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Effects of A Standardized, Freeze-Dried Grape Powder in Adults with Metabolic Syndrome

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Master of Science Thesis

Effects of A Standardized, Freeze-Dried, Grape Powder in Adults with Metabolic Syndrome

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List of Abbreviations

Advanced Oxidation Protein Products (AOPPs)
Alanine Amino Transferase (ALT)
American Association of Clinical Endocrinology (AACE)
Blood Pressure (BP)
Body Mass Index (BMI)
Cardiovascular Disease (CVD)
C-jun N-terminal Kinase (JNK)
Coronary Heart Disease (CHD)
Endoplasmic Reticulum (ER)
European Group for the Study of Insulin Resistance (EGIR)
Fatty Acid Binding Protein (FABP)
Free Fatty Acid (FFA)
High-Density Lipoprotein Cholesterol (HDL-C)
Homeostatic Assay for Insulin Resistance (HOMA-IR)
Impaired Fasting Glucose (IFG)
Impaired Glucose Tolerance (IGT)
Insulin Receptor Substrate 1 (IRS-1)
Insulin-Dependent Glucose Transporter Type 2 (GLUT2)
Insulin-Dependent Glucose Transporter Type 4 (GLUT4)
Interleukin 10 (IL-10)
Interleukin 1β (IL-1β)
Interleukin 6 (IL-6)
International Diabetes Federation (IDF)
Malondialdehyde (MDA)
Metabolic Syndrome (MetS)
National Cholesterol Education Program- Third Adult Treatment Panel (NCEP-ATP III)
National Institutes of Health (NIH)
Nuclear Magnetic Resonance Spectroscopy (NMR)
Omega-3 Fatty Acid (OMEGA 3)
Peripheral Blood Mononuclear Cells (PBMCs)
Phenyl methane sulfonyl Fluoride (PMSF)
Polyunsaturated Fatty Acid (PUFA)
Proliferator-Activated Receptor Alpha (PPARα)
Reactive Oxygen Species (ROS)
Saturated Fatty Acid (SFA)
Soluble Intracellular Adhesion Molecule One (sICAM-1)
Standardized Grape Powder (GRAPE)
Thiobarbituric acid (TBA)
Thiobarbituric Acid Reactive Substances (TBARS)
Toll-Like Receptors (TLRs)
Trans-fatty Acid (TRANS)
Triglyceride (TG)
Tumor Necrosis Factor alpha (TNF-a)

Two-way Analysis of Variance (ANOVA)
Type 2 Diabetes Mellitus (T2DM)
Waist Circumference (WC)
World Health Organization (WHO)

Abstract

Diets rich in fruits and vegetables have been correlated to lower disease risk. This is theorized to be due to food bioactive components such as: vitamin C, vitamin E, carotenoids, and polyphenols. Polyphenols are of particular interest in dietary research do to their abundance in the human diet. The effects of whole grapes, in the form of a standardized, freeze-dried grape powder (GRAPE) were tested in adults with metabolic syndrome (MetS).

Subjects entered a randomized, placebo-controlled, crossover intervention for 13 weeks. This included a 2-week run-in, 4-week intervention (GRAPE or placebo), 3-week washout, and final 4-week intervention (alternate powder). Qualifying subjects (n = 20; 12 male, 8 female) were instructed to consume 60 g/day of GRAPE or nutritionally matched placebo. Subjects were consuming the equivalent of 2.5 cups of fresh grapes a day, which contains roughly 200 mg of polyphenols. The placebo did not contain polyphenols found in grapes. Subjects did not significantly alter their diets when comparing GRAPE and placebo.

The first aim of the study examined the effect of GRAPE on cardiometabolic risk factors. Plasma lipids, glucose, and alanine aminotransferase (ALT) were not significantly different between GRAPE and placebo periods. Interestingly, GRAPE slightly, but significantly, decreased BMI compared to placebo (-0.22 kg/m^2, p = 0.048). Additionally, a trend in weight reduction was apparent in the GRAPE group (-0.62 kg, p = 0.081). When stratifying for sex, plasma triglycerides (TG) (-43.45 mg/dL, p = 0.033) and pulse (-3.94 bpm, p = 0.003) were significantly reduced in men. In the female group a significant increase in TC was observed (5.25 mg/dL, p = 0.003) as well as Non-HDL-C (7.50 mg/dL, p = 0.010) with GRAPE ingestion compared to placebo.

The second aim of the study examined the effects of GRAPE on oxidative stress, inflammation and insulin resistance. Markers of oxidative stress, measured as advanced oxidative protein products (AOPPs), or thiobarbituric acid reactive substances (TBARS), were unaltered when comparing GRAPE to placebo. Plasma cytokines TNF-α, IL-6, and MCP-1 did not change significantly when comparing GRAPE to placebo. Finally, when examining plasma insulin changes and HOMA insulin resistance score, no significant differences were seen between groups. However, when stratifying for sex, significant differences were seen in the female subset. Plasma AOPPs were seen to be trending toward a significant increase compared to placebo (-24.35 µM vs, $p = 0.085$). Plasma TBARS were significantly increased with GRAPE compared to placebo (-0.19 µM, $p = 0.042$). It was seen that GRAPE significantly increased plasma insulin (9.82 ± 3.94 pmol/L, $p = 0.042$) and HOMA-IR Score (0.40 ± 0.16, $p = 0.043$) in the female subset.

These results suggest that GRAPE may influence body weight or composition, but not plasma lipids or underlying comorbidities, in a mixed sex population of adults with MetS. Further research is warranted on sex-dependent effects.

Introduction

In 2012, roughly 39 million people died from non-communicable, chronic, yet preventable, diseases (World Health Organization, 2015). The four main diseases were: diabetes, cancer, chronic lung disease, and cardiovascular disease (CVD). Throughout the world, CVD alone claimed 17.5 million lives in 2012, amounting to 30% of total deaths. Another chronic condition which warrants concern is type 2 diabetes mellitus (T2DM). Recent research estimates 9.3% of individuals in the United States are afflicted with T2DM, and more noteworthy, it is frequently listed as a cause of death (American Diabetes Association, 2017). However, in 2010, roughly only 35 to 40% of deaths of Americans with diabetes mentioned it on the death certificate. This suggests that diabetes may be an underreported cause of death. Interestingly, some of the identified risk factors for CVD overlap with those of T2DM, which has led to the recognition of metabolic syndrome (MetS) (National Institutes of Health, National Heart, Lung, and Blood Institute, 2016). Metabolic syndrome is a constellation of symptoms that together, increases the risk for development of T2DM and CVD.

The symptoms of MetS include: decreased concentrations of high-density lipoprotein cholesterol (HDL-C), and elevated plasma triglycerides (TG), fasting blood glucose, systolic blood pressure (SBP) or diastolic blood pressure (DBP), and elevated waist circumference (WC). Having three of the five previously mentioned symptoms qualifies an individual as having MetS (National Institutes of Health, National Heart, Lung, and Blood Institute, 2016).

In a state of high plasma FFA or TG, which can be present in excess central adiposity characteristic of MetS, the excess may be redirected to non-adipose tissues. Hepatic uptake of FFA is unregulated. If circulating levels of FFA are high, the liver will take them into its cells (Malhi and Gores, 2008). In the fatty liver, circulating FFA can activate many intracellular responses such

as: C-jun N-terninal kinase (JNK) activation, toll-like receptor (TLR) activation, and endoplasmic reticulum (ER) stress. The downstream result of these activations is insulin resistance (Shoelson, 2006). Additionally, the activity of tumor necrosis factor (TNF)-α increases the release of FFA from adipocytes and blocks the synthesis of adiponectin, which sensitizes adipose tissue to insulin. TNF-α also can activate NF-κB signal transduction, leading to the production of cellular adhesion molecules, and an inflammatory state in the adipocyte (Fernandez-Sanchez et al., 2011). It can also stimulate the expression of IL-6 and MCP-1 (Kern et al., 2001) These molecules attract inflammatory cells to the afflicted area, which generates a larger inflammatory response. Inflammation is not the sole contributor to insulin resistance. Oxidative stress can result in the dysfunction of insulin-related pathways (Keane et al., 2015). Excessive generation of reactive oxygen species (ROS) can lead to the damage of DNA, protein, and lipid membranes. Oxidative stress via ROS can be the result of over nutrition in the form of high glucose and lipid diets (Keane et al., 2015). This over nutrition can lead to the dysfunction of the electron transport chain, which produces increased ROS. Another pathway that ROS may be generated is via superoxide dismutase. Superoxide can be converted to H_2O_2, which is much more stable. However, H_2O_2 itself can react with Fe^{2+} to produce a highly reactive hydroxyl radical ($OH^{\bullet}$) (Keane et al., 2015). The consequences of all this damage is ultimately cell death and an inflammatory response.

Insulin resistance, inflammation, and oxidative stress are states commonly associated with MetS however, MetS is preventable by lifestyle interventions such as smoking cessation, increasing exercise, or modifying diet (National Institutes of Health, National Heart, Lung, and Blood Institute, 2016).

Disease prevention through dietary modifications is of particular interest. Data has shown that increased consumption of fruit and vegetables is correlated with reduced risk for all-cause

mortality and CVD (Wang et al., 2014). This appears to be due to the bioactive components of these foods, such as vitamin C, vitamin E, carotenoids, and polyphenols (Perez-Jimenez and Saura-Calixto, 2008). Polyphenols have garnered great interest in research due to their abundance in the human diet, as well as antioxidant properties (Manach, et al., 2004). One whole food that is rich in polyphenols is the grape (*Vitis vinifera)*. Anthocyanins are the dominant species of polyphenol found in red grapes, whereas flavan-3-ols are predominant in white grapes (Perez-Jimenez and Saura-Calixto, 2008). In previous research, the effectiveness of grape seed, skin, stem, and extracts on CVD risk have all been studied (Perez-Jimenez and Saura-Calixto, 2008). However, the consumption of whole grapes as a dietary intervention has not been fully evaluated. (Mozaffarian et al., 2015)

The objective of this study was to examine the effects of GRAPE on adults with MetS. We hypothesized that whole grape consumption would positively impact the metabolic profile of male and female adults with MetS. For this study, we had two specific aims. Aim 1 attempted to determine the effect of whole grape consumption on cardiometabolic risk factors pivotal to MetS assessment. It was our hypothesis that ingestion of whole grapes would positively impact the cardiometabolic profile of adults with MetS when compared to a placebo. Aim 2 attempted to determine the effect of whole grape consumption on inflammation, oxidative stress, and insulin resistance in adults with MetS. It was our hypothesis that ingestion of whole grapes will positively impact markers of inflammation, oxidative stress, and insulin resistance when compared to a placebo.

Chapter 1: Review of Literature

CHRONIC DISEASE

In the United States, cardiovascular disease (CVD) accounted for 30.8% of all deaths in 2013 (American Heart Association Statistical Update, 2016). The number one cause of death in adults over 65 years of age are diseases of the heart. Approximately 80% of CVDs can be prevented through lifestyle modification. These modifications include: not smoking/cessation, engaging in routine physical activity, controlling high blood pressure and elevated blood lipid concentrations, and maintaining a healthy diet (American Heart Association Statistical Update, 2016).

Diabetes is another chronic disease of concern. It is a cluster of metabolic diseases that are characterized by hyperglycemia (American Diabetes Association, 2014). This is the result of defects in insulin secretion, insulin action, or both processes. The chronic hyperglycemia seen in type 2 diabetes mellitus (T2DM) can lead to long term damage of: kidneys, nerves, the eyes, and heart and blood vessels. Overall, T2DM accounts for ~90-95% of total cases of diabetes in the U.S. (American Diabetes Association, 2014). Type 2 diabetes mellitus is a form of diabetes which is characterized by insulin resistance, rather than absolute insulin deficiency (Type 1, auto-immune disorder). As of 2012, in the United States, an estimated 21.1 million adults had been diagnosed with T2DM, with an estimated 8.1 million going undiagnosed (American Heart Association Statistical Update, 2016). An even larger number of U.S. adults are estimated to have prediabetes (fasting plasma glucose 100-126 mg/dL) at 80.8 million (35.3%). One risk factor that links both CVD and T2DM is obesity. Over the last ~30 years, obesity in the United States has increased in adults above the age of 20 from 23% (1988-1994) to 34% (2007-2008) (Ogden et al., 2010).

OBESITY

Obesity is the excess accumulation of body fat. As shown in the previous definitions, central obesity is an important risk factor to consider when examining MetS. Obesity is defined by WHO and the National Institutes of Health (NIH) as an individual having a BMI >30kg/m^2 (Nguyen et al., 2010). Those with obesity have a low-level of chronic inflammation. This can lead to an increase in risk for several chronic diseases including: T2DM, hypertension, CVD, and cancer (Nguyen et al., 2010 and National Institutes of Health, National Cancer Institute, 2017). Obesity can also be related to a host of digestive disorders, including: gastroesophageal reflux disease, colorectal polyps, and liver disease.

When cells accumulate more free-fatty acids (FFA) than those required for energetic processes, they are esterified and stored as triglyceride (TG) in lipid droplets. Adipocytes have a unique capacity to mobilize these fatty acid stores through the action of lipases (Schaffer, 2003). Non-adipose cells have a limited capacity for such storage, and when this capacity is exceeded dysfunction or cell death may occur; this is termed lipotoxicity.

LIPOTOXICITY- THE INDUCTION OF DYSFUNCTION

In a state of high plasma FFA or TG, the excess may be redirected to non-adipose tissues. In the pancreas, FFA dysregulate insulin secretion through many potential mechanisms, including effects on peroxisome proliferator-activated receptor alpha (PPARα), glucokinase, and insulin-dependent glucose transporter 2 (GLUT2) expression (Shaffer, 2003). It has been shown *in vitro* in primary rat pancreatic β-cells, as well as in human β-cell lines that excess FFA can lead to cellular apoptosis (Prentki et al., 2002). The extent of caspase activation increases with larger glucose concentrations. This suggests that the toxic effects of FFA and glucose are synergistic.

In the liver, TG and FFA accumulation is associated with non-alcoholic steatohepatitis (NASH). Non-alcoholic steatohepatitis is usually found in obese subjects and can progress to cirrhosis (Garg and Misra, 2002). Metabolic abnormalities associated with insulin resistance are also seen in high prevalence in those with NASH. Hepatic uptake of FFA is unregulated, and if circulating levels of FFA are high, then the liver will take them into its cells. Liver fatty acid binding protein (FABP) is present in the cytosol of hepatocytes. In the absence of FABP, hepatocytes are seemingly protected against saturated-fat induced TG deposition (Malhi and Gores, 2008). In the fatty liver, circulating FFA can activate many intracellular responses such as: C-jun N-terninal kinase (JNK) activation, toll-like receptors (TLRs) activation, and endoplasmic reticulum (ER) stress.

JNK is activated by FFA, in accordance with their toxicity. Free-fatty acid activation of JNK can lead to improper phosphorylation of insulin receptor substrate 1 (IRS-1), with the substitution of serine for tyrosine; resulting in insulin resistance (Shoelson, 2006). FFA also serve as ligands for TLRs in times of metabolic dysregulation, this represents an external cellular stress. An internal cellular stressor comes in the form of ER stress. Both TLR activation and ER stress can activate IKKβ, leading to NF-κB translocation to the nucleus (Shoelson, 2006). The products of NF-κB are often shown to induce or promote insulin resistance and include pro-inflammatory cytokines. These cytokines include, but are not limited to: TNF-α, interleukin 6 (IL-6), and monocyte chemoattractant protein-1 (MCP-1).

TNF-α was one of the first pro-inflammatory cytokines identified in the systemic response to be linked to the development of insulin resistance (Fernandez-Sanchez et al., 2011). The activity of TNF-α increases the release of FFA from adipocytes and blocks the synthesis of adiponectin, which sensitizes adipose tissue to insulin. It also interferes with the tyrosine phosphorylation of

IRS-1. TNF-α also can activate NF-κB signal transduction, leading to the production of cellular adhesion molecules, and an inflammatory state in the adipocyte (Fernandez-Sanchez et al., 2011). TNF-α can also stimulate the expression of IL-6 and MCP-1 (Kern et al., 2001) These molecules attract inflammatory cells to the afflicted area and generate a larger inflammatory response.

Inflammation is not the sole contributor to insulin resistance. Oxidative stress can result in the dysfunction of insulin-related pathways (Keane et al., 2015). Excessive generation of reactive oxygen species (ROS) can lead to the damage of DNA, protein, and lipid membranes. Oxidative stress via ROS can be the result of over nutrition in the form of high glucose and lipid diets (Keane et al., 2015). This over nutrition can lead to the dysfunction of the electron transport chain, which produces increased ROS. Another pathway that ROS may be generated is via superoxide dismutase. Superoxide can be converted to H_2O_2, which is much more stable. However, H_2O_2 itself can react with Fe^{2+} to produce a highly reactive hydroxyl radical ($OH^{\bullet}$) (Keane et al., 2015). The consequences of all this damage is ultimately cell death and an inflammatory response. However, the body has endogenous antioxidant systems to help off-set H_2O_2 build-up. Glutathione peroxidase and catalase are endogenous peroxidases that facilitate the decomposition of H_2O_2 to H_2O.

METABOLIC SYNDROME CLASSIFICATION

Metabolic Syndrome is considered to be a constellation of symptoms that increases the risk for T2DM and CVD (Fernandez, 2007). The rate of MetS in the United States is ~34% (American Heart Association 2016). The criteria and cut-offs for the classification of MetS vary between health agencies. Some common health agency definitions are listed below in **Table 1.4.1**. The most common criteria used for MetS classification are: reduced high-density lipoprotein cholesterol (HDL-C) concentrations, as well as increased plasma triglyceride (TG), blood glucose,

blood pressure (BP), and waist circumference (WC). The World Health Organization (WHO) definition of MetS assumes that insulin resistance is one of the major underlying contributors to the condition (World Health Organization, 1999). Thus, there is no fasting plasma glucose requirement; it requires instead a surrogate for insulin resistance such as glucose intolerance, impaired glucose tolerance (IGT), insulin resistance, or diabetes. This definition is somewhat controversial. To include insulin resistance makes it difficult to determine the lowest quartile of insulin sensitivity, which must be done using a clamp procedure, and is not feasible in clinics and epidemiological studies (Alberti et al, 2006). This definition also includes waist-to-hip ratio (WHR) as a measure of central obesity, which is relevant as a simple estimator of disease outcome. However, waist circumference is a more relevant index of central obesity, which correlates with negative metabolic variables (Janssen et al., 2004).

The European Group for the Study of Insulin Resistance (EGIR) proposed a modified model after WHO released its version in 1999 (Balkau et al., 1999). This modified method discontinued the physical measure of insulin using a euglycemic clamp. Instead, the EGIR proposed using a fasting plasma insulin level to estimate insulin resistance. To qualify, individuals would have to be within the top 25% of fasting plasma insulin values in a non-diabetic population. Additionally, the EGIR called for impaired fasting glucose (IFG) to replace IGT. This new definition also slightly altered the cut-off values for TG and HDL-C as well as being defined on treatment for dyslipidemia or hypertension as inclusion criteria.

The National Cholesterol Education Program- Third Adult Treatment Panel (NCEP-ATP III) proposed its definition of MetS in 2001 as a prevention method for coronary heart disease (CHD). Unlike previous methods of diagnosis, it does not include an insulin resistance component. The NCEP-ATP III definition does not require a glucose component like the previous definitions.

Instead, each risk factor is weighted evenly and an individual must have three of the five to have MetS. This definition also includes waist circumference as its measure of central obesity, similar to EGIR.

The American Association of Clinical Endocrinology (AACE) released its position on *insulin resistance syndrome* in 2002. The AACE statement does not include a specified number of risk factors to qualify, and allows for clinical judgement when identifying MetS in individuals (Einhorn, 2003). However, when diagnosed with diabetes, the individual is no longer considered to have *insulin resistance syndrome.*

Taking these definitions into account, the International Diabetes Federation (IDF) also released its own stance on MetS. This is considered to be a world-wide definition to be used in epidemiological and clinical practice (Alberti, et al., 2006). This definition was produced out of clinical practicality. The IDF used the NCEP-ATP III as a starting point because this definition did not include measurements of insulin resistance, which requires a very involved process. Insulin resistance is a key component of MetS, however the IDF definition focuses on WC. This is because it is an easy measure for clinical purposes and provides an accurate assessment of central obesity (Janssen et al., 2004).

DIETARY INTERVENTION

Increasing evidence suggests that eating a diet rich in fruit and vegetables plays a key role in the prevention of chronic diseases, such as T2DM, CVD, and certain cancers (Liu, 2013). Bioactive components of these plant-based foods are theorized to explain the association between fruits and vegetables and decreased risk for chronic disease. Phytochemicals are an example of a class of bioactive molecules exclusive to plant-based foods (Liu, 2013). Polyphenols are a complex and diverse group of phytochemicals with numerous subclasses, present in many plant-based

foods. It has been shown that many fruits contain high amounts of polyphenols; wild blueberry (429 ± 10 mg of GAE /100 g), blackberry (412 ± 6 mg of GAE/100 g), cranberry (287 ± 5 mg of GAE/100 g), and red grape (161 ± 7 mg of GAE/100g) were among the top ten fruits of 25 analyzed in one particular study (Wolfe et al., 2008). Grapes are an important contributor of polyphenols to the American diet (12.8% of total phenolics). Grapes also show high antioxidant capacity in cell studies, ranking 4th among 25 common fruits analyzed (Wolfe et al., 2008). Thus, whole grapes and grape products could be used as a dietary intervention when attempting to combat a chronic disease such as MetS.

For the benefit of research, the California Table Grape Commission has developed a standardized, freeze-dried, whole grape powder (GRAPE). Within this powder are seeded and seedless, red, green, and black table grapes in proportion with the annual crops (van Breeman et al., 2016). Chemical analysis is carried out on the powder to quantify the polyphenols within the powder. These polyphenols include: catechin, epicatechin, flavanols (quercetin, kaempferol, isorhamnetin), anthocyanidins (malvidin, peonidin, cyanidin), and resveratrol. Separate powders have been developed for human clinical trials and animal trials. For human clinical trials, a placebo powder has been developed that matches the GRAPE in kcals, color, texture, and flavor. In animal studies only a sugar-matched powder is used as a control. Long-term, easily reproducible, studies on the health benefits of whole grapes may be carried out with an appropriately matched placebo powder as a result of this development.

USE OF GRAPE AS INTERVENTION IN HUMANS

The standardized, freeze-dried, grape powder has been used in trials relating to cardiometabolic health in humans. Zern et al., (2005) conducted a study in women (24 pre-menopausal, 20 post-menopausal) to evaluate the effectiveness of GRAPE on plasma lipids,

inflammatory cytokines, and oxidative stress. The study design was randomized, single-blind, placebo-controlled, crossover. Subjects were instructed to consume 36g/day of GRAPE or placebo powder for four weeks, then entered a three-week washout. After the washout, subjects began the second arm, for four more weeks of the study on the opposite powder. Intake of GRAPE significantly decreased TG concentrations in both pre- and post-menopausal women by 15 and 6%, respectively ($p < 0.002$) compared to placebo. Menopausal status had no effect on plasma cytokine concentrations. However, ingestion of GRAPE significantly decreased TNF-α in both pre- and post-menopausal groups ($p < 0.05$).

Barona et al., (2012) conducted a study in men evaluate the effectiveness of GRAPE on MetS. Twenty-four men, aged 30-70 years were randomly assigned to this double-blind, placebo-controlled, crossover study. Subjects were instructed to consume 46g/day of GRAPE or placebo powder for thirty days, then entered a three-week washout. After the washout, subjects began the second arm of the study for thirty more days, on the opposite powder. Intake of GRAPE significantly reduced systolic blood pressure compared to placebo (122 ± 11 mmHg vs. 128 ± 10 mmHg, ($p < 0.025$). Plasma soluble vascular cell adhesion molecule-1 (sICAM-1) was lowered in the GRAPE group (142 ± 50 µg/L) compared to placebo (151 ± 51 µg/L) ($p < 0.025$). The decrease in sICAM-1 suggests function is being restored to the endothelium.

Upon further analysis of the same cohort, Barona et al., (2012) examined the effectiveness of GRAPE on MetS in the presence or absence of dyslipidemia. At baseline, these subjects were classified as dyslipidemic (HDL-C ≤40mg/dL and TG ≥150mg/dL) and non-dyslipidemic. All subjects significantly decreased their systolic blood pressure after ingestion of GRAPE compared to placebo ($p <0.0025$). In the dyslipidemic group adiponectin ($p <0.05$) and interleukin 10 (IL-10) ($p <0.005$) were decreased. The non-dyslipidemic group the opposite, with an increase in

adiponectin ($p < 0.05$) and IL-10 ($p < 0.005$). The GRAPE increased IL-10 and adiponectin, two anti-inflammatory proteins, in the non-dyslipidemic group. It was the conclusion of this author that people with dyslipidemic MetS have a lower number of anti-inflammatory HDL and more pro-inflammatory small dense LDL; this may provide a more inflammatory environment; thus, cells may be less responsive than their non-dyslipidemic counterparts.

Zunino et al. conducted a study to evaluate the effectiveness of GRAPE on obese individuals. Twenty-four obese adults (BMI 30-45kg/m^2), aged 20-60 years, were randomly assigned to this double-blind, placebo-controlled, crossover study. Subjects were instructed to consume 46g/day of GRAPE or placebo powder for three weeks, then entered a three-week washout. After the washout, subjects began the second arm of the study for three more weeks, on the opposite powder. Intake of GRAPE significantly reduced systolic blood pressure compared to placebo (117.5mmHg vs. 122.6mmHg, $p < 0.049$) over the first two weeks of the intervention. At week three, change was no longer significant. Using nuclear magnetic resonance spectroscopy (NMR), lipoproteins can be quantified into subclasses (Jeyarajah et al., 2006). It was observed that GRAPE significantly reduced NMR-large LDL and NMR-LDL-C concentrations in plasma after the three-week intervention ($p < 0.05$). Additionally, GRAPE significant reduced VLDL size compared to placebo after two weeks (52.9nm vs. 56.9nm, $p = 0.041$). This significance was not apparent after final week of the three-week intervention. This group also showed that interleukin 1β (IL-1β) ($p = 0.041$) and IL-6 ($p = 0.018$) increased significantly in LPS-stimulated peripheral blood mononuclear cells (PBMCs) in the GRAPE group. The combination of IL-1β and IL-6 will work in concert to stimulate phagocytosis of pathogens. This data suggests that GRAPE may sensitize the immune cells in obese individuals.

CONCLUSIONS

The American heart association has stated the rate of MetS in the United States is ~34% (American Heart Association 2016). Metabolic syndrome can result in a multitude of complications because of the wide range of inclusion. Central obesity is a major contributing factor to the development of lipotoxicity. Lipotoxicity can result in the production of pro-inflammatory cytokines, ER stress, and insulin resistance. These are just three underlying factors that can contribute to the advancement of MetS. As a dietary intervention, grapes and grape products have shown promising results in cell, animal, and human research to provide relief of these comorbidities. The California Table Grape Commission has developed a standardized, freeze-dried, grape powder for use in clinical and animal trials. This allows for more reliable, reproducible, studies to be conducted on long-tern whole grape ingestion. However, more research is needed in areas related to humans with whole grape consumption. The GRAPE has been used in some clinical trials with male and female subjects, and even in those with MetS. These studies have shown promising results. However, few studies exist to date that examine the effects of whole grape consumption on MetS and its underlying drivers, inflammation, oxidative stress, and insulin resistance.

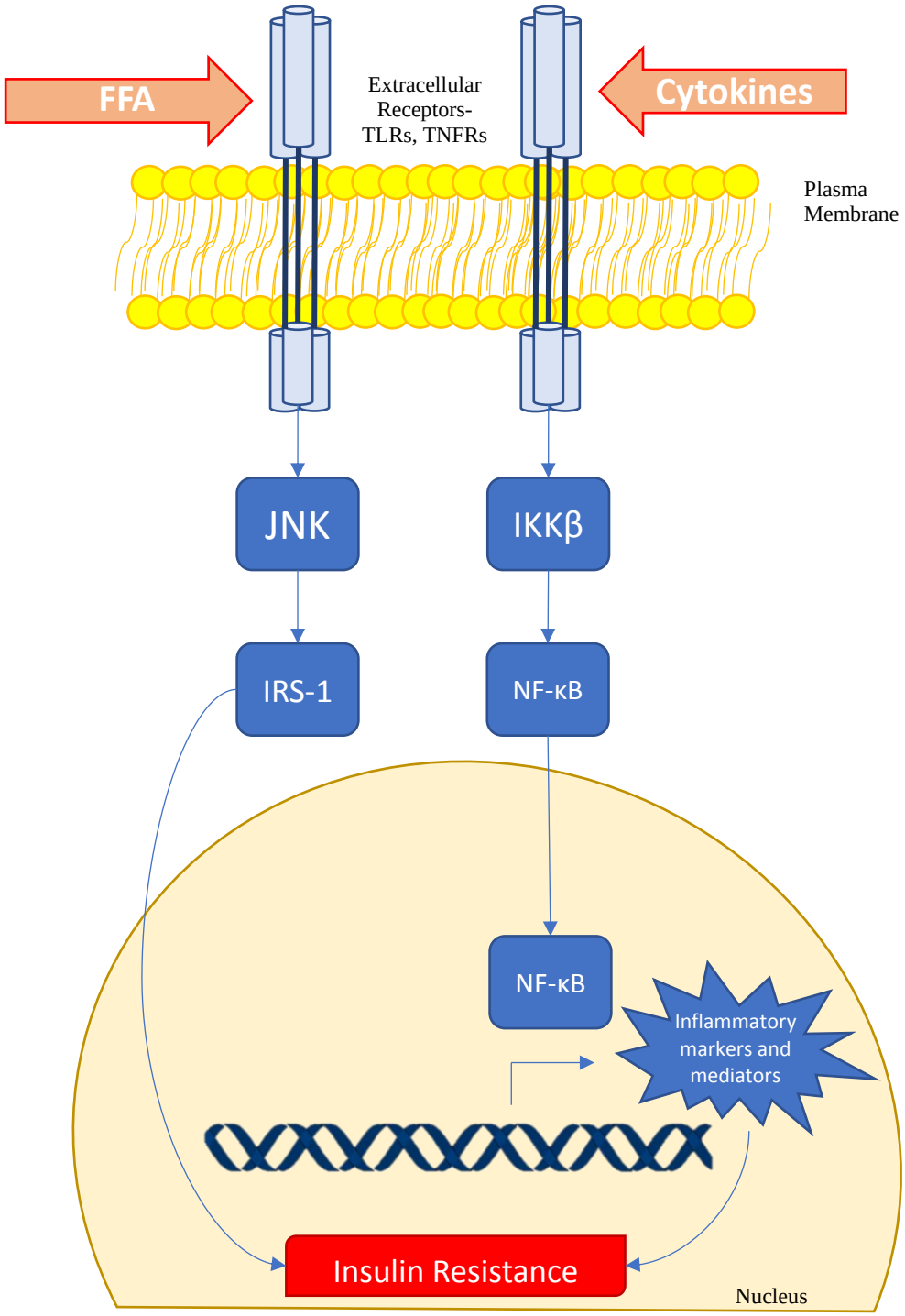

Figure 1.3.1. Potential cellular mechanisms of inflammatory signaling that may lead to insulin resistance

Table 1.4.1. Differences in agencies when establishing a definition for MetS. Adapted from Alberti *et al.* (2006)

	WHO		EGIR	NCEP ATP III	AACE	IDF
	Glucose intolerance, IGT, diabetes, and/ or IR with 2 of the following:		Insulin resistance plus 2 of the following:	Three of the following:	No defined number of risk factors	Elevated WC (ethnicity specific) plus 2 of the following:
Fasting Plasma Glucose			>110 mg/dL, non-diabetic	>100 mg/dL	>110-126 mg/dL	>100 mg/dL or T2DM
Blood Pressure	≥140/90 mmHg		≥140/90 mmHg or treatment	≥130/≥85 mmHg	≥130/≥85 mmHg	≥130/≥85 mmHg
Triglycerides	≥150 mg/dL		>178 mg/dL or treatment	≥150 mg/dL	≥150 mg/dL	≥150 mg/dL
HDL Cholesterol **Men:** **Women:**	<35 mg/dL <39 mg/dL		<39 mg/dL or treatment	<40 mg/dL <50 mg/dL	<40 mg/dL <50 mg/dL	<40 mg/dL <50 mg/dL
Central Obesity **Men:** **Women:**	Waist: hip >0.90 >0.85	BMI (and/or) >30 kg/m²	Waist circumference ≥94 cm ≥80 cm	Waist circumference ≥102 cm ≥88 cm	BMI ≥25 kg/m²	Europids Male ≥94 cm Female≥80 cm South Asians Male ≥94 cm Female≥80 cm Chinese Male ≥94 cm Female≥80 cm Japanese Male ≥94 cm Female≥80 cm Other ethnicities not defined due to lack of data. Use Europid data.
Other	Urinary albumin excretion rate: ≥20 ug	Albumin: Creatinine ≥30 mg/g			2-hour Post glucose challenge >140 mg/dL	

Chapter 2: Effects of GRAPE on Cardiometabolic Risk Factors in Adults with Metabolic Syndrome

Introduction

Some of the identified risk factors for CVD overlap with those of T2DM. Some of these risk factors include: obesity, dyslipidemia, and hypertension. In the United States, 69% of adults are overweight and 35% are obese. The burden of cost per capita for obesity in the United States is $1160 for men and $1525 for women (American Heart Association, 2016). Approximately 25% of U.S. have high TG (>150 mg/dL). Roughly 80 million U.S. adults have hypertension, which equates to 32.6%. The very high rates of risk factors for T2DM and CVD has led to the recognition of metabolic syndrome MetS (National Institutes of Health, National Heart, Lung, and Blood Institute, 2016). Metabolic syndrome is a constellation of symptoms that together, increases the risk for development of T2DM and CVD.

The symptoms of MetS include: decreased concentrations of high-density lipoprotein cholesterol (HDL-C), and elevated plasma triglycerides (TG), fasting blood glucose, systolic blood pressure (SBP) or diastolic blood pressure (DBP), and elevated waist circumference (WC). Having three of the five previously mentioned symptoms qualifies an individual as having MetS (National Institutes of Health, National Heart, Lung, and Blood Institute, 2016).

The management of these factors is treated similar to those in the chronic disease state. However, when disease in in full-blown progression dietary intervention is often forgone. Metabolic syndrome is simply a cluster of risk factors that increases one's risk for disease. According to Grundy et al., (2005), addressing physical inactivity and nutritional status are first

line defenses in MetS. It is recommended to avoid a more atherogenic profile, a diet roughly 30% fat is taken by the individual, in order to reduce the risk of decreasing HDL further. It is also recommended to intake a high amount of fruits and vegetables.

Data has shown that increased consumption of fruit and vegetables is correlated with reduced risk for all-cause mortality and CVD (Wang et al., 2014). This is theorized to be due to the bioactive components of these foods, such as vitamin C, vitamin E, carotenoids, and polyphenols (Perez-Jimenez and Saura-Calixto, 2008). Polyphenols have garnered great interest in research due to their abundance in the human diet, as well as antioxidant properties (Manach, et al., 2004). One whole food that is rich in polyphenols is the grape (*Vitis vinifera)*. In previous research, the effectiveness of grape seed, skin, stem, and extracts on CVD risk have all been studied (Perez-Jimenez and Saura-Calixto, 2008). However, the consumption of whole grapes as a dietary intervention has not been fully evaluated (Mozaffarian et al., 2015). It is our hypothesis that ingestion of whole grapes will positively impact the cardiometabolic profile of adults with MetS when compared to a placebo.

Materials and Methods

OVERVIEW OF STANDARDIZED GRAPE POWDER AND PLACEBO POWDER

Standardized Grape Powder

Standardized grape powder (GRAPE) is used within clinical research and animal studies. The GRAPE is made from a combination of green, red, and black grapes of the seeded and seedless variety. The proportion of green, red, and black grapes in the GRAPE is in accordance with annual production, as noted by the California Table Grape Commission (van Breemen, R. B et al., 2016; California Table Grape Commission, 2016). These grapes are frozen and ground with food grade dry ice and reground to ensure a fine consistency (National Foods Lab; Livermore, CA). The

GRAPE also contains silicon dioxide as an anti-caking agent (van Breemen, R. B et al., 2016; California Table Grape Commission, 2016). A standard serving of fresh grapes is ~126 g. It has been calculated previously that 100 g of fresh grapes is equal to 18.2 g of GRAPE. Since one serving of fresh grapes is ~126 g, one serving of grapes is equal to 23 g of GRAPE. (California Table Grape Commission, 2016) Nutrient composition of GRAPE according to the California Table Grape Commission can be found in **Table 2.2.1**.

Placebo Powder

The placebo powder, for use in human clinical trials, is produced to mimic the characteristics of the GRAPE. Also produced by the California Table Grape Commission, the placebo is matched to the GRAPE in certain characteristics including: dietary fiber, sugar profile, organic acid profile, and other sensory characteristics. Fresh grapes contain 0.2-0.8% organic acids, including tartaric, malic, and citric acids, these were added in proportion to the placebo powder. Silicon dioxide was also added in the same quantity as the GRAPE for anti-caking (van Breemen, R. B et al., 2016; California Table Grape Commission, 2016).

Table 2.2.1. Summary of Nutrients in the Grape Powder

Nutrient	Amount (per 100g)
Calories	371 kcal
Total Fat	0.299 g
Total Carbohydrate	88.6 g
Protein	3.58 g
Beta-Carotene	0.127 mg
Vitamin A	212 IU
Vitamin C	2.7 mg
Calcium	50 mg
Iron	1.43 mg
Sodium	11.8 mg
Potassium	973 mg
Thiamine HCl	0.17 mg
Folic Acid	49 mcg
Phosphorus	104 mg
Magnesium	33.3 mg
Zinc	0.416 mg
Copper	0.450 mg
Manganese	0.379 mg
Moisture	4.52 g
Ash	3.02 g

Table 2.2.2. Summary of Phytochemical Composition of Grape Powder

Compounds	Total (mg/kg)	Individual (mg/kg)
Catechins	135.4	
Catechin		77.4
Epicatechin		58.9
Anthocyanins	533.65	
Peonidin		47.63
Cyanidin		266.7
Malvidin		219.32
Flavonols		
Quercetin		148.7
Kaempferol		7.38
Isorhamnetin		13.95
Stilbenes		
Resveratrol		13.6
Total Polyphenols (Gallic Acid Equivalents)	3260	

HUMAN SUBJECTS AND INTERVENTION

Recruitment

Adults aged 30-70 were recruited using flyers, email, and in-person methods. Flyers were posted throughout the University of Connecticut campus (Storrs, Connecticut) and surrounding area in approved locations. Local and university events were also used to recruit subjects. Study overview sheets and flyers were brought to local and university events in order to assist study staff in recruitment.

Enrollment Totals

The total number of individuals prescreened for the study was 53. Of those 53 individuals, 43 continued with an in-person screening. Twenty-nine individuals qualified for the study after the screening process, six chose not to participate. Of the remaining 23 participants, three dropped

while on study. Twenty participants completed the study (n = 20). Of the 20 subjects finished, 12 subjects were male and 8 were female.

Pre-screening

Potential subjects were emailed or called on the telephone to follow-up and gauge interest and eligibility. Pre-screening questions addressed broad inclusion and exclusion criteria, as well as their ability to comply with the study and its time commitments. After these questions, if the individual was still interested in the study, and appeared to be eligible, a screening visit would be scheduled.

Screening

Once recruited, individuals were screened. During the screening visit the experimental procedure and time commitments of the study were explained. Consent was obtained before any personal information was collected. Inclusion criteria for the study was based on a modified version of the NCEP ATP-III criteria for metabolic syndrome. Potential subjects had to meet these criteria, or be taking blood pressure medication as an alternative for the hypertension criteria. Exclusion criteria included: not having metabolic syndrome, having a history of major chronic disease (e.g. cancer, diabetes, CVD), weight fluctuation greater than ± 10% over the previous four weeks, taking lipid or glucose altering medication, taking anti-inflammatory medication, exceeding "extreme risk" for any of the inclusion criteria [TG (>500 mg/dL), TC (>300 mg/dL), blood glucose (>126 mg/dL), WC (>200cm), or BP (>160/100 mmHg)]), or allergy to the powder or its ingredients. After obtaining consent, the individual filled out a medical history form for the study staff to keep on file. Once paperwork was completed, anthropometric data was collected. Blood pressure and pulse were measured using an electronic sphygmomanometer (Omron,

Healthcare Inc., Bannockburn, IL). Body weight was measured and recorded in kilograms. Height was measured and recorded in inches then converted to meters. Waist circumference was measured using a non-flexible waist measuring tape above the iliac crest and recorded in centimeters. After recording the anthropometric measures, a finger prick was conducted and blood was collected via capillary tube. Individuals were fasted for twelve hours previous to the blood collection to assure accurate values. Blood samples were immediately analyzed using an Alere Cholestech LDX Analyzer (MedTeck, Salt Lake City, UT). Individuals were notified immediately of qualification or disqualification. If their values did not fit within the specified inclusion criteria, they were informed they did not qualify. However, if any of their values were within the "extreme risk" range, they were not enrolled and advised to see a doctor. If the individual being screened met the study inclusion criteria and was willing to continue with enrollment they were given required study materials and scheduled a start date.

Table 2.2.3. Summary of NCEP/ATP-III Criteria for MetS.

NCEP ATP III	
Three of the following:	
Fasting Plasma Glucose	>100 mg/dL
Blood Pressure *Systolic* *Diastolic*	 ≥130 mmHg ≥85 mmHg
Triglycerides	≥150 mg/dL
HDL Cholesterol *Men:* *Women:*	 <40 mg/dL <50 mg/dL
Waist circumference *Men:* *Women:*	 ≥102 cm ≥88 cm

Intervention

The intervention was a randomized double-blind, placebo-controlled, crossover study. Neither the subjects, nor the investigators, were aware of the identity of the placebo or GRAPE

throughout the duration of the intervention. Subjects were assigned a number according to their entry into the study. Once qualified for the study, subjects were randomly assigned to an intervention powder. Subjects were instructed to ingest one 30 g packet of powder approximately 30 minutes after the morning meal and another packet of powder approximately 30 minutes after the evening meal. Total powder ingestion was 60 g/day (prepackaged powder prepared by the California Table Grape Commission, Fresno, CA). Sixty grams/day of grapes is roughly 2.5 cups, and provides ~200 mg polyphenols. Upon starting the study, subjects entered a run-in phase. The run-in phase consisted of two weeks of avoiding restricted foods that were provided in a list to the subject. These restricted foods were to be avoided throughout the duration of the intervention. During the second week of the run-in phase, subjects recorded their diet and exercise habits. After the run-in phase, subjects began their first intervention powder for the first four weeks of the intervention. For this arm, subjects were instructed to continue avoiding the restricted foods. After the first arm of the intervention, subjects entered a three-week washout, to normalize any potential metabolic changes. The second arm of the study mirrored the first, the exception being subjects consumed the opposite intervention powder.

BLOOD PROCESSING AND STORAGE

Twelve- hour fasting blood samples were collected at baseline and after four weeks for both arms of the study. During the two baseline visits, four tubes of blood were collected to use for plasma and two were collected for serum. During the two end-point visits, two tubes were collected for plasma and serum, respectively. Blood samples were collected using BD Vacutainer serum-separator, and EDTA plasma tubes (Becton, Dickinson, and Company, Franklin Lakes, NJ). Blood samples were centrifuged at 2400 RPM for 11 minutes at 4°C. Once finished centrifuging, plasma was collected from EDTA tubes and aliquoted into a separate 15mL tube. Bovine aprotinin

from a stock of 7500-13,000 U/mL was added to the plasma to inhibit serine proteases. A final concentration of ~1000 U/L was desired, thus 1 µL of stock was added per 1 mL of plasma. Phenyl methyl sulfonyl fluoride (PMSF) from a stock of 100 mM was also added to inhibit serine and cysteine proteases. A final concentration 100 nM was desired, thus 1 µL of PMSF was added per 1 mL of plasma. The final ratio of plasma to protease inhibitors was 1mL plasma: 1 µL aprotinin: 1 µL PMSF. The contents of this tube were transferred to 1.5mL Eppendorf tubes (Eppendorf North America, Hauppauge, NY) in 500 µL aliquots and stored at -80°C for later analysis. Once finished centrifuging, serum from serum-separator tubes was transferred neat into 1.5 mL Eppendorf tubes in 500 µL aliquots and stored at -80°C for later analysis.

DIETARY ANALYSIS, EXERCISE RECORDS, AND COMPLIANCE

Upon enrollment in the study, subjects were provided with a series of dietary and exercise records. Subjects were provided with a 5-day diet record, and a 7-day exercise record. The purpose of these records was to review and ensure compliance as well as consistency in the diet and exercise patterns. Subjects were to fill out the 5-day and 1-day diet records in the six days prior to the baseline blood draws for each arm of the study. Subjects were only required to fill out the 5-day diet record for the end-point blood draws. Subjects had were to fill out the exercise records the week prior to each blood draw. Using NDSR dietary analysis software, (University of Minnesota, Minneapolis, MN) subject diet records were analyzed for significant changes.

Compliance to the study powders was measured in several ways. First, subjects were given hard copy records to fill out daily and weekly. Included within these records were questions about adhereance and compliance in additon to the the foods to be avoided inn the restricted list. Next, study participants were asked to bring empty packets to their end-point visits. Study staff then

counted these packets to ensure the number of empty packets matched up with the hard copy records.

SERUM BIOCHEMICAL ANALYSIS

Plasma Lipids, Glucose, ALT

Total cholesterol, HDL-C, TG, glucose, and alanine amino transferase (ALT), were measured using enzymatic and photometric assays via a COBAS c111 Analyzer (Roche Diagnostics International Limited, Rotkreuz, Switzerland). Non-HDL-C was calculated by subtracting HDL-C from TC. LDL-C was calculated using the Friedewald method. Samples from baseline and end-point for each arm were run simultaneously to minimize variance.

STATISTICAL ANALYSIS

Differences between end-points of groups were evaluated by independent *t* tests (significance = $p < 0.05$). Two-way repeated measures ANOVA was used to determine the effect of the intervention for variables with baseline measures, with the within subject variables as treatment (tests GRAPE vs. placebo effect) and between subjects-variable as sequence order (tests carryover effect). All statistical analysis was done using IBM SPSS Statistics software. Data are reported as mean ± SEM.

Results

DIETARY INTAKE DURING INTERVENTION

During the intervention, subjects were instructed not to change their habitual dietary patterns. To ensure this, a dietary analysis was performed using NDSR software. Subjects did not alter their diets from one arm of the study to another in any of the observed categories.

Table 2.3.1. Summary of Dietary Intake of Subjects. n = 15. *$p < 0.05$.

Variable	End-Point	GRAPE vs. Placebo	*P*-value
Total Energy, *kcal*			
GRAPE	1803.73 ± 249.30	133.93 ± 168.61	0.440
PLACEBO	1669.80 ± 156.55		
Glycemic Load			
GRAPE	102 ± 26.84	14.93 ± 18.28	0.428
PLACEBO	88.00 ± 10.87		
Total Fat, *g*			
GRAPE	78.49 ± 9.39	1.42 ± 8.86	0.875
PLACEBO	77.08 ± 8.74		
Total Carbohydrate, *g*			
GRAPE	191.10 ± 43.75	24.51 ± 25.98	0.361
PLACEBO	166.59 ± 21.65		
Total Protein, *g*			
GRAPE	83.66 ± 5.49	9.68 ± 5.65	0.109
PLACEBO	73.98 ± 4.13		

Variable	End-Point	GRAPE vs. Placebo	*P*-value
Total Alcohol, *g*			
GRAPE	3.87 ± 1.78	-1.32 ± 1.31	0.332
PLACEBO	5.19 ± 2.39		
Cholesterol, *mg*			
GRAPE	335.67 ± 35.18	-3.13 ± 36.36	0.933
PLACEBO	338.80 ± 41.10		
SFA, *g*			
GRAPE	26.01 ± 3.29	1.81 ± 3.38	0.602
PLACEBO	24.20 ± 2.76		
PUFA, *g*			
GRAPE	17.48 ± 3.07	-0.76 ± 3.05	0.807
PLACEBO	18.24 ± 2.69		
TRANS, *g*			
GRAPE	2.21 ± 0.36	0.21 ± 0.30	0.496
PLACEBO	2.00 ± 0.29		
OMEGA 3, *g*			
GRAPE	2.03 ± 0.29	0.09 ± 0.38	0.807
PLACEBO	1.93 ± 0.28		
Total Dietary Fiber, *g*			
GRAPE	17.84 ± 2.59	1.24 ± 1.42	0.397
PLACEBO	16.60 ± 2.09		
Vitamin E, *IU*			
GRAPE	16.53 ± 3.16	2.13 ± 2.25	0.358
PLACEBO	14.40 ± 2.12		

Variable	End-Point	GRAPE vs. Placebo	*P*-value
Vitamin C, *mcg*			
GRAPE	70.75 ± 9.05	5.02 ± 12.33	0.690
PLACEBO	65.72 ± 12.30		
Selenium, *mcg*			
GRAPE	110.59 ± 7.47	5.12 ± 6.66	0.455
PLACEBO	105.47 ± 6.90		
β-Carotene, *mcg*			
GRAPE	3305.13 ± 775.94	-1765.47 ± 1415.45	0.233
PLACEBO	5070.60 ± 1580.30		
α-Carotene, *mcg*			
GRAPE	473.07 ± 156.09	-972.93 ± 556.76	0.102
PLACEBO	1446.00 ± 698.59		
Lutein & Zeaxanthin, *mcg*			
GRAPE	3930.67 ± 1414.78	1102.87 ± 1542.33	0.486
PLACEBO	2827.80 ± 570.36		
Lycopene, *mg*			
GRAPE	7441.20 ± 1639.42	1840.20 ± 1781.44	0.319
PLACEBO	5601.00 ± 589.01		
Daidzein, *mg*			
GRAPE	0.20 ± .05	-0.04 ± 0.05	0.489
PLACEBO	0.23 ± 0.07		
Genistein, *mg*			
GRAPE	0.21 ± 0.07	-0.06 ± 0.09	0.463
PLACEBO	0.28 ± 0.11		

Abbreviations: Saturated fatty acid (SFA), Polyunsaturated fatty acid (PUFA), Trans-fatty acid (TRANS), Omega-3 fatty acid (OMEGA 3)

EFFECTS OF GRAPE ON ANTHROPOMETRIC VARIABLES

When analyzed using two-way ANOVA, it was observed that body weight in the GRAPE group was trending toward a minor decrease compared to placebo (0.03 ± 0.33 kg vs. 0.65 ± 0.21 kg, p = 0.081). Consequently, BMI was observed to be slightly, but significantly (0.01 ± 0.11 kg/m^2 vs. 0.23 ± 0.07 kg/m^2, p = 0.048), decreased in the GRAPE group over the 4-week intervention period. When stratifying groups into male and female it was seen that GRAPE did have a significant effect on anthropometrics. In men, pulse was significantly reduced (-0.64 ± 1.22 bpm vs. 3.30 ± 1.15 bpm, p = 0.003).

Table 2.3.2. Summary of Anthropometrics. n=19, male n=11, female n=8

Variable	Baseline	Week 4	Change	*P*-value
Body weight, *kg*				
GRAPE	93.82 ± 3.92	93.85 ± 3.97	0.03 ± 0.33	0.081*
Male	103.71 ± 4.38	103.86 ± 4.51	0.15 ± 0.55	0.213#
Female	79.79 ± 4.36	79.70 ± 4.29	-0.09 ± 0.38	0.227$
PLACEBO	92.93 ± 3.72	93.55 ± 4.00	0.65 ± 0.21	
Male	102.58 ± 4.12	103.26 ± 4.51	0.68 ± 0.35	
Female	79.59 ± 4.11	80.19 ± 4.09	0.60 ± 0.21	
BMI, *kg/m^2*				
GRAPE	32.93 ± 1.08	32.94 ± 1.07	0.01 ± 0.11	***0.048****
Male	33.58 ± 1.70	33.62 ± 1.71	0.04 ± 0.17	0.184#
Female	32.06 ± 1.40	32.03 ± 1.35	-0.03 ± 0.16	0.238$
PLACEBO	32.64 ± 1.01	32.92 ± 1.08	0.23 ± 0.07	
Male	33.20 ± 1.60	33.42 ± 1.65	0.22 ± 0.11	
Female	31.99 ± 1.33	32.24 ± 1.32	0.25 ± 0.09	

Variable		Baseline	Week 4	Change	*P*-value
Waist Circumference, *cm*					
GRAPE		109.07 ± 2.94	108.42 ± 3.10	-0.65 ± 0.52	0.382*
	Male	112.58 ± 4.10	112.69 ± 4.27	0.11 ± 0.41	0.990#
	Female	103.21 ± 4.15	101.52 ± 4.23	-1.70 ± 1.14	0.315$
PLACEBO		107.78 ± 2.72	107.58 ± 2.99	0.02 ± 0.49	
	Male	111.18 ± 3.72	111.31 ± 4.06	0.12 ± 0.60	
	Female	102.57 ± 4.08	102.45 ± 3.97	-0.62 ± 0.99	
Systolic Blood Pressure, *mmHg*					
GRAPE		129.04 ± 2.58	129.41 ± 3.10	0.37 ± 2.10	0.516*
	Male	132.21 ± 3.92	133.82 ± 4.52	1.61 ± .19	0.672#
	Female	124.49 ± 3.17	122.07 ± 3.41	-2.42 ± 4.23	0.414$
PLACEBO		131.27 ± 2.98	133.02 ± 3.84	1.74 ± 2.24	
	Male	136.06 ± 4.08	136.85 ± 6.04	0.79 ± 3.47	
	Female	124.71 ± 4.10	127.75 ± 3.38	3.03 ± 2.58	
Diastolic Blood Pressure, *mmHg*					
GRAPE		85.16 ± 1.64	86.37 ± 1.88	1.21 ± 1.23	0.485*
	Male	86.94 ± 2.28	88.64 ± 2.46	1.70 ± 1.43	0.216#
	Female	83.24 ± 2.58	82.75 ± 3.01	-0.49 ± 2.14	0.700$
PLACEBO		87.32 ± 1.75	87.05 ± 1.90	-0.49 ± 1.25	
	Male	89.70 ± 1.98	88.70 ± 2.64	-1.00 ± 1.56	
	Female	84.58 ± 3.24	84.79 ± 2.67	0.21 ± 2.17	
Pulse, *beats/min*					
GRAPE		67.34 ± 2.39	66.93 ± 2.53	-0.42 ± 1.39	0.533*
	Male	68.00 ± 3.63	67.36 ± 4.43	-0.64 ± 1.22	***0.003#***
	Female	65.78 ± 3.44	67.29 ± 1.93	1.50 ± 2.59	0.444$
PLACEBO		66.35 ± 2.36	67.70 ± 2.25	1.17 ±1.09	
	Male	64.27 ± 3.40	67.57 ± 3.54	3.30 ± 1.15	
	Female	69.63 ± 3.53	67.87 ± 2.52	-1.76 ± 1.60	

* = comparison of GRAPE vs. placebo in entire population, # = comparison of GRAPE vs. placebo in male subset, $ = comparison of GRAPE vs. placebo in female subset.

EFFECTS OF GRAPE ON PLASMA VARIABLES

Using a two-way ANOVA, plasma lipids, fasting glucose, and plasma ALT were compared over the 4-week period. None of the plasma variables measured significantly changed after four weeks in the GRAPE or placebo groups. However, when stratified for sex, male subjects in the GRAPE group had a significant decrease in TG (-35.00 ± 15.79 mg/dL vs. 8.45 ± 11.57 mg/dL, $p = 0.033$). Female subjects had a significant increase in TC (-0.75 ± 6.55 mg/dL vs -6.00 ± 5.94 mg/dL, $p = 0.003$), Non-HDL-C (3.75 ± 5.55 mg/dL vs. -3.75 ± 3.97 mg/dL, $p = 0.010$), and LDL-C (1.43 ± 5.21 mg/dL vs. -6.30 ± 4.67 mg/dL, $p = 0.049$) with GRAPE ingestion compared to placebo.

Table 2.3.3. Summary of plasma lipids, glucose, and ALT. n=19, male n=11, female n=8.

Variable	Baseline	Week 4	Change	*P*-value
Total Cholesterol, *mg/dL*				
GRAPE	197.21 ± 10.72	190.47 ± 10.98	-6.74 ± 4.77	0.374*
Male	187.73 ± 16.43	176.64 ± -11.09	-11.09 ± 6.66	0.206#
Female	210.25 ± 11.36	209.50 ± 15.87	-0.75 ± 6.55	***0.003$***
PLACEBO	196.89 ± 9.14	196.95 ± 11.04	0.05 ± 4.37	
Male	186.82 ± 12.51	191.27 ± 16.42	4.45 ± 6.07	
Female	210.75 ± 12.46	204.75 ± 14.13	-6.00 ± 5.94	
HDL-C, *mg/dL*				
GRAPE	46.63 ± 4.78	46.11 ± 4.12	-0.53 ± 0.93	0.490*
Male	37.27 ± 2.27	38.00 ± 1.73	0.73 ± 1.07	0.474#
Female	54.75 ± 6.22	56.75 ± 7.46	2.00 ± 1.56	0.558$
PLACEBO	45.53 ± 3.33	46.05 ± 3.84	0.53 ± 1.16	
Male	38.82 ± 2.00	38.27 ±180	-0.55 ± 1.64	
Female	59.50 ± 9.40	57.25 ± 8.20	-2.25 ± 1.52	
Non-HDL-C, *mg/dL*				
GRAPE	150.58 ± 11.18	144.37 ± 10.86	-6.21 ± 4.86	0.458*
Male	153.45 ± 17.75	144.91 ± 16.34	-8.55 ± 7.70	0.340#
Female	151.50 ± 12.90	155.25 ± 16.66	3.75 ± 5.55	***0.010$***
PLACEBO	151.37 ± 9.53	150.89 ± 11.18	-.47 ± 4.17	
Male	153.82 ± 13.92	159.00 ± 17.23	5.18 ± 5.74	
Female	154.50 ± 13.10	151.25 ± 14.88	-3.75 ± 3.97	

Variable	Baseline	Week 4	Change	*P*-value
LDL-C, *mg/dL*				
GRAPE	122.51 ± 8.59	120.32 ± 9.44	-2.19 ± 3.33	0.807*
Male	119.16 ± 12.97	114.91 ± 12.65	-4.82 ± 4.35	0.405#
Female	127.10 ± 10.74	128.53 ± 14.63	1.43 ± 5.21	***0.049$***
PLACEBO	125.12 ± 7.61	124.38 ± 9.22	-0.737 ± 3.92	
Male	119.81 ± 10.09	123.13 ± 1349	3.31 ± 5.72	
Female	132.40 ± 11.86	126.00 ± 12.71	-6.30 ± 4.67	
Triglycerides, *mg/dL*				
GRAPE	140.37 ± 19.78	120.26 ± 12.59	-20.11 ± 11.01	0.147*
Male	156.45 ± 30.34	121.45 ± 18.05	-35.00 ± 15.79	***0.033#***
Female	118.25 ± 21.47	118.63 ± 18.11	0.38 ± 12.11	0.540$
PLACEBO	131.26 ± 15.81	132.58 ± 16.21	1.32 ± 7.86	
Male	140.91 ± 23.12	149.36 ± 24.21	8.45 ± 11.57	
Female	118.00 ± 20.80	109.50 ± 17.94	-8.50 ± 9.53	
Glucose, *mg/dL*				
GRAPE	99.53 ± 2.88	100.74 ± 2.94	1.21 ± 1.63	0.577$
Male	99.09 ± 3.89	100.73 ± 3.42	1.64 ± 1.59	0.886#
Female	100.13 ± 4.56	100.75 ± 5.45	0.63 ± 3.36	0.808*
PLACEBO	99.32 ± 2.83	99.37 ± 3.01	0.05 ± 1.36	
Male	100.27 ± 4.10	102.27 ± 3.85	2.00 ± 1.87	
Female	98.00 ± 3.93	95.38 ± 4.73	-2.63 ± 1.63	
ALT, *U/L*				
GRAPE	33.00 ± 3.11	34.63 ± 2.60	1.63 ± 1.46	0.841*
Male	33.64 ± 3.49	35.00 ± 2.45	1.36 ± 2.02	0.662#
Female	32.13 ± 5.91	34.13 ± 4.88	2.00 ± 2.24	0.770$
PLACEBO	34.32 ± 3.23	35.32 ± 3.54	1.00 ± 1.92	
Male	35.27 ± 3.19	34.55 ± 2.55	-0.73 ± 1.42	
Female	33.00 ± 6.58	36.38 ± 7.97	3.38 ± 4.14	

* = comparison of GRAPE vs. placebo in entire population, # = comparison of GRAPE vs. placebo in male subset, $ = comparison of GRAPE vs. placebo in female subset.

Discussion

The current study has shown that GRAPE, slightly, but significantly reduced BMI in subjects with MetS compared to placebo (0.01 ± 0.11 kg/m^2 vs. 0.23 ± 0.07 kg/m^2, p = 0.048). Accompanying this significant reduction in BMI, was a decreasing trend in body weight in the GRAPE group (0.03 ± 0.33 kg vs. 0.65 ± 0.21 kg, p = 0.081). In a C57BL/6J mouse model of obesity, it has been shown that purified anthocyanins from strawberries and blueberries showed a significant decrease in body and fat pad weight (Prior et al., 2014). This group also examined the effects of the whole berries, and did not observe significant changes in body or fat pad weight. This brings about the question of what other parts of the fruit can positively or negatively impact obesity.

Hollis et al., (2009), published a study in 2009, showing similar results to the current study. This study examined the effects of Concord grape juice (CGJ) vs. a polyphenol-free substitute juice (SGJ). Anthropometrics remained the same in most aspects of the study, however a significant reduction in WC and body weight in the CGJ group was seen. This study was 12 weeks, three times the length of the current intervention. At week six of this intervention these effects of CGJ had not yet become apparent. Many studies have been conducted on resveratrol, a common grape polyphenol. In 3T3-L1 pre-adipocytes it is observed across many studies that PPARγ, C/EBPα, SREBP-1c mRNA and protein expression is decreased (Kwon et al., 2012, Chen et al., 2011, Rayalam et al., 2008, Kang et al., 2012). The result of reduction in such mRNA/ protein expression was decreased adipogenesis. In Sprague-Dawley rats, it has been shown that resveratrol reduces expression of heparin releasable lipoprotein lipase (HR-LPL), which is the active form of this enzyme (Alberdi et al., 2011). When LPL and PPARγ (the transcriptional factor for LPL) were

measured, no changes were seen. This suggested a post-translational modification of LPL which led to a reduction in uptake of TG in this particular study.

Other clinical studied on dried fruits (including freeze-dried grapes and raisins) have showed minimal effects on plasma lipids. Raisins have shown no impact TC, LDL-C, and TG in studies that included men and women (Puglisi et al., 2008, Anderson et al., 2014, Kanellos et al., 2014). Figs have shown no impact on HDL-C, LDL-C, or TG; but increased TC in men and women with elevated LDL-C (Peterson et al., 2011). Freeze-dried grapes showed no changes in plasma lipids in a study that included men with MetS at a dose of 46 g/day (Barona et al., 2012). Freeze-dried grape powder has been shown to decrease LDL-C and TG in pre- and post-menopausal women at a dose of 36 g/day (Zunino et al., 2014). Taking these results into account, along with the results of the current study (60 g/day), it may be reasonable to theorize a sex-related dose dependency may be responsible for the changes observed in the literature. In males, 46 g/day showed no improvement in lipoprotein profiles. When the dose was increased to 60 g/day, a significant reduction in TG was observed. In females, a 36 g/day dose showed improvements in the lipoprotein profile, regardless of menopausal status. When the dosage was increased to 46 g/day in another study, these improvements were no longer seen. The current study examined 60 g/day of GRAPE supplementation, and saw a significant increase in LDL-C and TC, respectively. This seems to suggest that there is a sex-dependent mechanism, with dose-dependency, that contributes to the results seen.

Chapter 3: Effects of GRAPE on Oxidative Stress, Inflammation, and Insulin Resistance in Adults with Metabolic Syndrome

Introduction

Throughout the world, CVD claimed 17.5 million lives in 2012, amounting to 30% of total deaths. Another chronic condition of concern is T2DM. Recent research estimates 9.3% of individuals in the United States are afflicted with T2DM, and it is frequently listed as a cause of death (American Diabetes Association, 2017). Interestingly, some of the identified risk factors for CVD overlap with those of T2DM, which has led to the recognition of MetS (National Institutes of Health, National Heart, Lung, and Blood Institute, 2016). Metabolic syndrome is a constellation of symptoms that together, increases the risk for development of T2DM and CVD.

In the excess central adiposity characteristic of MetS, a state of high plasma FFA or TG may be present. The excess may be redirected to non-adipose tissues. Hepatic uptake of FFA is unregulated. If circulating levels of FFA are high, the liver will take them into its cells (Malhi and Gores, 2008). In the fatty liver, circulating FFA can activate many intracellular responses such as: C-jun N-terninal kinase (JNK) activation, toll-like receptor (TLR) activation, and endoplasmic reticulum (ER) stress. The downstream result of these activations is insulin resistance (Shoelson, 2006). Additionally, the activity of TNF-α increases the release of FFA from adipocytes and blocks the synthesis of adiponectin, which sensitizes adipose tissue to insulin. Tumor necrosis factor-α also can activate NF-κB signal transduction, leading to the production of cellular adhesion molecules, and an inflammatory state in the adipocyte (Fernandez-Sanchez et al., 2011). It can

also stimulate the expression of IL-6 and MCP-1 (Kern et al., 2001). These molecules attract inflammatory cells to the afflicted area and generate a larger inflammatory response.

Inflammation is not the sole contributor to insulin resistance. Oxidative stress can result in the dysfunction of insulin-related pathways (Keane et al., 2015). Excessive generation of ROS can lead to the damage of DNA, protein, and lipid membranes. Oxidative stress via ROS can be the result of over nutrition in the form of high glucose and lipid diets (Keane et al., 2015). This over nutrition can lead to the dysfunction of the electron transport chain, which produces increased ROS. Another pathway that ROS may be generated is via superoxide dismutase. Superoxide can be converted to H_2O_2, which is much more stable. However, H_2O_2 itself can react with Fe^{2+} to produce a highly reactive hydroxyl radical ($OH^{\bullet}$) (Keane et al., 2015). The consequences of all this damage is ultimately cell death and an inflammatory response.

Insulin resistance, inflammation, and oxidative stress are states commonly associated with MetS however, MetS is preventable by lifestyle interventions such as smoking cessation, increasing exercise, or modifying diet (National Institutes of Health, National Heart, Lung, and Blood Institute, 2016). Data has shown that increased consumption of fruit and vegetables is correlated with reduced risk for all-cause mortality and CVD (Wang et al., 2014). This is hypothesized to be linked with bioactive food components. The bioactive components can include: vitamin C, vitamin E, carotenoids, and polyphenols (Perez-Jimenez and Saura-Calixto, 2008). Polyphenols have garnered great interest in research due to their abundance in the human diet, as well as antioxidant properties (Manach, et al., 2004). One whole food that is rich in polyphenols is the grape (*Vitis vinifera)*. In previous research, the effectiveness of grape seed, skin, stem, and extracts on CVD risk have all been studied (Perez-Jimenez and Saura-Calixto, 2008). However, the consumption of whole grapes as a dietary intervention has not been fully evaluated

(Mozaffarian et al., 2015). It is our hypothesis that ingestion of whole grapes will positively impact markers of inflammation, oxidative stress, and insulin resistance when compared to a placebo.

Materials and Methods

Plasma Insulin and HOMA-IR

Plasma insulin was measured using a Luminex/xMAP magnetic bead-based multiplexing assays using MAGPIX instrumentation (EMD Millipore, Billerica, MA). Samples from baseline and end-point for each arm were run simultaneously to minimize variance. Insulin resistance was calculated via the homeostatic assay for insulin resistance (HOMA-IR) using the formula established by Matthews et al. (1985); HOMA-IR = $\left[\frac{\text{glucose (nmol/L)} * \text{insulin } \mu\text{U/mL)}}{22.5}\right]$.

Plasma Inflammatory Markers

Serum inflammatory markers were measured using a Luminex/xMAP magnetic bead-based multiplexing assays using MAGPIX instrumentation (EMD Millipore, Billerica, MA). Analytes measured include: IL1β, IL-6, MCP-1, and TNF-α. Samples from baseline and end-point for each arm were run simultaneously to minimize variance.

Serum TBARS

Malondialdehyde (MDA) is a compound formed when lipids in the body become oxidized. Thiobarbituric acid (TBA) reacts with MDA to form a compound (TBA-MDA) that will fluoresce at 532nm. The fluorescence measure of this reaction is one of the most common procedures to quantify lipid peroxidation (Draper et al., 1993).

Serum lipid peroxidation products were assessed by thiobarbituric acid reactive substances (TBARS) assay (Farrell et al. 2015). Briefly, 50 µL of samples were added to a 1.5 mL tube. Non-

lipid TBARS were precipitated out using 100 µL of ice-cold 10% TCA solution. Samples were then incubated on ice for 15 minutes. Next, the samples were centrifuged at 2200 x *g* for 15 minutes at 4°C. Following centrifugation, 100 µL of supernatant was transferred to fresh tubes, and 0.67% TBA (w/v) was added. The solution was then boiled for 10 minutes at 95°C and subsequently cool on ice for an additional 10 minutes. A UV/vis fluorescence microplate was loaded 75 µL of standards/ samples. Absorbance was read at 530 nm and plotted against an MDA standard. TBARS are expressed in µM of MDA equivalents.

Serum AOPPs

Advanced oxidation protein products (AOPP) are a novel marker of protein oxidation in plasma, primarily composed of altered albumins (Witko-Sarsat et al., 1996). Modifications to the albumin-lysine residues by myeloperoxidase product hypochlorous acid can affect the interaction with SR-B1 (Marsche et al., 2009) The interference with SR-B1 could impair reverse cholesterol transport, thus increasing risk for CVD. It is known that serum TG can interfere with the AOPP assay in highly lipemic samples (Anderstam et al., 2008).

Plasma was depleted of apoB-containing lipoproteins via dextran sulfate/Mg2+ method. Then, 200 µL of 5-fold diluted plasm or chloramine-T standards (0–100 µmol/L) were mixed with 20 µL of glacial acetic acid and 10 µL of 1.16 M potassium iodide solution in a 96-well plate. The plate was mixed on a clinical rotator for one minute and absorbance read at 340 nm with a spectrophotometer. AOPP concentrations are expressed in µmol/L of chloramine-T equivalents.

STATISTICAL ANALYSIS

Differences between end-points of groups were evaluated by independent *t* tests (significance = $p < 0.05$). Two-way repeated measures ANOVA was used to determine the effect of the intervention for variables with baseline measures, with the within subject variables as treatment (tests GRAPE vs. placebo effect) and between subjects-variable as sequence order (tests carryover effect). All statistical analysis was done using IBM SPSS Statistics software. Data are reported as mean ± SEM.

Results

EFFECTS OF GRAPE ON PLASMA MARKERS OF OXIDATIVE STRESS

Using two-way ANOVA, it was determined that no significant differences were present in serum markers of oxidative stress. When stratifying for sex, changes were seen the female subset. Plasma AOPPs were seen to be trending toward a significant increase compared to placebo (9.50 ± 10.64 μM vs. -14.85 ± 13.06 μM, $p = 0.085$). Plasma TBARS were significantly increased with GRAPE compared to placebo (0.08 ± 0.10 μM vs. -0.11 ± 0.07 μM, $p = 0.042$).

Table 3.3.1. Summary of Plasma Markers of Oxidative Stress. n=19, male n=11, female n=8

Variable	Baseline	Week 4	Change	*P*-value
AOPPs, *μM*				
GRAPE	72.90 ± 27.94	87.25 ± 25.92	14.35 ± 24.21	0.148*
Male	63.71 ± 7.20	81.94 ± 6.42	18.23 ± 6.04	0.799#
Female	84.39 ± 11.11	93.88 ± 10.23	9.50 ± 10.64	0.085$
PLACEBO	80.80 ± 31.05	78.60 ± 37.48	-2.20 ± 36.65	
Male	71.75 ± 9.11	79.68 ± 9.69	7.93 ± 11.04	
Female	92.11 ± 11.25	77.26 ± 16.66	-14.85 ± 13.06	
TBARS, *μM*				
GRAPE	0.43 ± 0.20	0.53 ± 0.30	0.10 ± 0.32	0.194*
Male	0.42 ± 0.07	0.54 ± 0.11	0.12 ± 0.11	0.454#
Female	0.44 ± 0.07	0.52 ± 0.08	0.08 ± 0.10	***0.042$***
PLACEBO	0.48 ± 0.24	0.45 ± 0.20	-0.03 ± .28	
Male	0.41 ± 0.06	0.44 ± 0.06	0.03 ± 0.10	
Female	0.57 ± 0.10	0.46 ± 0.08	-0.11 ± 0.07	

* = comparison of GRAPE vs. placebo in entire population, # = comparison of GRAPE vs. placebo in male subset, $ = comparison of GRAPE vs. placebo in female subset.

EFFECTS OF GRAPE ON PLASMA MARKERS OF INSULIN RESISTANCE

Using a paired samples *t* test, it was determined that no significant differences were present in plasma insulin or HOMA-IR score. When stratifying for sex, it was seen that GRAPE significantly increased plasma insulin (9.82 ± 3.94 pmol/L, $p = 0.042$) and HOMA-IR Score (0.40 ± 0.16, $p = 0.043$) in the female subset.

Table 3.3.2. Summary of Plasma Insulin Resistance Markers. n=19, male n=11, female n=8

Variable	Endpoint	GRAPE vs. PLACEBO	*P*-value
Plasma Insulin, *pmol/L*			
GRAPE	72.71 ± 12.54	1.03 ± 6.38	0.874*
Male	80.82 ± 12.20	-9.64 ± 9.12	0.317#
Female	63.38 ± 10.76	9.82 ± 3.94	***0.042***$
PLACEBO	73.74 ± 8.33		
Male	90.45 ± 19.24		
Female	53.56 ± 8.42		
HOMA-IR Score			
GRAPE	3.13 ± 0.43	0.03 ± 0.27	0.917*
Male	3.56 ± 0.64	-0.34 ± 0.41	0.419#
Female	2.58 ± 0.49	0.40 ± 0.16	***0.043***$
PLACEBO	3.10 ± 0.59		
Male	3.90 ± 0.92		
Female	2.18 ± 0.39		

* = comparison of GRAPE vs. placebo in entire population, # = comparison of GRAPE vs. placebo in male subset, $ = comparison of GRAPE vs. placebo in female subset.

EFFECTS OF GRAPE ON PRO-INFLAMMATORY CYTOKINES

Using a paired samples *t* test, it was determined that no significant differences were present in pro-inflammatory cytokines. However, TNF-α was trending toward a decrease when comparing GRAPE to placebo (-0.42 ± 0.23 pg/mL). When stratifying for sex, no significance was shown.

Table 3.3.3. Summary of Pro-Inflammatory Cytokines. n=19, male n=11, female n=8

Variable	Endpoint	GRAPE vs. PLACEBO	*P*-value
TNFα, *pg/mL*			
GRAPE	4.56 ± 0.37	-0.42 ± 0.23	0.081*
Male	4.79 ± 0.47	-0.21 ± 0.23	0.397#
Female	4.40 ± 0.63	-0.56 ± 0.46	0.283$
PLACEBO	4.98 ± 0.52		
Male	4.99 ± 0.55		
Female	4.95 ± 1.01		
MCP-1, *pg/mL*			
GRAPE	151.56 ± 8.83	-9.06 ± 5.90	0.142*
Male	156.91 ± 11.42	-5.00 ± 9.41	0.606#
Female	160.92 ± 11.06	-7.03 ± 7.68	0.390$
PLACEBO	160.62 ± 10.40		
Male	161.92 ± 14.10		
Female	167.95 ± 11.59		
IL-6, *pg/mL*			
GRAPE	3.65 ± 0.65	-0.21 ± 0.63	0.742*
Male	3.32 ± 0.70	-0.58 ± 1.05	0.590#
Female	3.94 ± 1.30	0.20 ± 0.48	0.689$
PLACEBO	3.86 ± 0.96		
Male	3.90 ± 1.56		
Female	3.74 ± 0.97		

* = comparison of GRAPE vs. placebo in entire population, # = comparison of GRAPE vs. placebo in male subset, $ = comparison of GRAPE vs. placebo in female subset.

Discussion

After the 4-week intervention, no effects were seen on oxidative stress markers, plasma insulin, HOMA-IR, or pro-inflammatory cytokines with GRAPE ingestion. Zern et al. (2005)

assessed the effects of GRAPE on a female population. The results of that study showed a significant decrease in TNF-α and no change in IL-6. In the current study both TNF-α and IL-6 were not significantly reduced by GRAPE when compared to placebo. Hollis et al. (2009) assessed the effect of CGJ on insulin compared to a placebo. In this study, over a 12-week intervention, insulin showed no significant change. Glucose also showed no significant change. It follows that HOMA-IR would also remain unchanged. In a large review, Wang et al. (2013), examined novel insights of dietary polyphenols in relation to obesity. It was observed that animal studies showed promising results in relation to oxidative stress, however these data did not translate to human studies.

It has been shown previously that polyphenols can be used to induce a pro-oxidative and pro-inflammatory state (Lee et al, 2006). The results of the current study, in combination with previous literature suggest that 60 g/day of GRAPE may induce a pro-oxidative and pro-inflammatory state in females. Results in females showed a significant increase in plasma TBARS, plasma insulin, and HOMA-IR; as well as a trend in increasing AOPPs. The increase in markers of oxidative stress may be a result of increased oxidation due to GRAPE directly, which may lead to further insulin resistance in tissues such as adipose and muscle. This further insulin resistance may then account for the increase in plasma insulin and HOMA. An alternative hypothesis relates to the ability of polyphenols to stimulate insulin secretion from pancreatic β-cells (Dall'Asta et al., 2015). In this instance, polyphenols may have increased insulin secretion from pancreatic β-cells, leading to the increase in plasma insulin and HOMA-IR in an already moderately insulin-resistant population. The increased insulin resistance could have contributed to significant and trending increases markers of oxidative stress.

Summary and Conclusions

It was the objective of this thesis to evaluate the effectiveness of standardized, freeze-dried, grape powder on adults with MetS. Subjects were consuming the equivalent of 2.5 cups of fresh grapes a day, which contains roughly 200 mg of polyphenols. The placebo did not contain polyphenols found in grapes. Subjects entered a randomized, placebo-controlled, crossover intervention for 13 weeks. This included a 2-week run-in, 4-week intervention (GRAPE or placebo), 3-week washout, and final 4-week intervention (alternate powder). Subjects did not significantly alter their diets when comparing GRAPE and placebo. Aim 1 focused on cardiometabolic risk factors. We hypothesized that GRAPE ingestion would improve cardiometabolic risk factors. Overall, GRAPE improved BMI significantly, was trending toward an improvement in weight, but did not improve WC (surrogate for central obesity). When stratifying for sex an improvement was seen in men in TG and pulse. However, when stratifying for sex, TC and Non-HDL-C was seen to increase in female subjects with GRAPE ingestion. The increase in TC can be accounted for by the increase in Non-HDL-C, since HDL-C remained the same. This is the opposite of what was hypothesized. Aim 2 focused on the underlying comorbidities of MetS; oxidative stress, inflammation and insulin resistance. It was hypothesized that GRAPE would have a positive impact on markers of oxidative stress, inflammation, and insulin resistance. Overall, no significance was reached between groups when comparing GRAPE to placebo in these categories. When stratifying for sex, the male subset had no significant differences in any of these variables. However, the female subset presented significant increases in TBARS, plasma insulin, and HOMA-IR with GRAPE ingestion. The significant increase in

HOMA-IR can be accounted for by the increase in plasma insulin, since glucose remained the same. This was the opposite of what was hypothesized.

Future Directions

Future human research is needed to examine the effects of polyphenols on adipocyte regulation. Mechanistic studies have presented evidence that polyphenols can inhibit the growth of adipocytes (Kwon et al., 2012, Chen et al., 2011, Rayalam et al., 2008, Kang et al., 2012). Animal studies have built the case even stronger with reduced body weight and smaller fat pad size (Prior et al., 2014 and Alberdi et al., 2011). Now, the current study suggests that GRAPE ingestion has reduced BMI significantly, with a trend in decreasing body weight. Additionally, plans to measure HDL functionality and NMR for lipoprotein particle size are planned. This research may offer further insight into the effects of GRAPE on the lipoprotein profile of adults with MetS. Future research is also needed to assess sex differences in metabolic dysfunction. The current study observed markedly different responses between male and female subsets. Further data analysis with these subjects may elucidate the mechanisms responsible. Possible studies examining the dose-dependency of GRAPE in men and women could provide more evidence of a sex relation. Previous studies that have examined the doses of 36 g/day (Zern et al., 2005) and 46 g/day (Zunino et al, 2014 and Barona et al., 2012), and the current study show marked differences between males and females when consuming these doses of GRAPE. Administering these doses to men and women in a randomized, placebo-controlled, crossover design may provide answers to questions that have arisen in this area. Measures of lipoprotein particle size, oxidation state, and associated apolipoproteins could also elucidate potential mechanisms that account for sex differences

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