulin, glucose, HOMA, and change in METs were controlled in all analyses). Effect size is Cohen's *d* (0.2=small; 0.5=medium; 0.8=large).

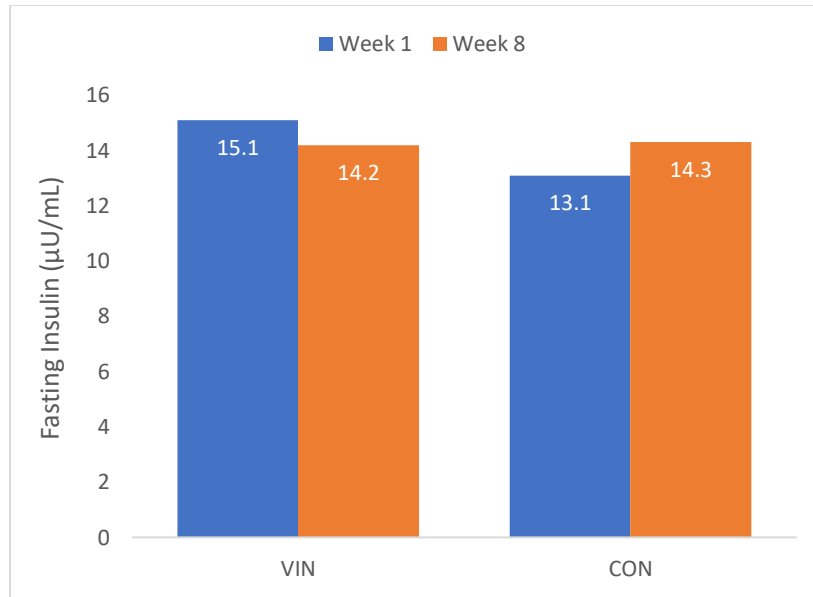


Figure 5: Comparison of mean fasting insulin ( $\mu\text{U}/\text{mL}$ ) between groups at week 1 and week 8 ( $P < 0.001$ ,  $d = 0.618$ ). Data considered significant at  $P < 0.05$ . Data represents all participants who completed the trial and provided blood samples ( $n = 43$ ). VIN:  $n = 20$ , CON:  $n = 23$ .

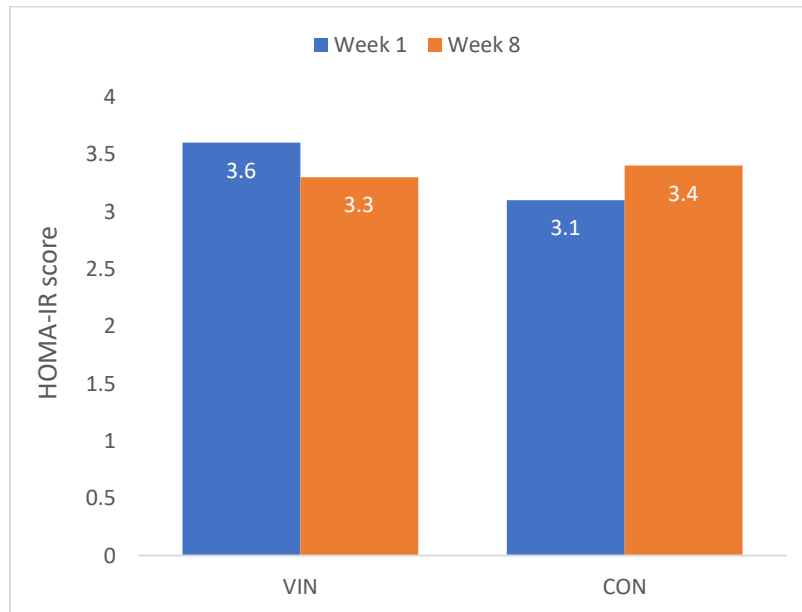


Figure 6: Comparison of mean HOMA-IR scores between groups at week 1 and week 8 ( $P < 0.001$ ,  $d = 0.607$ ). Data considered significant at  $P < 0.05$ . Data represents all participants who completed the trial and provided blood samples ( $n = 43$ ). VIN:  $n = 20$ , CON:  $n = 23$ . HOMA-IR: Homeostatic Model Assessment of Insulin Resistance.

Twenty-four-hour dietary recall data was obtained from each participant before and after the trial to confirm diet was maintained throughout the study period. There were no significant changes in mean energy and macronutrient intake (Table 4). Mean energy intake among the VIN group at week 1 was 1860 ±813 kcals with 50%, 13%, and 37% of energy from carbohydrates, protein, and fats respectively. At week 8, the VIN group had a mean energy intake of 1947 ±1025 kcals with 47%, 17%, and 37% of energy from carbohydrates, protein, and fats. Mean energy intake among the CON group at week 1 was 2089 ±887 kcals with 43%, 17%, and 34% of energy from carbohydrates, protein, and fats respectively. At week 8, the CON group had a mean energy intake of 2201 ±1220 kcals with 49%, 15%, and 37% of energy from carbohydrates, protein, and fats.

Table 4: Dietary Recall Data Between Week 1 and Week 8<sup>a</sup>

		VIN (n=21)	CON (n=24)	P value	Effect Size
Calories (kcal)	Week 1	1860 ±813	2089 ±887	0.947	0.000
	Week 8	1947 ±1025	2201 ±1220		
Carbohydrate (g)	Week 1	234 ±124	225 ±95	0.259	0.030
	Week 8	227 ±104	270 ±162		
Protein (g)	Week 1	62 ±29	90 ±73	0.234	0.033
	Week 8	81 ±85	83 ±46		
Total fat (g)	Week 1	77 ±38	78 ±39	0.633	0.005
	Week 8	79 ±50	90 ±63		
Total fiber (g)	Week 1	20 ±13	23 ±11	0.639	0.005
	Week 8	19 ±13	21 ±11		

<sup>a</sup>Data are mean±SD. P value for 8-week change between groups (repeated measures ANOVA). Data considered significant at P<0.05. Effect sizes >0.140 are considered large.

Participant responses to the exit survey were quantified by scoring their responses on a 0, 1, 2, 3 scale, with positive responses earning a score of 0 or 1 and negative responses earning a score of 2 or 3. Chi square statistic was used to determine that a slight but insignificant association (approaching significance,  $P=0.059$ , with moderate effect size, Cramer's  $V= 0.282$ ) was observed: the type of vinegar supplementation received was slightly, but insignificantly, associated with the negative or positive side effects experienced with a moderate measured effect.

Table 5. Subjective Side Effects from Participant Exit Survey

	Negative Side Effects	Positive Side Effects	$P$ value <sup>1</sup> (effect size) <sup>2</sup>
VIN, % [ $n$ ]	52% [11]	48% [10]	0.059 (0.282)
CON, % [ $n$ ]	25% [6]	75% [18]	

<sup>a</sup>Data are mean%. <sup>1</sup> $P$  value determined using Chi square statistic. Data considered significant at  $P<0.05$ . <sup>2</sup>Cramer's  $V$  effect size.

## CHAPTER 5

### DISCUSSION

In the present study, we investigated the effect of daily vinegar consumption on fat metabolism, specifically visceral fat, and fasting blood glucose and insulin in healthy overweight and obese adults. The findings from this study failed to show any significant differences in waist circumference ( $P=0.694$ ) or visceral fat ( $P=0.368$ ) between VIN and CON groups after 8 weeks of vinegar treatment.

The great majority of research demonstrating a link between vinegar ingestion and body fat content have been shown in rat samples. Animal studies have found significant reductions of body fat with vinegar consumption, including weight, visceral fat, total fat, and blood fat levels.<sup>6,8,11</sup> In their rat model, Ok and colleagues<sup>6</sup> explored the link between acetic acid metabolism and the oxidation of fat. Rats were fed a high fat diet yet experienced reduced body weight and fat, and serum triglycerides with increased intakes of vinegar.<sup>6</sup> In addition, elevated levels of phosphorylated AMPK were seen as a result of the vinegar intake, resulting in lipolysis and hepatic fatty acid oxidation, and ultimately providing further support for a possible mechanism to explain the effects vinegar may have on obesity.<sup>6</sup> However, with the exception of Kondo and colleagues<sup>4</sup>, human studies on vinegar consumption have not shown statistically significant reductions in body fat measurements. A systematic review that compared the effects of treatment between animal and human studies reported that there may be limitations to the conclusions drawn from animal experiments as they may not translate well to human biological systems as previously thought.<sup>64</sup> Thus, these findings may provide a rationale for the inconsistent conclusions between animal studies showing fat reductions with

vinegar treatment and the human studies that show no effect on fat content with vinegar treatment.

In their large scale study of 155 participants, Kondo and colleagues were the first to directly study this relationship between vinegar and fat metabolism in humans and found significant dose response reductions in mean fat assessments including body weight, BMI, body fat ratio (BFR), waist circumference, and serum triglyceride levels.<sup>4</sup> These reductions in fat were seen as early as four weeks and it was on this basis that the treatment period for the present study was set at 8 weeks. However, the results from the present study do not agree with their findings. In their study, a CT scan was used to assess body fat changes. CT scanning is the gold standard in body fat analysis, producing highly accurate results.<sup>4</sup> Assessment of visceral and total fat in the present study was measured using a DXA scanner, which can produce results for body fat content that are less accurate than that of CT scans.<sup>58</sup> In the Gradmark and colleagues study that found DXA measurements to be no more accurate at estimating visceral fat content than anthropometric measurements compared to CT scanning, investigators noted that the data from previous obesity studies using DXA for assessing body fat may have yielded unfavorable conclusions due to poor accuracy of this method.<sup>60</sup> Also in the Kondo and colleagues study, the body weight variance between measurements (collected at weeks 0, 4, 8, 12, and post treatment week 16) equated to less than three pounds over a 12-week period in all dose vinegar groups which may not realistically translate well to the general population in terms of cost-benefit for fat loss.<sup>4</sup>

In other vinegar studies that investigated the effects of vinegar consumption, fat reduction was not observed in accordance with our findings. Johnston and colleagues

found no significant changes in body weight with a vinegar drink (containing 750mg acetic acid) provided twice daily at meals<sup>65</sup>, nor in body weight or blood lipids<sup>66</sup> after a 12-week vinegar treatment period for both studies. Further, Lim and colleagues reviewed over 20 human intervention trials that examined vinegar consumption and glycemic control, and found no significant reductions in body weight or in the lipid profile as a result of vinegar consumption.<sup>10</sup>

A significant reduction between groups was found in all glycemic blood analyses in the present study; fasting blood glucose ( $P=0.003$ ), fasting insulin ( $P<0.001$ ), and HOMA-IR scores ( $P<0.001$ ). This was further substantiated with a moderate to high measured effect (Cohen's  $d$ ) in all blood indices. A HOMA-IR score of  $< 3.0$  is considered a normal insulin resistance measurement. The mean HOMA scores for both groups at baseline and at week 8 was  $> 3.0$ . Participant measures show an overall resistance to insulin with normal glucose values. Considering the inclusion criteria for waist circumference in the present study, the above average HOMA scores are indicative of the association between large waist size and insulin resistance.

Attenuated postprandial glucose and insulin blood levels related to regular vinegar consumption has been demonstrated in several short-term studies with convincing evidence. A recent meta-analysis concluded that postprandial glucose and insulin response may be reduced with regular ingestion of vinegar, and the overall effect size was stronger in subjects with impaired glucose tolerance, insulin resistance, and diabetes when compared to healthy subjects without metabolic disorders.<sup>25</sup> However, longer term trials are needed to determine safe, continuous consumption of vinegar as a means to promote glycemic control in various metabolic disorders.

All participants who completed this study reported their experience with any symptoms, positive or negative, they may have had at any point over the 8-week trial related to their vinegar supplementation (Appendix F,G). Participant responses to the exit survey were quantified using Chi square statistic. The type of vinegar supplementation received was slightly, but insignificantly, associated with the negative or positive side effects experienced. Based on participant responses overall, the negative side effects of the vinegar supplementation were predominately characterized by nausea or sickness, and adverse stomach or bowel issues with moderate effect size (Cramer's  $V=0.3$ ); while the positive side effects were predominately characterized by appetite suppression, decreased heart burn or acid reflux, or improved overall vitality.

The primary negative response among VIN group participants involved symptoms of nausea or stomach issues after intake of their liquid supplement; while the primary positive response was overall decreased appetite throughout the day and feeling of weight loss. Single negative responses by individuals in the VIN group include: burning of esophagus or stomach, frequent bowel movements (first day only), vinegar-smelling sweat, feeling of eroded front teeth, or reflux if taken too late. One participant experienced diarrhea after ingestion and reduced daily liquid vinegar supplementation by half. Single responses to “positive symptoms” among VIN group participants include: improved or increased bowel movements, reduced stomach bloating, increased water consumption, and feeling better overall.

CON group participants had individual responses to “negative symptoms” that were unspecific or isolated occurrences with no common complaints between group participants. Single negative responses by CON group participants include: stomach pain



or bloating, sometimes mild nausea, or tired eyes right after ingestion, and stomach ache if taken too early or late in the day. The majority of CON group participants indicated more positive responses to their supplement compared to the VIN group. The primary positive response among the group involved the feeling of weight loss or feeling leaner with pill intake, and some experienced improved digestion, or decreased symptoms of acid reflux or heart burn. Other single responses to “positive symptoms” by the CON group include decreased appetite, and improved digestion.

As noted, some participants experienced a decrease in heart burn or acid reflux symptoms. This may be explained by preliminary research proposing that acid ingestion may alleviate heart burn symptoms related to gastroesophageal reflux disease (GERD). A crossover pilot study (unpublished) examined the efficacy of vinegar on reducing heartburn symptoms that were related to GERD.<sup>67</sup> GERD occurs when the lower esophageal sphincter (LES) is weakened, which ultimately allows for the acidic contents of the stomach to flow back up into the esophagus.<sup>67</sup> Severity of this condition is dependent on how weak the LES is and the effect of saliva to buffer bolus acidity.<sup>67</sup> Saliva secretion increases when acidic foods and beverages are ingested, buffering the acidity as the bolus makes its way down the GI tract via primary peristalsis initiated by swallowing muscle movements.<sup>67</sup> Secondary peristaltic waves can be triggered by stretch receptors in the esophageal lining as well as pH receptors.<sup>67</sup> The proposed mechanism to which this pilot study was designed was based on the acid sensitive receptors in the esophageal mucosa that are triggered when the intraluminal pH drops. Stimulation of these receptors induces esophageal peristaltic muscle contractions, propelling a food bolus down the esophagus into the stomach.<sup>67</sup> Additionally, the acidity of vinegar may

also reduce the symptoms of heart burn related to GERD by amplifying the primary peristalsis movements and stretching out its duration.<sup>67</sup>

Vinegar has received substantial marketing attention as a result of reports that vinegar influences appetite suppression, lending to its popularity as a weight loss supplement.<sup>22</sup> Subjective reports from several participants in this study also agree with the appetite suppression side effects of vinegar. In a previously discussed study, Darzi and colleagues<sup>22</sup> investigated the palatability and tolerability of vinegar and their influence on appetite, both physiologically and gustatorily (taste sensation). They found that appetite was influenced by the palatability of vinegar and by subsequent feelings of nausea.<sup>22</sup> Inasmuch as palatability decreased (becoming less pleasurable), nausea increased resulting in reduced appetite, prolonged fullness, and a reduction in subsequent energy intake.<sup>22</sup> Based on these findings (appetite suppression and nausea related to the liquid vinegar ingestion) and the self-reported side effects from the VIN group in the present study, it would be expected that energy intake (as determined from the 24-hour dietary recalls) over the trial period would have decreased. However, data from dietary recalls obtained before and after the trial were not significantly different in the VIN group between weeks 1 and 8. Mean energy intakes were fairly consistent, despite several reports of suppressed appetite or induced nausea. Each participant received a calendar to track their vinegar supplementation each day. The mean compliance percent among the VIN group was  $92.7 \pm 13.3\%$  ( $n=18$ ; 3 lost their compliance calendars), therefore it is unlikely that a lack of compliance to the liquid vinegar supplement would have explained the inconsistency between the unchanged pre and post energy intakes and self-reported side effects of liquid vinegar ingestion that included nausea or appetite suppression.

Participants were instructed to maintain their dietary intakes throughout the trial period. It is possible that participants strictly followed study protocols despite vinegar-induced appetite cues to suppress hunger.

It is also interesting to note that among the VIN group there was a slight reduction in carbohydrate intake, -3%, and a slight increase in protein intake, +4%. In contrast, the CON group had a 6% increased intake of carbohydrates and a 3% increase in fat intake. A finding from the Darzi and colleagues study suggests that vinegar may have influence on curbing sweet cravings (which are primarily satisfied by carbohydrates) however, this was shown with a trend approaching significance ( $P=0.058$ ) among treatment groups.<sup>22</sup> The present study examined the relationship between vinegar and visceral fat, therefore it was not in the nature of the study design to place more specific controls to fully assess dietary intake. It may be worthwhile for future research to study the impact of daily vinegar ingestion on appetite, macronutrient composition of meals, and on respiratory quotient (RQ) variances.

This study had several limitations. The use of DXA scanning to assess body fat and distribution may generate conclusions based on results that may not be the most accurate compared to other methods of fat assessment. Eight weeks may be too short a time span to see a significant change in visceral fat. Finally, dietary intake was self-reported via 24-hour recall and may be not reflect true compliance to maintain normal dietary habits.

We conclude that the findings from this study did not support our hypothesis that daily red wine vinegar consumption will reduce visceral fat and waist circumference in healthy adults with central obesity. Further research is indicated on the association

between vinegar ingestion and appetite suppression, macronutrient distributions within the diet, and substrate utilization, as well as the safety of long-term daily vinegar consumption.

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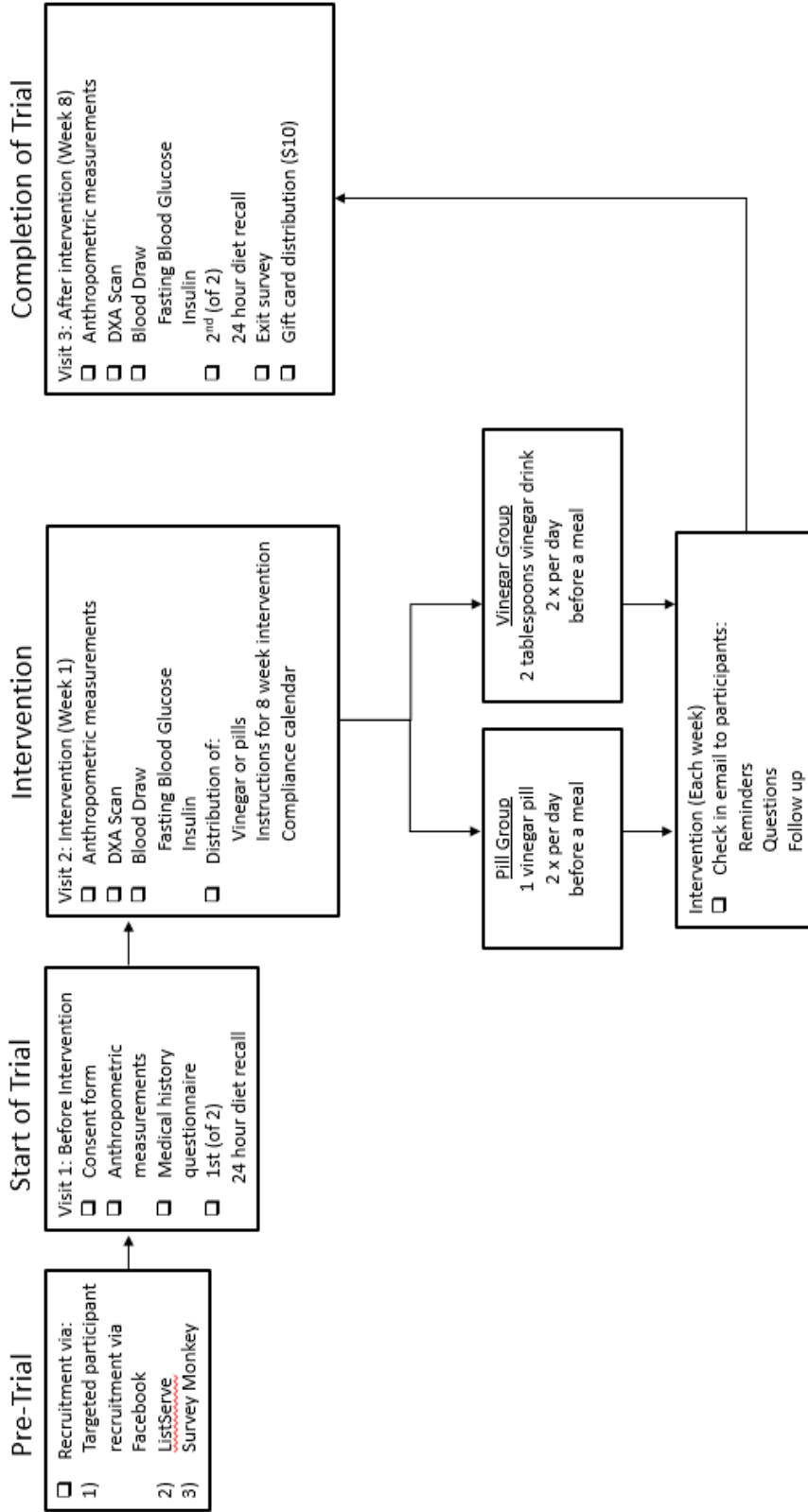
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APPENDIX A  
STUDY DESIGN FLOW CHART



APPENDIX B  
IRB APPROVAL

APPROVAL:CONTINUATION

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

Dear Carol Johnston:

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

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

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

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

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

Sincerely,

IRB Administrator

APPENDIX C  
ONLINE RECRUITMENT SURVEY

I am a graduate student in the School of Nutrition and Health Promotion at Arizona State University and am conducting a research study to examine health benefits of vinegar supplementation. I am inviting your participation in the screening process, which will consist of answering questions regarding health history, demographics, and scheduling availability. You have the right to not answer any question, and to stop participation at any time. We are recruiting health adults who have a little extra belly fat. Your participation in this survey and study is voluntary. If you choose not to participate or to withdraw from the study at any time, there will be no penalty. Your responses to this survey will be confidential. If you meet the criteria for this study, you will be contacted to schedule an in-person appointment at the downtown campus of Arizona State University.

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

By selecting “I agree” below you are agreeing to continue forward with the survey and be contacted by investigators (via e-mail) to schedule an appointment, should you qualify.

1. What is your email?

2. Are you an ASU student, staff, or faculty on the downtown campus?

- Yes  
 No

3. How old are you?

Age (years)

4. Are you male or female?

- Male  
 Female

5. Do you smoke cigarettes?

- Yes  
 No

6. Do you consider yourself to have extra belly fat?

- Yes



- No
- Unsure

7. How tall are you?

Height (inches) (Note: 5 feet=60 inches)

8. How much do you weigh?

Weight (pounds)

9. Are you generally healthy? (e.g., not seeing a doctor for a medical condition)

- Yes
- No

10. Do you take a prescription drug daily? (excluding birth control)

- Yes
- No

11. Are you willing to drink a diluted vinegar drink daily or consume a vinegar pill daily for 8 weeks?

- Yes
- No

12. Do you have any food allergies or diet restrictions?

- Yes
- No

13. Have you lost or gained more than 10 pounds in the last six months?

- Yes
- No
- Unsure

14. If female, are you pregnant, or hope to get pregnant, in the next few months?

- Yes
- No

15. Are you ok with providing a small blood sample on two occasions during the research trial?

- Yes
- No

16. Are you willing to meet with investigators at the ABC1 building on the ASU Downtown campus on 3 occasions?

- Yes
- No

APPENDIX D  
CONSENT FORM

# **The Impact of Vinegar on Fat Metabolism and Blood Values**

## **INTRODUCTON**

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

## **RESEARCHERS**

Dr. Carol Johnston, a Nutrition professor, and Lisa Gonzalez, a graduate student, at Arizona State University Downtown, Phoenix, have requested your participation in a research study.

## **STUDY PURPOSE**

The purpose of this 8-week long research study is to evaluate the impact of vinegar ingestion on fat metabolism and blood glucose concentrations.

## **DESCRIPTION OF RESEARCH STUDY**

You have indicated to us that you are 18-45 years of age and healthy. If female, you are not currently pregnant or planning a pregnancy. Also, you have not had abdominal surgery or chronic conditions involving your digestive track. This study will involve the completion of a brief health history questionnaire to demonstrate the absence of other conditions that may contraindicate participation.

This research entails that you visit our test facilities on three occasions on ASU's Downtown Phoenix campus. At your **first visit** (lasting ~30 minutes) you will complete a health history questionnaire and a 24-h diet recall to confirm your eligibility. Your height, weight, and waist circumference will be measured. You will be scheduled for the **second visit** which is the start of the 8-week study. You will need to fast for this visit (no food or drink with the exception of water for >8 hours). A blood sample will be taken from an arm vein (~2 tablespoons), and the DXA scan will take place. For the DXA scan (performed using an FDA-approved Dual-energy X-ray Absorptiometry machine) you will be asked to lie face up on an open, padded table for 7 minutes while the scanner arm of the DXA machine passes over the entire body. You can wear regular clothing but any metal (clothing or accessory) must be removed. You will be exposed to a small amount of radiation (1-4 microSieverts) that is within an acceptable range per the FDA. For comparison, you would be exposed to approximately 80 microSieverts on a transatlantic airline flight of 8 hours, 50 microSieverts living in Denver, Colorado, at an elevation of 5,000 feet for approximately 4 weeks, or 30 to 40 microSieverts during a typical chest x-ray. (For test accuracy, you will be asked about test procedures using barium/isotopes in the recent past and be scheduled for your visit with an adequate lapse of time.) At this visit you will also receive your vinegar supplement which is to be consumed daily as instructed. This visit is expected to last ~1 hour. The third visit will mirror visit 2, and you will receive a \$10 gift card.

## **RISKS**

Mild discomfort due to the venous blood draw may occur. Blood sampling may be associated with nausea, dizziness, faintness, and bruising at the site of needle insertion. A trained phlebotomist will collect the blood and manage participant reaction as appropriate. Regarding the DXA scan, anytime you are exposed to radiation there is potential risk. Females will take a urine pregnancy test prior to the DXA scan to demonstrate the absence of a pregnancy. A certified X-ray technician will complete all DXA scans.

## **BENEFITS**

You may not benefit from this study, but once the study is complete you will be provided with your test results if desired including bone mineral density and body fat composition.

## **NEW INFORMATION**

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

## **CONFIDENTIALITY**

All information obtained in this study is strictly confidential unless disclosure is required by law. The results of this research study may be used in reports, presentations, and publications, but your name or identity will not be revealed. In order to maintain confidentiality of your records, Dr. Johnston will use subject codes on all data collected, maintain a master list separate and secure from all data collected, and limit access to all confidential information to the study investigators.

**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.

**COSTS AND PAYMENTS**

You will receive a \$10 gift card at the completion of data collection.

**COMPENSATION FOR ILLNESS AND INJURY**

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

**VOLUNTARY CONSENT**

Any questions you have concerning the research study or your participation in the study, before or after your consent, will be answered by Dr. Carol Johnston, 500 N. 3<sup>rd</sup> St., Phoenix, AZ 85004. [602-827-2265]

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

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

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

Subject's Signature	Printed Name	Date
Contact phone number	Email	

**INVESTIGATOR'S STATEMENT**

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

Signature of Investigator \_\_\_\_\_ Date \_\_\_\_\_

APPENDIX E  
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: Have you ever been pregnant? \_\_\_\_\_  
If yes, date of last pregnancy? \_\_\_\_\_  
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:*

<u>Medication</u>	<u>Dosage</u>	<u>Frequency</u>

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

<u>Supplement</u>	<u>Dosage</u>	<u>Frequency</u>

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		

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

---

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

---

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

---

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

---

20. Are you willing to consume vinegar supplements daily for 8 weeks?    Yes    No



APPENDIX F

EXIT SURVEY

## Exit Survey - *The Impact of Vinegar on Fat Metabolism and Blood Values*

1. 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+

2. Did you have **problems consuming your vinegar supplement daily** during the study? Circle the most appropriate answer:

Never                      Occasionally                      Weekly                      Daily

3. Did you like taking your supplement? Circle the most appropriate answer:

No (terrible taste)

Not really (it was hard to remember to take the supplement)

Neutral (I did it since I was in this study and said I would)

Yes (the supplement was easy to take)

Yes (great taste)

4. Will you continue to take vinegar supplements after the study is over?      Yes      No

5. Did you experience any negative symptoms during the study? Yes No

If yes, please explain: \_\_\_\_\_

6. Did you experience any positive symptoms during the study? Yes No

If yes, please explain: \_\_\_\_\_

APPENDIX G  
EXIT SURVEY RESPONSES

## Exit Survey Responses

<b>Symptoms</b>	<b>VIN Freq.</b>	<b>CON Freq.</b>	<b>Total</b>	<b>%</b>
Nausea or sick after intake *(sometimes)	8	*1	9	20
Decreased appetite	4	1	5	11
Overall: felt better, leaner	1	4	5	11
During ingestion: burning esophagus or stomach burned/ grumbled	4		4	9
Shortly after ingestion: stomach pain/bloating	3	1	4	9
Improved digestion/bloating * (improved acid reflux/heart burn)	1	*3	4	9
Improved bowel movements	2		2	4
Diarrhea or increased frequency of bowel movements $\geq$ 1 day	2		2	4
Improved dietary habits as a result of vinegar supplement	2		2	4
Weight loss	2		2	4
Reflux if taken too late at night	1		1	2
Front teeth felt eroded	1		1	2
Sweat smelled like vinegar	1		1	2
Didn't notice any changes	1		1	2

**Symptoms: Break Down by Group**

<b>Positive Symptoms VIN:</b>	<b>Freq.</b>	<b>Negative Symptoms VIN:</b>	<b>Freq.</b>
Decreased appetite	4	Nausea	5
Weight loss <i>*1 subject "more room in clothes"</i>	2	Sick <i>*1 subject "Very sick, had to cut down to once per day in evening before big meal"</i>	3
Increase in bowel movements	1	Experienced stomach pain right after intake of supplement; lasted ~20 minutes each time	2
Easier to defecate	1	Stomach burned or grumbling after intake	2
Stomach was not bloated as usual	1	Burning esophagus	2
Increased overall daily water consumption	1	Bad taste	1
Became more conscious of what was being consumed	1	Frequent bowel movements the first day	1
Body felt better, more refreshed overall	1	Stomach bloated	1
		Diarrhea	1
		Reflux if taken too late at night	1
		Front teeth felt eroded	1
		Sweat smelled like vinegar	1
		Didn't notice any changes	1
<b>Positive Symptoms CON:</b>	<b>Freq.</b>	<b>Negative Symptoms CON:</b>	<b>Freq.</b>
Felt loss of weight or more lean <i>*1 subject "Felt as if I lost weight while still eating the same"</i>	3	Stomach ache if taken too early/late	1
Decreased appetite	1	Sometimes experienced nausea	1
Decreased symptoms of acid reflux	1	"Eyes felt tired after taking it"	1
Reduced heart burn	1		
Overall, felt better	1		
Improved digestion	1		

**Did you have problems consuming supplement daily during study:**

	Never	Occasionally	Weekly	Daily
VIN	10	5	2	4
CON	17	5	0	0

**Did you like taking supplement:**

	Frequency
No (terrible taste)	VIN: 8 CON: 0
Not really (it was hard to remember to take the supplement)	VIN: 2 CON: 3
Neutral (I did it since I was in this study and said I would)	VIN: 9 CON: 3
Yes (the supplement was easy to take)	VIN: 3 CON: 16
Yes (great taste)	VIN: 2 CON: 1

*Comments on problems consuming supplement daily:**Hard to take with you if you have to go somewhere**Hard to fit into a busy day**Hard to remember to take each day**Timing is often a problem**Hard to fit into schedule**Felt sick after taking it**Tasted bad, had to psyche self into taking it**Burned going down*