THE EFFECTS OF RED WINE AND GRAPE JUICE CONSUMPTION IN OVERWEIGHT INDIVIDUALS ON MULTIPLE HEALTH PARAMETERS

A Thesis
by
WENG ON HO

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Department of Nutrition and Health Care Management

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ABSTRACT

THE EFFECTS OF RED WINE AND GRAPE JUICE CONSUMPTION IN OVERWEIGHT INDIVIDUALS ON MULTIPLE HEALTH PARAMETERS
(December 2010)

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INTRODUCTION: Red wine consumption may have potential health benefits due to the presence of polyphenolic compounds. However, the literature is equivocal regarding specific mechanisms for positive health outcomes. The purpose of this research was to investigate the effects of muscadine red wine and grape juice on weight, body fat, lipids, inflammation, and antioxidant capacity in overweight individuals. **METHODS:** In a randomized crossover design, 19 subjects consumed 300 mL of wine (WG) or grape juice (JG) for two weeks and acutely upon returning to the lab. Blood was drawn at baseline, post two weeks, and acutely. The statistical design was a 2 (treatments) x 3 (times) repeated measures ANOVA. A paired *t*-test was used to compare differences in diet. Results were analyzed using SPSS 16.0. **RESULTS:** Overall weight gain occurred in both groups with treatment effect (P=0.044) and time effect (P=0.018). Significant weight gain was found in WG (P=0.027). Total fat mass percentage was not significantly different after treatments. C-reactive protein (CRP) and lipids were not affected by red wine or grape juice. Ferric reducing ability of plasma (FRAP) significantly increased after acute, but not chronic, consumption of red wine (P<0.001). Oxygen radical absorptive capacity of plasma (ORAC) did not change significantly for either treatment. **CONCLUSION:** Adding wine or grape juice to the diets of overweight sedentary individuals, with no other dietary alterations,

resulted in significant weight gain. Neither acute nor chronic consumption of red wine or grape juice altered blood lipids or reduced inflammation. However, acute consumption of red wine resulted in significant changes in antioxidant capacity which may confer potential benefits on health variables other than ones examined in the present study.

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INTRODUCTION

It is well known that France has lower rates of obesity and reduced incidence of mortality due to cardiovascular disease despite having a diet high in saturated fat. This observation is known as the "French Paradox" (1). A possible explanation for this phenomenon may be associated with the consumption of red wine by the French. Studies show that moderate red wine consumption may have potential health benefits due to its alcohol content and the substantial amounts of phenolic compounds present (2-4). Research has shown individuals with moderate alcohol consumption, including red wine, reduce the risk of dying from heart disease by 40 %, increase high density lipoprotein (HDL) cholesterol, and promote other cardio-protective effects (5). Polyphenols are a class of compounds found in plants that exhibit antioxidant characteristics. Red wine polyphenolic compounds have been found to act as powerful vasodilators and help preserve the integrity of endothelial tissue (6). Polyphenols, such as resveratrol and quercetin, have been shown to increase serum antioxidant capacity, which may protect against damage caused by free radical production in the body, and prevent the increase of inflammatory agents and the oxidation of low density lipoprotein (LDL) (7, 8). Excessive production of radical species can lead to atherosclerotic conditions which can promote development of multiple cardiovascular diseases (9). Compared to other types of juices, such as pomegranate juice

and acai juice, red wine has a higher antioxidant capacity (10). The objective of this research was to examine outcome effects on anthropometric measures, blood lipids, inflammation, and blood antioxidant capacity associated with chronic and acute consumption of muscadine grape juice and muscadine red wine.

LITERATURE REVIEW

Health Benefits of the Components of Red Wine

Ethanol

Ethanol has been shown to influence chronic illness risks (11). In recent studies, moderate alcohol consumption has been consistently shown to exert protective effects on heart disease (5, 12, 13). An increase in HDL cholesterol, caused by moderate alcohol consumption, was shown to reduce atherosclerotic disease risk (14). For diabetic patients, moderate ethanol intake is not generally contraindicated (15). Although moderate intake of ethanol was shown to have no effect on the glycemic control of patients with type 1 diabetes, it reduced fasting blood glucose concentrations of patients with type 2 diabetes (16).

Conversely, the relationship between moderate ethanol intake and cancer risk is still very unclear. For example, in the Framingham Study (17), breast cancer risk was not particularly associated with wine, beer, or spirits consumption, while a study by Longnecker et al. (18) revealed that breast cancer risk was increased by one or two drinks of ethanol. Therefore, the relationship between ethanol consumption and chronic illness risks such as atherosclerotic cardiovascular disease, diabetes, and cancer varies qualitatively (15).

More than 50 epidemiological studies suggest that the effects of ethanol on overall mortality follow a J-shaped curve (15). The low mortality rate given by moderate doses of ethanol is believed to be attributed to its protective effect on heart disease, while the high mortality rate resulting from high ethanol intake is attributed to its exacerbating effect on cancer and cirrhosis (19). In addition, the three types of alcoholic beverages affect mortality in a different way. Low-to-moderate intake of hard liquor implies an increased risk of all-cause mortality, while the consumption of beer does not have a significant effect on mortality risk (11). However, moderate intake of red wine is associated with lower rates of mortality and appears to be protective in all populations studied (11, 15, 19).

In short, although ethanol does not clearly indicate benefits against chronic diseases such as cancer and diabetes, regular consumption of any alcohol in moderate amounts seems to be beneficial to cardiovascular health, with the strongest evidence of this trend seen among wine-drinking populations (15). Populations that consume a moderate amount of red wine also have been observed to have lower rates of cancer, compared to those who consume similar quantities of other alcohol-containing beverages (15). In addition, epidemiological studies suggest there are other significant health properties of drinking red wine (19, 20). Obesity, one of the biggest health concerns in America, may be negatively associated with red wine consumption. Conditions and diseases associated with obesity include hypertension, hyperlipidemia, type II diabetes, coronary heart disease, stroke, obstructive sleep apnea, asthma, orthopedic disorders, and certain cancers (21). The rising demand for safe and effective anti-obesity drugs has made red wine appealing to overweight individuals because it may reduce weight and body fat naturally without the side effects of prescription medications (22). Moderate red wine intake was shown to prevent an increase of body weight in rats (23).

A very recent study concluded that moderate amounts of red wine can elevate adiponectin concentration *in vivo* by 29.8 % (24). An increase in plasma concentration of adiponectin (an adipose-tissue-specific protein) has been shown to correlate negatively with body mass index, glucose, insulin, and triglyceride plasma levels and positively with HDL and insulin-stimulated glucose disposal (25). Adiponectin also has anti-inflammatory and anti-atherogenic properties (25). The discovery of a significant increase in adiponectin levels by moderate red wine consumption strengthens the concept of red wine's ability to exert health benefits. In addition, in populations where fruits and vegetables were limited, wine consumption showed a clear benefit over other forms of ethanol (19). Aside from the ethanol component in red wine, there are other constituents which may contribute health properties.

Phenolics

Wine is not simply grape juice with added alcohol (15). The fermentation process needed for wine making produces a variety of other chemically active ingredients (26). During the production of wine, organic acids and phenolics in the freshly pressed grape juice are converted into polyphenolics (phenolic acids and polyphenols) (26). Polyphenolics are responsible for the different tastes, colors, and mouthfeels of wine (26). According to the calculations of Paganga et al. (27), the antioxidant activity in 1 glass of red wine (150 mL) is equivalent to 12 glasses of white wine, 2 cups of tea, 5 apples, 5 portions (100 g) of onions, 5.5 portions of eggplant, 3.5 glasses of black currant juice, 500 mL of beer, 7 glasses of orange juice, or 20 glasses of apple juice. The large content of phenolic compounds in red wine contributes to its properties of reducing disease risk, which is separate from the already protective effect of ethanol (15).

Polyphenolics can be classified as either flavonoids or non-flavonoids (28). Polyphenols are well known for contributing to the health benefits of red wine. Flavonols, a subcategory of flavonoids, were shown to reduce damage on white blood cell DNA among type 2 diabetics (29). However, the flavonol studied was extracted from tea and onions instead of wine. Since the phenolic profiles of onion and red wine are different, distinguished differences may exist in their antioxidant properties or capacities (15). Little information exists on the effects of wine polyphenols on diabetes (15).

Quercetin, which is a well-known red wine flavonol, has a wide spectrum of bioactive effects (30). These include its positive influences as an anti-inflammatory, antimicrobial, and antioxidant agent as well as its ability to regulate the immune system and the central nervous system (31-35). Quercetin also has specific anticancer properties including the inhibition of the growth of cancer cells derived from the stomach (36), colon (37), and breast (34) in humans. In mice, quercetin suppressed the growth and development of uterine cervical cancer (38), melanomas (39), and intestinal tumors (40).

More recently, resveratrol, a non-flavonoid, has also been studied extensively. Resveratrol has been found to have protective effects on the cardiovascular system due to its ability to inhibit platelet aggregation (22) and to promote vasodilation (41). Resveratrol also possesses anti-carcinogenic properties (42). Results of *in vitro* studies using pharmacologic doses of resveratrol indicate protection of heart (43) and brain tissue (44) from oxidative stress while eliminating cancer cells (45, 46). In addition, resveratrol also improves insulin sensitivity in mice that are fed a high caloric diet by activating the SIRT 1 gene which mimics the effects of calorie restriction (47). Heavy doses of resveratrol are shown to be beneficial in decreasing obesity-related death by up to 31 % in mice (48). Obese mice that

received resveratrol extract not only remained as agile as lean mice but also lived longer than the obese mice that received the same high-calorie diet without resveratrol, with organs that also appeared normal. Researchers believe that resveratrol lowers the rate of diabetes, liver problems, and other diseases linked to obesity (48). Another possibility of why treated mice may have had longer life expectancy is that resveratrol possesses anti-viral properties which combat illness (49). Resveratrol was found to restrict the ability of viruses to replicate, reduce production of viral DNA, and increase the potency of some antiviral drugs (50-52). Resveratrol also possesses anti-inflammatory properties (53) which directly influence arthritis, Crohn's disease, psoriasis, and has an association with the development of both cardiovascular disease and cancer (22). The anti-inflammatory properties of resveratrol have proven to be an effective inhibitor of the pro-inflammatory enzymes cyclooxygenase and 5-lipoxygenase in mice (54, 55). These anti-inflammatory properties have shown a reduction of acute and chronic chemically-induced edema, lipopolysaccharide-induced airway inflammation, and inflammation associated with osteoarthritis (20, 22, 56, 57). A single dose up to 5 mg of resveratrol was shown to have no serious adverse health effects (58). These findings provide strong evidence of resveratrol's biochemical capabilities, but the question is whether wine polyphenols exert the same effects in humans.

A central issue relevant to the interest in wine phenolics is the production of reactive oxygen species (ROS), including free radicals, an essential part of human metabolism (59). ROS can damage vital biological systems and are relevant in more than 100 disease states (60). The production of ROS, in amounts that overwhelm the antioxidant defense system, is known as oxidative stress (59). Chronic oxidative stress, caused by production of ROS, is strongly linked to numerous diseases which include cardiovascular disease (35). Therefore, it

is of great interest to know whether the plasma antioxidant effects of red wine consumption would be correlated with the changes in blood oxidative stress in the human body.

Most red wine antioxidant effects have been observed *in vitro* (26, 61, 62). Several studies examined measures *in vivo* and also demonstrated these antioxidant effects after a single ingestion of red wine and dealcoholized red wine (2-4, 63). However, the same antioxidant capabilities have not been demonstrated in other studies when the similar red wine beverages are consumed for a longer duration between 10 to 30 days (64-69). Therefore, it is still unclear whether red wine and/or dealcoholized red wine intake can increase plasma antioxidant capacity and prevent oxidation *in vivo.* Also, the effect of acute red wine or dealcoholized red wine consumption may be significantly different from the effect of chronic consumption.

Summary of Current Literature

Various clinical and experimental data suggest red wine components exert desirable effects on lipid profiles, inflammation markers, and other disease risk factors. Red wine contains an abundant amount of polyphenolic compounds which have been shown to provide numerous health benefits based on antioxidant properties. It is important to determine whether the health benefits are derived from ethanol, the polyphenols, or a synergistic combination of the two components in wine. It is also important to examine the difference between the health effects of acute ingestion of beverages with respect to the health effects of chronic ingestion of these beverages.

Development of the Research Design

Subjects

Overweight or obese individuals, aged 40 and over, were recruited in this research experiment to determine the effects of chronic (daily consumption for 2 weeks) and acute (1-h after consumption) consumption of 300 mL muscadine wine or grape juice on parameters of weight and body fat, blood lipids, C-reactive protein, and blood antioxidant capacity and phenols. The basis of this study is that older individuals exhibit more oxidative stress and inflammation than younger ones due to decreased immune function (70, 71). Subjects of at least 40 years of age were expected to display increased health benefits when compared to younger subjects. Individuals selected for participation in this study were overweight or obese because high body mass index (BMI) and obesity have been shown to be associated with early mortality (72).

Wine and Grape Juice

Muscadine wine was chosen because muscadine grapes may have a higher level of antioxidants and other nutrients than other grape species (73). Therefore, muscadine wine may have a greater potential for health benefits when compared to other wines. Three-hundred mL of red wine or grape juice were consumed because most studies suggested that moderate consumption of red wine exerts optimal benefits (15). The U.S. Department of Agriculture defines a “moderate” consumption of wine to be 150 mL for women and 300 mL for men on a daily basis (74).

Procedure

The subjects were randomly assigned to receive 14 days of daily consumption of either 300 mL red wine or 300 mL grape juice, followed by an identical acute amount of the initial drink. Subjects then stopped drinking for 2 weeks during a washout period. The whole cycle was repeated with the opposing treatment (wine treatment switched to grape juice treatment and vice versa). By doing this, the subjects served as their own control. Therefore, confounding variables were significantly reduced.

Outcome Measures

Plasma antioxidant activities were examined due to the relationship with the development of atherosclerosis, inflammatory diseases, and cancer (46). Biomarkers of inflammation were examined because inflammation has been shown to be a direct cause of diseases such as arthritis, Crohn's, and psoriasis, and may have a role in the development of cancer and cardiovascular disease (22). Changes in body weight and composition were also investigated along with red wine consumption to determine the physical effects, if any, of red wine consumption.

Antioxidant Capacity

Oxygen radical absorbance capacity (ORAC) and ferric reducing ability of plasma (FRAP) were used to measure plasma antioxidant capacity. The antioxidant activity in wine depends on various factors which include the physical properties of the substrates, stages and conditions of oxidation, and the different phases of antioxidants (75). ORAC and FRAP correlated significantly when used for measuring the antioxidant capacity of phenolics in processed commodities such as rapeseed and olive oils (76). Since red wine is processed

from grapes, this combined method would seem advantageous. The ORAC method, initially developed by Cao et al. (60), measures the antioxidant activities on hydrophilic chain-breaking antioxidant capacity against peroxyl radical. The ORAC assay was significantly improved by Ou (77) and co-workers when fluorescein was used as the fluorescent probe. The absorbance value given by the high intensity of fluorescence from fluorescein will be used as the quantified units in this assay. During the antioxidant capacity assessment, a peroxyl-radical-generating chemical called AAPH will be introduced to samples, blank solutions with no antioxidant capacity, and Trolox with known antioxidant capacity (78). When the fluorescein is oxidized by the peroxyl radicals generated by the AAPH, its fluorescence will be lost and the absorbance measured will be lowered and a decreased reading of absorbance will result (77). Antioxidants, if present in the sample, will provide an inhibition of the radical action by the AAPH and create a the lag period before onset of peroxidation (60). By comparing the area under the fluorescence decay curve (AUC) of the sample with that of the blank, the protective effect of the antioxidant present in the sample can be quantified after the assay has been calibrated with the Trolox (77). The advantage of the ORAC assay is that it accounts for both inhibition percentage and the length of inhibition time of the free radical action by antioxidants (60). On the other hand, Benzie and Strain developed the FRAP assay which measures the direct ability of antioxidants to reduce a ferric complex to the ferrous complex in acidic conditions (79). The resulting reduced ferrous complex (blue in color) is measured spectrophotometrically at 593 nm. This value reflects the total reducing capacity of electron-donating antioxidants (53). Both ORAC and FRAP together allow for an effective way of measuring the antioxidant capacity of red wine. The advantages seen in one method adjust for the inadequacy of the other method.

Inflammation Status

C-reactive protein (CRP) was used as the main indicator of inflammation status. CRP is an acute phase protein that is elevated during inflammatory stress (80). It has been suggested that testing CRP levels in the blood may be an additional way to assess cardiovascular disease risk. Studies indicate that an increase in CRP is associated with increases in chronic disease (81). This correlation is most commonly seen in individuals who smoke, have hypertension, atherogenic lipoproteins, and hyperglycemia (81). In healthy young adults, high CRP levels are positively correlated with oxidative stress (82, 83).

Body Composition

A Dual Energy X-ray Absorptiometry (DEXA) machine was used in our study for analyzing potential changes in body composition. Compared to skin fold measurements, DEXA, the most commonly used anthropometric assessment of body fat, provides a more accurate measurement of body composition because of its precision (76).

Significance of the Study

Compared to previous studies which looked at plasma antioxidant status and/or indices of oxidative damage in red wine and/or dealcoholized wine, this study was the first to incorporate all the stated parameters (4, 84, 85). The design of the study will strengthen and expand the scope of the body of knowledge about red wine consumption.

METHODOLOGY

This study was approved by the Institutional Review Board and the University Research Council at Appalachian State University (Appendix A).

Subjects:

Twenty-six volunteers who were overweight or obese (BMI >24.9), at least 40 years old, non-smoking, and were not on prescription medications were recruited (Appendix B). All volunteers were infrequent consumers of alcohol, i.e. they consumed less than three alcoholic drinks per every two weeks. Volunteers were randomized to a wine consumption (WG) group or grape juice (JG) group.

Study Design

A diagram of the study design can be found in Appendix C. Volunteers were instructed to stop consuming fruits and fruit juices three days before coming in for testing and orientation. Drug and alcohol use were prohibited 24 hours prior to reporting for each visit. Volunteers were also instructed to fast for at least eight hours prior to reporting to the lab. All volunteers visited the ASU lab four times throughout the study. At baseline orientation, health questionnaires (Appendix D) and consent forms (Appendix A) were obtained. Height and weight were measured, and body composition was assessed by

bioelectrical impedance analysis (BIA) and DEXA. Blood samples (blood sample A) were drawn.

Subjects were instructed on how to adhere to the dietary restrictions. Herbal medicines, medications, or large-dose vitamin/mineral supplements (above 100 % of Recommended Dietary Allowances) were not allowed during the study period. Volunteers were encouraged to maintain normal dietary patterns during the study. Red wine was given to the WG, and grape juice was given to the JG. At home, the WG group consumed two 150 mL servings of wine per day for two weeks, and the JG group consumed grape juice with the same number of servings, serving size, and duration as the WG group. Subjects were asked not to consume other alcoholic beverages, grapes, or grape products during the study. Subjects were also taught to use a 3-day diet record for two weekdays and one weekend day during the treatment weeks (Appendix E).

On their second visit, two weeks after treatment, 3-day diet records were collected to analyze the diet of each subject. Weight, percent body fat, and blood (blood sample B) were also obtained. Each subject then consumed a single 300 mL serving of either red wine or grape juice, depending on which group they belonged to. Another blood draw (blood sample C) was taken precisely one hour after consumption. To ensure safety, subjects remained in the lab for one hour after the acute consumption and were given a small meal before leaving. Subjects then had a washout period in which they stopped drinking wine or grape juice for two weeks.

On their third visit, weight, percent body fat, and blood were obtained. Then, the whole cycle was repeated with the opposing treatment for each subject (wine treatment switched to grape juice treatment and vice versa) for two weeks (blood samples D, E, and F

were then collected). Another set of 3-day diet records were obtained. The fourth visit's protocols were performed in the same way as the second visit.

Wine and Grape Juice Samples

Hatteras Red wine was provided by Duplin Winery (Rose Hill, NC). This type of red wine is made from muscadine grapes. Muscadine juice was provided by D'Vine Foods (Elizabethtown, NC). Heat and direct sunlight were avoided when storing the red wine and grape juice. Once opened, the wine and juice were stored in a refrigerator.

Assessment Protocol

Height

Height was measured by a wall-mounted stadiometer. All shoes were taken off. The subject was asked to stand with heels together, arms to the side, legs straight, shoulders relaxed, and head in the Frankfort horizontal plane. The subject had to touch his/her buttocks, shoulder blades, and back of the head against the vertical surface of the stadiometer as much as possible. The subject was asked to inhale a deep breath, hold the breath and maintain an erect posture. Measurement was taken at this time with the headboard lowered on the highest point of the head. Enough pressure was applied to compress the subject's hair during measurement. All readings were corrected to the nearest inch.

Weight and Body Composition

BIA was used to assess weight and body fat percentage. Before arriving to the lab for testing, subjects were advised to drink enough water to prevent dehydration. Subjects stood on the scale barefoot and with minimal clothing. All readings were measured in pounds and

converted to kilograms for interpretation. DEXA was used to further assess total fat mass percentage and abdominal fat percentage.

Blood

Blood draws were done by venipuncture by trained technicians. Needle disposal boxes were available for all technicians. Two 5 mL EDTA tubes of blood were drawn for FRAP and ORAC. One 5 mL serum-separator tube (SST) of blood was collected for assessing C-reactive protein (CRP). The SST tubes were allowed to sit for 20 minutes to allow blood to clot. Blood tubes were then centrifuged within two hours at 3000 rpm for ten minutes at 3 ˚C. Plasma collected for analysis of FRAP and ORAC was aliquoted into snap-top tubes (0.5 mL) and frozen using liquid nitrogen. The tubes were then stored at –80 ˚C for further analysis. All blood collected was disposed in red biohazard bags after analyses.

Blood lipids and CRP

Blood tubes were delivered to the biochemistry laboratory at Charles A. Cannon Jr., Memorial Hospital located in Linville, North Carolina to assess CRP and blood lipids using automated procedures.

Oxygen Radical Absorbance Capacity (ORAC)

The ORAC method works by reacting the samples with a fluorescent probe to form a non-fluorescent product that directly measures the antioxidant activities of chain-breaking antioxidants (76). The materials and methods for the ORAC assay were adapted from the method of Ou et.al (77). Twenty µL of sample, blank, and Trolox standard solutions were pipetted into appropriate wells on a microplate. Two hundred µL of fluorescein working solution were also pipetted into one of the wells. The microplate was then placed into a BMG Flurostat Galaxy/Optimal Plate Reader for analysis. Fluorescence readings at cycle *i* (*fi*) were

obtained from the machine. Relative Area under the Curve (relative AUC) was calculated by using the equation [AUC = $(0.5 + f_5 / f_4 + f_6 / f_4 + f_7 / f_4 + \ldots + f_i / f_4) \times$ Cycle], while net Area Under the Curve (net AUC) was obtained by subtracting the AUC of the blank from that of a sample. The final ORAC values were calculated by using a quadratic regression equation ($y = a + b\chi + c\chi^2$) between the Trolox concentration (μM) and the net AUC. The quadratic regression was used in the range of 6.25-50 μM Trolox. Data were expressed as micromoles of Trolox Equivalents per liter of sample (μmol TE/L).

Ferric Reducing Ability of Plasma (FRAP)

FRAP assay measures the direct ability of antioxidants to reduce a ferric complex to the ferrous complex in acidic conditions (75). The materials and methods for the FRAP assay were adapted from the method of Benzie and Stain (79). Blank, sample, and ascorbate standard tubes were made by adding 100 μL of plasma sample, deionized water, and ascorbate standard, respectively, to 3 mL of FRAP reagent and vortexed. Tubes were incubated at 37 ˚C for four minutes. The absorbance was then measured at 593 nm immediately after incubation using a spectrophotometer. Plasma reducing potential was reported as ascorbate acid equivalents (μmol Ascorbate/L) based on the reference curve of ascorbate concentration. One μmol ascorbate was expressed as one reducing equivalent.

Data Analyses

Statistical tests were performed using SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA). The design was a 2 (treatments) x 3 (times) repeated measures ANOVA. ORAC and FRAP values were expressed as mean ± standard estimated mean (SEM), and other data were expressed as mean ± standard deviation (SD). A *P*-value of ≤ 0.05 was considered

statistically significant. A paired *t*-test was employed to determine if there were differences between subject characteristics. The three-day diet records were analyzed using Food Processor SQL software (ESHA Research, Salem, OR, USA). Any data with a *z*-score ± 2.5 were considered outliers and were excluded from statistical analyses.

RESULTS

Of the 27 original subjects, 19 completed all stages of the study. Subject characteristics are presented in Table 1. Subjects ranged in age from 40 to 58, with six overweight and 13 obese. Table 2 shows the weight change in the wine and grape juice groups. Overall weight gain occurred in both groups with treatment effect (P=0.044) and time effect (P=0.018). Subjects who drank wine gained significantly more weight (P=0.027). However, Table 3 shows that total fat mass percentage was not significantly different after treatments. CRP and blood lipids were also not significantly changed by either treatment as seen in Table 4 and Table 5. Figure 1 shows that plasma ORAC was unchanged by either treatment. However, Figure 2 shows that FRAP significantly increased after acute, but not chronic, consumption of red wine (P<0.001). All raw data are presented in Appendix F.

Table 1. Baseline Characteristics of Subjects

Variable	Treatment
Number	19
Gender	8 Males, 11 Females
Age (yr)	48.79 ± 5.29
Height (m)	1.68 ± 0.09
Body mass (kg)	90.90 ± 15.10
BMI (kg/m^2)	32.01 ± 3.85

Values are means ± standard deviations.

Table 2. Weight Change after Two Weeks of Daily Consumption of Red Wine and Grape Juice

	Baseline Weight (kg)	End Weight (kg)	Change in Weight (kg)	Post Hoc *t*-test: End Weight (kg) -Baseline Weight (kg) (Sig. 2-tailed)
Wine Group	90.97 ± 15.06	91.56 ± 15.20	0.59± 1.08	0.027
Grape Juice Group	91.65 ± 15.33	91.88 ± 15.49	0.23 ± 0.84	0.249
	Treatment	Time	Interaction	
P-value	0.044	0.018	0.256	

Values are means ± standard deviations

Table 3. Percent Total Fat Mass Change after Two Weeks of Daily Consumption of Red Wine and Grape Juice

	Baseline Percent Total Fat Mass (%)	End Percent Total Fat Mass (%)	Change in Percent Total Fat Mass (%)
Wine Group	36.78 ± 6.89	36.33 ± 6.36	- 0.45 ± 1.56
Grape Juice Group	36.51 ± 6.70	36.82 ± 6.34	+0.31 ± 1.11
	Treatment	Time	Interaction
P-value	0.565	0.759	0.074

Values are means ± standard deviations

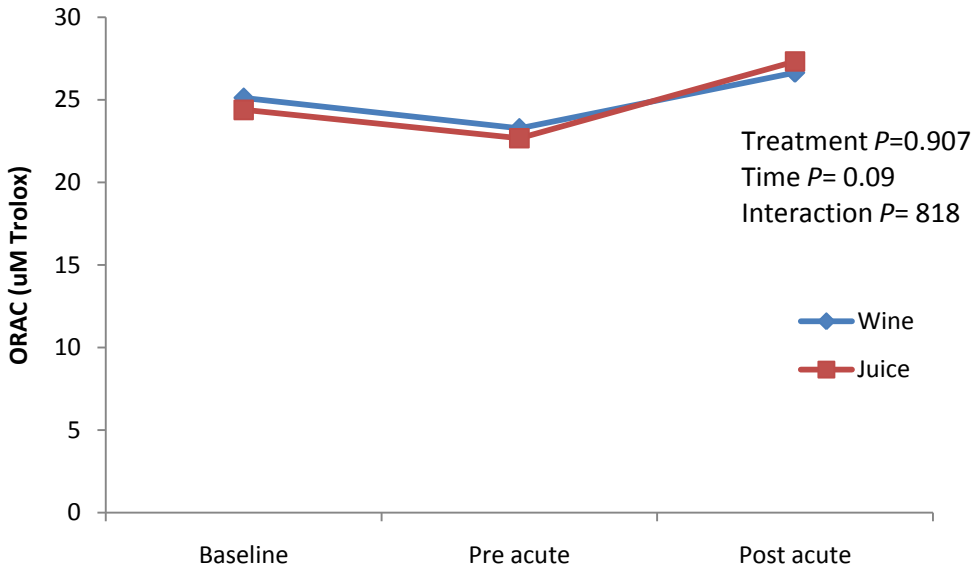
Table 4. Changes in C-Reactive Protein and Lipid Profile after Two Weeks of Daily Consumption of Red Wine and Grape Juice

	Wine Group	Grape Juice Group	*P*-value
CRP (mg/L)			Treatment = 0.774
Baseline	3.77 ± 2.61	3.84 ± 2.68	Time = 0.194
After Two Weeks of Consumption	4.14 ± 2.96	4.31 ± 3.21	Interaction = 0.894
Total Cholesterol (mg/dL)			Treatment = 0.219
Baseline	216.54 ± 34.71	211.15 ± 36.42	Time = 0.735
After Two Weeks of Consumption	215.31 ± 32.49	209.38 ± 35.29	Interaction = 0.932
HDL (mg/dL)			Treatment = 0.572
Baseline	47.31 ± 14.12	47.54 ± 14.25	Time = 0.440
After Two Weeks of Consumption	47.00 ± 15.66	45.69 ± 13.77	Interaction = 0.373
LDL (mg/dL)			Treatment = 0.906
Baseline	141.82 ± 28.89	139.36 ± 29.99	Time = 0.101
After Two Weeks of Consumption	133.09 ± 24.76	136.73 ± 30.31	Interaction = 0.418
Triglycerides (mg/dL)			Treatment = 0.028
Baseline	135.60 ± 32.87	121.10 ± 42.79	Time = 0.262
After Two Weeks of Consumption	147.90 ± 45.75	121.00 ± 31.95	Interaction = 0.544

Values are means ± standard deviations.

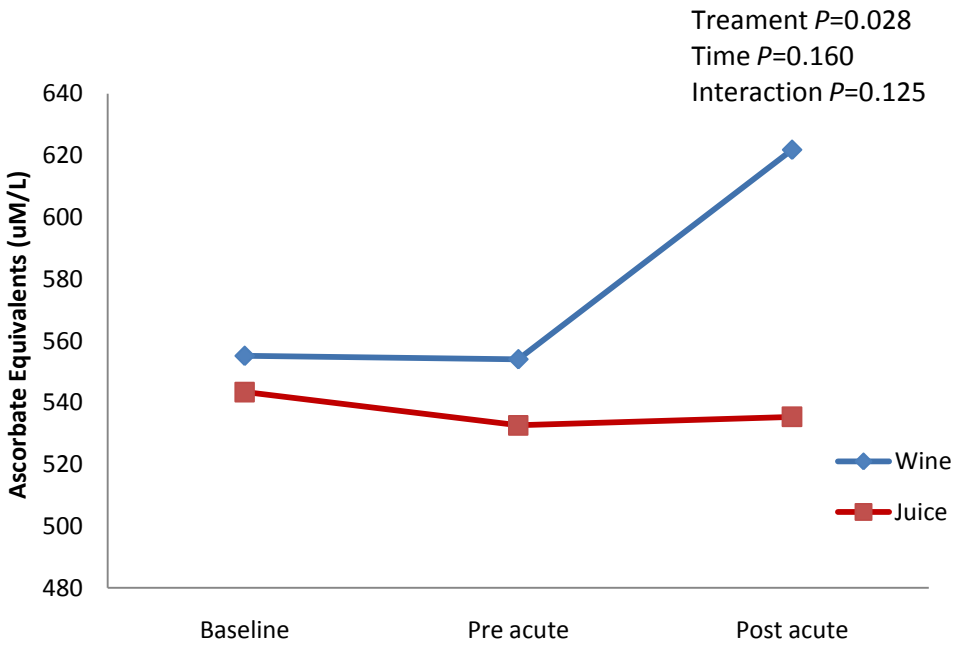
Table 5. Post Hoc *t*-test of Significant Treatment Effects on Triglycerides

		Paired Differences		*t*	Sig. (2-tailed)
		Mean	Std. Deviation		
Wine Group	End Triglycerides - Baseline Triglycerides (mg/dL)	12.45	31.44	-1.314	0.218
Grape Juice Group	End Triglycerides - Baseline Triglyceride (mg/dL)	0.29	31.05	-0.039	0.969
Wine Group Baseline Triglycerides - Grape Juice Group Baseline Triglycerides (mg/dL)		10.50	38.25	1.027	0.323
Wine Group End Triglycerides - Grape Juice Group End Triglycerides (mg/dL)		18.57	40.79	1.703	0.112
Absolute Mean Change of Triglycerides of Wine Group - Absolute Mean Change of Triglycerides of Grape Juice Group (mg/dL)		12.40	62.17	-0.631	0.544



Values are means ± standard errors of the means

Figure 1. Changes in Oxygen Radical Absorbance Capacity (ORAC).



Values are means ± standard errors of the means

Figure 2. Changes in Ferric Reducing Ability of Plasma (FRAP).

DISCUSSION

According to the Centers for Disease Control and Prevention, coronary heart disease (CHD), remains the leading cause of death in both men and women in the United States (86). Both epidemiological and experimental studies have shown that mild-to-moderate alcohol consumption, particularly red wine, can reduce incidence of mortality from CHD. The possible protective effects of red wine consumption on other leading causes of death, including various cancers and diabetes, have been well documented in research. The majority of research attributes the health benefits of red wine consumption to the alcohol and phenolic compounds present in red wine. Similarly, grape juice contains many of the same biologically active phenolic compounds found in red wine and may also contribute to the prevention of many diseases related to oxidative stress, such as atherosclerosis and Parkinson's disease (87).

The present study sought to investigate the effects of red wine and grape juice consumption on blood antioxidant capacity, blood lipids, inflammatory biomarkers, weight change, and body composition. We hypothesized that, following the consumption of both beverages, there would be increased plasma antioxidant capacity through the increased presence of polyphenolic compounds. It follows that increased antioxidant capacity would reduce the chance of lipoprotein oxidation and in turn ultimately reduce atherogenesis.

Further, we expected a decrease in CRP due to reduced inflammatory reaction. Additionally, as alcohol was introduced into the subject's diet, we hypothesized that there would be an increase in HDL in the wine group.

The Effect of Red Wine and Grape Juice Consumption on Plasma Antioxidant Capacity

Utilizing the FRAP assay, this study demonstrated that acute consumption of red wine, but not grape juice, resulted in a significant increase in plasma antioxidant capacity. This is in agreement with the majority of research which shows that red wine increases antioxidant capacity compared to grape juice. The difference in antioxidant capacity between red wine and grape juice consumption may be due to the amount of antioxidant present. Therefore, it is crucial to examine the antioxidant content of both beverages. The compositional information of the red wine and the grape juice used is available for review in Appendix G. A 300 mL serving of red wine provides approximately 87 mg of resveratrol. We were, unfortunately, unable to obtain the antioxidant or resveratrol information about the grape juice from the manufacturer.

The phenolic composition of the grape juice can be significantly affected during grape juice production, especially when grapes are crushed and pressed (88). Besides, the commercial grape juice-making process employs clarifying agents and filters which can decrease resveratrol levels (89). Resveratrol is also likely to be destroyed during commercial pasteurization because it is unstable to light, heat, and wet-heating (90). In contrast, total phenolic content of wine was shown to increase rapidly as it underwent the fermentation

process and continued to increase gradually as the wines aged in barrels (91). Furthermore, the occurrence of phenolic substances in wines was not only a consequence of their extraction from grapes during winemaking (92). During fermentation, the complex polymeric and glycosidic phenolic substances present in grapes break down into monomeric forms and thus become relatively easier to be degraded by digestive juices in human intestine (93). For all of the above reasons, wine is very likely to have an increased antioxidant capacity when compared to grape juice.

However, it is not yet clear to what extent these compounds are absorbed in humans in a metabolically active form and affect plasma antioxidant capacity (88). Hence, further research needs to be conducted to determine the relationship between the phenolic profile in grape-derived beverages and their effect on plasma antioxidant capacity. Dietary polyphenolics are substrates for a number of enzymes in the small intestine and in the liver (94). They are usually ingested in larger forms as glycosides (94). Some glycosides can be absorbed intact in the small intestine using the sodium-dependent glucose transporter 1, although most dietary polyphenolics need to be transformed into smaller aglycones by various intestinal enzymes before passing the gut barrier. Conjugation will then immediately occur in the gut barrier (95). These conjugates are further metabolized in the liver (95). On the other hand, the polyphenols left in the intestinal lumen are subjected to hydrolysis and degradation in the colon due to the activity of enzymes of the colonic microflora, causing the destruction of the flavanol structure and the formation of simpler phenolic compounds (94). These complex breakdown, conjugation, and degradation activities that happen after ingestion of dietary polyphenolics influence the bioactivities of the compound and their subsequent effect on plasma antioxidant capacity. Careful examination should be done in

future studies to investigate the relationship between the types and amount of polyphenolics in grape-derived beverages and their effect on plasma antioxidant capacity.

A possible explanation for different effects observed between the two beverages could be due to the presence of alcohol in red wine. Antioxidant status is the result of the interaction of many different compounds and systemic metabolic interactions (96). The absorption of antioxidants, their metabolic endpoint, and availability for antioxidant protection in humans are not fully understood (96). A review by Manach and co-workers (97) highlighted that polyphenols found in the fruits and vegetables most commonly consumed in the human diet are not necessarily the most active within the body due to the absence of ethanol. Our study demonstrated the different effects observed following grape juice and wine consumption. Since the main difference between red wine and grape juice is the presence of alcohol, we speculate that the remarkable increase in the antioxidant capacity followed by acute red wine consumption is due to the presence of alcohol in the wine, which might have enhanced the absorption and/or mechanism of the phenolic compound.

It is important to note that although this study utilized both the ORAC and FRAP assays to assess serum antioxidant capacity, only FRAP changed significantly. It is not surprising to see different results in the ORAC and FRAP assays. The results of these two assays cannot be compared directly, as the assays are based on different underlying mechanisms utilizing different radical or oxidant sources, and thus generate different values (98). In short, the ORAC assay has more biological relevance to the antioxidant efficacy *in vivo.* ORAC is able to assay both the hydrophilic as well as lipophilic antioxidants including enzymes, large molecules such as albumin, small molecules including ascorbic acid, uric acid, tocopherol, polyphenols, and hormones such as estrogen (98). Therefore, a change in

the antioxidant activity provided by a single specific antioxidant, such as a wine polyphenolic, may not be detected by ORAC due to the activity other antioxidants. On the other hand, the FRAP assay is used to measure hydrophilic and non-enzymatic antioxidants such as ascorbic acid, alpha-tocopherol, beta-carotene and uric acid. Because of this property, the increase in antioxidant capacity measured in our study can be attributed to more specific antioxidants that can only be detected by FRAP. Furthermore, the FRAP assay can underestimate antioxidant capacity as it assumes that redox reaction can be completed within the assay time (99). Possible underestimation by FRAP strengthens our conclusion about the ability of red wine consumption on increasing plasma antioxidant capacity.

As physical activity was not monitored for the study duration, it represents a potential limitation in the study design. Previous research has suggested that physical activity can improve antioxidant capacity (100, 101). It is important that future research take physical activity into account.

The Effect of Red Wine and Grape Juice Consumption on Inflammatory Biomarkers of Atherosclerosis

According to the American Heart Association, CRP is an acute phase protein that is elevated during inflammatory stress (81). Therefore, testing for elevated CRP levels in the blood may be an additional way to assess cardiovascular disease risk, as atherosclerosis is an inflammatory disorder (102). Another research study found that serum concentrations of CRP were reduced by 21 % after adding 30 g of ethanol from wine for 28 days (103). The decrease in CRP level was attributed to the non-alcoholic compounds in the wine, mainly

polyphenols (103). In contrast to these results, this study showed that serum concentration of CRP was not significantly changed. Instead of a decrease in the CRP level, there was actually a slight non-significant increase in CRP in both treatment groups. The increase of CRP levels is likely related to the weight gain contributed by the added calories. Weight gain was shown to be positively correlated with CRP level in other studies (104, 105). Besides, as low exercise levels are associated with slightly elevated CRP (106), the physical activity of the subjects represents a potential confounding factor in determining if CRP levels are significantly affected through alcohol consumption.

The Effect of Red Wine and Grape Juice Consumption on Lipid Profiles

A previous study using red wine from the same winery demonstrated that chronic red wine consumption was effective in reducing triglyceride levels (107). However, in contrast to this study, triglyceride levels increased after two weeks of wine consumption (P=0.028). Careful consideration was taken to improve subject compliance. Subjects were instructed to fast for at least eight hours prior to reporting to the lab for each blood draw in an effort to eliminate confounding variables. However, study subjects did not always remember to fast prior to blood draws. Because triglyceride levels change dramatically in response to meals (108), a possible explanation that the non-significant post-hoc increase in triglyceride levels (Table 4 and 5) could be related to subject compliance rather than red wine or grape juice supplementation.

On the other hand, HDL, LDL, and cholesterol levels do not respond as quickly as triglycerides to diet or lifestyle changes. Changes may take up to months to be seen.

Research shows that cholesterol profile of each individual has different physiological responsiveness from diet and lifestyle (109). Cholesterol profile changes in 3-4 weeks through diet are considered to be highly responsive (109). Some changes may take up to 3-6 months to be seen.

HDL was not altered in our study. There are numerous studies that have demonstrated that moderate amounts of alcohol (10-40 g/day) are a protective factor against CHD (110-112). The mode is most likely due to its ability to increase protective HDL cholesterol (113). In our study, although no significant changes were seen on HDL, it is speculated that red wine or grape juice supplementation for a longer period of time may increase HDL.

A slight decrease in LDL and cholesterol values in both red wine and grape juice treatment groups after two weeks was observed in our study. These findings are in agreement with a study by Yugarani and coworkers (114) that found that a high fat diet with daily supplementation of tannic acid, a phenol found in high concentrations in muscadine grape and muscadine grape products, lowered LDL and total cholesterol by 29.6 % and 33.31 %, respectively, after 10 weeks of supplementation. Auger et al. (115) demonstrated that hamsters fed an atherosclerotic diet with a 8-week red wine phenolic extract supplementation had lower total cholesterol levels compared to those that were not under supplementation. Therefore, it is speculated that red wine or grape juice supplementation for a longer period of time may lower LDL and cholesterol to more significant levels.

The Effect of Red Wine and Grape Juice Consumption on Weight Change

Another observation of our study was the overall weight gain across both treatment groups. We asked the subjects to add the wine or the grape juice to the normal daily diet. Thus, it is not surprising to see this increase in weight gain as a result of added calories from the beverage. Also, when body composition was further examined, there was no significant change seen in the total body fat percentage. If subjects gained weight, but the total body fat percentages were the same, the weight gain could be related to lean body mass gain.

One interesting observation was that, when comparing the weight gain of WG with the weight gain in the JG, only WG was found to gain significantly more weight (P=0.027). However, when looking at the diet of each subject in both treatments, no significant difference was found on the macronutrients intake. Besides, the wine had slightly less calories than the grape juice. In addition, although not significant, a negative 0.45% change in total fat mass was found in the WG, while a positive 0.31% change was found in the JG. This suggested that red wine may have stronger ability in modulating body fat percentage compared to grape juice.

The Effect of Red Wine and Grape Juice Consumption on Overall Heart Health

One of the most interesting questions is whether drinking red wine and grape juice will pose health benefits to the human body. In our study, grape juice, although not significantly, conferred an increase on antioxidant capacity followed by acute consumption, as demonstrated by both FRAP and ORAC assays. The capability of grape juice to increase

antioxidant capacity was also clearly demonstrated by Day et al. (116). Red wine, on the other hand, was shown to significantly increase the plasma antioxidant capacity after consumption. These findings corroborate the research done by Whitehead and coworkers (4) who found a significant increase in antioxidant capacity of healthy adults after 1 hour and 2 hours of red wine consumption when compared to baseline. Although the effect caused by red wine consumption diminishes after several hours, this short-term increase in antioxidant capacity could have significant positive effect on human health. An increase in antioxidant capacity may help the human body combat oxidation and enhance blood circulation. This could potentially create better passage for white blood cells to fight acute infection, enhance oxygen transmission through red blood cells during aerobic exercise, modulate blood pressure, and even enhance overall brain function.

The French regularly consume wine with meals, typically 2-3 times a day. The reduced risk of cardiovascular disease in the "French Paradox" may be related to this drinking pattern. Our study only required subjects to consume 300 mL of red wine per day and did not specify a certain time interval. It is highly likely that the subjects had varying degrees of habits regarding the time the red wine was consumed. As antioxidant capacity is enhanced for a short period with each intake, it is possible that small doses of red wine intake during regular intervals throughout the day may further enhance potential health benefits which deserves further research.

In summary, our study supports that middle-aged or older individuals with increased risk of cardiovascular disease and no contraindications to alcohol use, may benefit from daily moderate red wine intake. Given that CHD is a major health concern, this finding could have significant implications for those suffering from diseases related to increased oxidative stress.

However, it is important that people with a history of alcohol abuse not be encouraged to continue their habit based on potential CHD benefits only. It is also important that people with significant weight issues be encouraged to reduce caloric intake and increase their physical activities if red wine is added to their diet. Health professionals should take care in evaluating every individual's health situation and discussing with the patient both the adverse and potentially beneficial aspects of moderate drinking.

CONCLUSION

In this study, acute consumption of 300 mL of red wine increased plasma antioxidant capacity as evidenced by the FRAP assay in middle-aged, sedentary, overweight, and obese individuals. This investigation supports the hypothesis that consuming red wine may confer some health benefits due to this increase. However, acute consumption of 300 mL of grape juice, or two weeks of red wine or grape juice consumption, did not significantly alter plasma antioxidant capacity.

Because neither chronic nor acute consumption of grape juice yielded significant changes in the plasma antioxidant capacity, this study suggests that some compound in the wine, such as the alcohol, may help the absorption or change the metabolism of the phenolic compounds in human bodies. It is questionable whether grape juice, or any beverages or food with phenolic compounds, when paired with other types of alcohol, will yield the same result as red wine because the main difference between the two beverages is the alcohol content. Future research should examine the optimal amount of alcohol, if any, that will alter the changes in plasma antioxidant capacity followed by consumption of phenolic compounds.

In addition, since this is one of the very first studies to investigate the difference between the effects of chronic versus acute red wine consumption in human subjects, and since our research showed that only acute consumption of red wine increased plasma

antioxidant capacity, more research needs to be done to elucidate the best frequency of red wine consumption in order to confer potential health benefits. Future research should look further at how the plasma antioxidant capacity peaks and decreases after consuming red wine as well as using a direct and sensitive marker of oxidative stress damage such as F2-isoprostanes.

SUGGESTIONS FOR FUTURE RESEARCH DESIGN

This research design could be strengthened by:

1. Adding another treatment group using a resveratrol supplement of 87 mg;
2. Adding another treatment group using the same grape juice with added alcohol;
3. Measuring the antioxidant concentration and antioxidant capacity of all beverages *in vitro* and correlate results with plasma antioxidant concentration and capacity;
4. Measuring plasma antioxidant capacity of the subjects for multiple times after acute consumption (30 minutes, 1 hour, 2 hours and 2.5 hours post consumption);
5. Monitoring physical activity;
6. Monitoring total body fat percentage as well as muscle mass changes;
7. Developing different drinking patterns for subjects (with and without meals);
8. Lengthening the duration of chronic consumption; and
9. Increasing sample sizes.

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APPENDIX A

Institutional Review Board Documents

APPALACHIAN STATE UNIVERSITY

Informed Consent for Subjects in Research Projects Involving Human Subjects

Title of Project: Effect of chronic and acute red wine consumption on selected risk factors for disease, oxidative stress, and plasma antioxidant capacities

Primary Investigator: Tim L. Radak, Dr.P.H., RD
Co-investigators: Lisa McAnulty, Ph.D., RD
Steven R. McAnulty, Ph.D.

Purpose of this Research/Project

The purpose of this novel study is to examine the effects of chronic (2 wks) and acute (within 1 hr) consumption of 300 mL of red muscadine wine or dealcoholized wine on weight loss, body fat, illness rate, blood lipids, blood antioxidant capacity and phenols, and blood oxidative stress in 35 obese individuals. This study is novel in that it will be the first study to incorporate all prior stated parameters in one study. Given the prevalence of obesity in our country and particularly within our state, and increased oxidative stress in obese individuals, this project is highly relevant and will target a sizable section of the population.

I. BRIEF REVIEW

Obesity

Individuals with high body mass index (BMI) and obesity are prone to early mortality. Obesity is a well-established risk factor for hypertension, hyperlipidemia, type II diabetes, coronary heart disease, stroke, obstructive sleep apnea, asthma, orthopedic disorders, and certain cancers. Despite this risk, the prevalence of obesity continues to increase worldwide, and there is a growing demand for safe and effective anti-obesity drugs. Previous anti-obesity drugs or anorexigens, particularly centrally acting agents, have poor safety records (13). Red wine naturally contains high amounts of polyphenolic compounds which may be effective at reducing body fat and weight without the dangerous side effects associated with prescription drugs (5,15,16).

Blood Antioxidant Capacity

Phenolic compounds in red wine can exert antioxidant effects. This has led to speculation that red wine consumption provides unique anti-atherosclerotic effects compared to other alcoholic beverages. However, studies assessing the effects of red wine consumption on plasma antioxidant capacity and oxidative stress in humans have not been conclusive regarding this issue (2,12,14,20,22). These inconsistencies in outcome may have been due to use of different wine types, duration of supplementation, and amounts of supplementation.

Blood Oxidative Stress and Red Wine Polyphenolic Compounds

There is evidence that at least part of the process responsible for several of the major diseases known to contribute to mortality in the United States (for example, heart disease, cancer, diabetes, and Alzheimer's) is related to oxidative stress and inflammation. Oxidative stress is derived from the formation of compounds known as reactive oxygen species (ROS). Research has shown supplementation with naturally occurring polyphenolic compounds to be safer than traditional antioxidants and exert many positive effects. Dietary bioactive compounds from different functional foods, herbs and nutraceuticals (resveratrol, ginseng, ginkgo, nuts, grains, tomato, soy phytoestrogens, curcumin, melatonin, polyphenols, antioxidant vitamins, carnitine, carnosine, ubiquinone, etc.) may ameliorate or possibly even prevent diseases The action of polyphenols involves antioxidant activities, mitochondrial stabilizing functions, metal chelating activities, inhibition of cell death, and the killing of cancer cells. Functional foods and nutraceuticals constitute a great promise to improve health and prevent aging-related chronic diseases (3,6,17-19).

Resveratrol, a natural antioxidant and polyphenol found in red wine and grapes, has been found to pharmacologically upregulate nitric oxide (NO), and possess anti-inflammatory, anticarcinogenic, and antioxidant activities (10). Studies have been conducted suggesting that pharmacologic doses of resveratrol protects heart (1), and brain tissue from oxidative stress(4), and is able to kill cancer cells (9). Resveratrol appears able to enhance cellular protection by inducing production of existing cellular antioxidant systems (10). Despite the quantity of work demonstrating beneficial effects of resveratrol, human studies are virtually non-existent. A human subject study by Zern et al. (23) found that grape polyphenols beneficially affected key risk factors for coronary heart disease such as lipoprotein metabolism, oxidative stress, and inflammatory markers in both pre- and postmenopausal women.

II. PROCEDURES

One to two weeks prior to beginning the study, subjects will report to the ASU Human Performance Lab (054 Holmes Convocation Center) for baseline blood draw, orientation, screening, obtain informed consent, body composition, height, weight, and blood pressure. Body composition (% body fat) will be determined using bioelectrical impedance (BIA). Body composition, including % body fat will also be assessed via dual energy x-ray absorptiometry (DEXA). These methods are administered routinely and safely within our lab. Changes in arterial flow and elasticity will also be measured at Baseline and pre acutely after two weeks consumption and post acute consumption for each group using the Sphygmacor Cardiovascular Management System Arterial Pulse Wave Velocity and Aortic Blood Pressure Waveforms. This assessment of blood flow is completely safe, painless, and non-invasive. All measurements will be conducted in accordance with guidelines set forth by the Clinical Application of Arterial Stiffness, Task Force III. Upon reporting to the lab, subjects will sit quietly for 10 min and be instructed not to consume alcohol or other drugs 24 hours prior to reporting and to avoid caffeine use the morning of reporting to the lab. Subjects must agree to avoid the use of large-dose vitamin/mineral supplements (above 100% of recommended dietary allowances) and all herbs and medications during the study period. Each subject will keep a 3-day food record prior to each test and be encouraged to maintain normal dietary patterns during the study. During orientation, a dietitian will instruct the subjects on how to adhere to the dietary restrictions and how to record intake in a food record.

Subjects will perform two trials in a randomized crossover design: 1) Wine (W) – Subjects will consume 300 mL of Duplin red wine each night for 2 weeks and then report to the

laboratory in the morning for an acute episode of drinking 300 mL and having blood obtained within 1-h after consumption. 2) Dealcoholized Wine (DW) - Subjects will consume 300 mL of dealcoholized Duplin red wine each night for 2 weeks and then report to the laboratory in the morning for an acute episode of drinking 300 mL and having blood obtained within 1-h after consumption. A two week wash out time period will separate trials. To insure safety, subjects will remain in the lab for 2-h after the acute consumption at the ASU physiology lab before being allowed to leave. This should allow blood alcohol levels to reach legal limits.

Blood samples will be drawn three times for each trial which includes Baseline, Post 2 wks chronic consumption, and 1-h post acute consumption. The following tubes of blood will be drawn: Two x 5 mL EDTA tubes to assess plasma antioxidant capacity (FRAP and ORAC), one x 5 mL serum separator for blood lipids, one x 5 mL serum separator for C-reactive protein, and one x 5 mL serum separator for iron binding capacities. This is a total of about 25 mL of blood per draw and is far beneath what is typically obtained during a blood donation.

III. RISKS

The risks related to blood sampling will be minimized by having trained technicians perform the procedures. Nonetheless, there is a small risk of infection or bruising. The procedures are identical to those successfully used in a prior study. Our collaborating physician is on call and available by phone. The local rescue squad is 1 mile from our lab, with a documented response time of 2-3 minutes. All subjects will be provided instructions for care as well as contact phone numbers for the physician involved in the study and the principal investigator. Universal precautions will be used throughout all blood collections. This refers to a "mindset" or "attitude" taken by the researchers that assumes all blood or body tissues are potentially infectious. In the case of exposure of an experimenter by your blood or tissue, that blood will be analyzed for HIV and hepatitis (a positive HIV or hepatitis test will be reported to the subject).

The measurement of your body composition via BIA and dual energy x-ray absorptiometry will expose you to a small dose of electricity and radiation, respectively. This study involves a small radiation exposure that is less than other diagnostic tests involving radiation exposure. For example, the amount of radiation exposure received in this study is below that which you would receive from a CT scan, and well below the amount that poses a significant risk of harm. The total amount of radiation exposure is equivalent to the exposure you would receive in a cross-country airplane flight and present no additional risk to you. Changes in arterial flow and elasticity measured using the Sphygmacor Cardiovascular Management System Arterial Pulse Wave Velocity and Aortic Blood Pressure Waveforms assessment of blood flow is completely safe, painless, and non-invasive. A small pen-like probe is placed over your carotid artery (side of your neck), over the femoral artery (top of your leg) and ankle artery. A transducer (like a microphone) uses ultrasound waves (sound waves which bounce off the blood in the blood vessel) to measure the speed and direction of blood flow through an artery. No physical discomfort should be experienced during this test. Your privacy will be upheld with great care during the assessment of the femoral artery, as this is best located near the pubic area. There are no known risks associated with the Doppler ultrasound used in this technique.

The alcohol consumed on a daily basis in this study is considered modest and has been safely used in a prior research project (11). However unlikely, we will ask subjects to report any adverse symptoms associated with the amount of wine used in this study such as unusual nausea, headache, vomiting, weakness, or mental confusion Subjects will be given the contact information for the lead investigator in case of questions or problems. Excessive alcohol consumption associated with alcoholism is well understood to be detrimental both psychologically and physically. Individuals identified to have alcohol problems will not be

included in this study. Furthermore, subjects will be required to remain in the ASU laboratory for 2-h following the acute wine consumption to insure a return to legal alcohol limits before being allowed to leave.

IV. BENEFITS

The subject will receive results of all tests, with counseling given regarding implications for nutrition. I understand that no promise or guarantee of benefits have been made to encourage participation. Larger societal benefits include potential progress toward methods to minimize detrimental effects from oxidative stress associated with certain disease processes.

V. EXTENT OF ANONYMITY AND CONFIDENTIALITY

The identity of subjects will not be disclosed in any published documents or shared with anyone but the experimenters.

VI. COMPENSATION

None. If as a result of a research project, the investigator determines that the subject should seek counseling or medical treatment, a list of local services will be provided. In the event of physical injury resulting from the research procedures, immediate first-aid is provided free of charge. No funds have been set aside for medical treatment of any injury or illness resulting from this project.

VII. FEEDOM TO WITHDRAW

The subject is free to withdraw from this study at any time without penalty.

VIII. Approval of Research

This research project has been approved, as required, by the Institutional Review Board of Appalachian State University.

3-4-09 IRB Approval Date

3-3-2010 Approval Expiration Date

09-0186 Study #:

IX. Subject's Responsibilities

I voluntarily agree to participate in this study. I have the following responsibilities:

1. The subject will attend an orientation session in the ASU Human Performance Laboratory prior to the start of the study.

2. The subject will eat a diet conforming to a food list (as described by the study dietitian) during the week period prior to and during the three weeks of the study period.

3. The subject agrees to be randomized into either: 1) Red wine – Drink 300 mL red wine each day for 14 days and come to the ASU laboratory on the 15th day and drink 300 mL of red wine. Then, remain in the lab for 2-h afterwards for safety reasons. 2) Dealcoholized red wine - Drink 300 mL dealcoholized red wine each day for 14 days and come to the ASU laboratory on the 15th day and drink 300 mL of dealcoholized red wine. Then, remain in the lab for 2-h afterwards for safety reasons. While a subject in this project, subjects agree to avoid the use of large-dose vitamin/mineral supplements (above 100% of recommended dietary allowances), herbs, and medications purported to be antioxidants or affect oxidative stress. Body composition (% body fat) will be determined using BIA and DEXA at Baseline and Pre-acute session. Blood pressure measurements will be obtained at each blood draw.

4. On the day of acute testing and prior to the test, subjects will not eat anything past midnight the previous day before coming to the lab.

7. Blood samples will be collected at baseline, 2 wks post, and 1-h after acute drink on each of the two test sessions.

8. Females will not be pregnant upon entering the study and throughout the study time period.

X. Subject's Permission *(May be modified in the case of minors or members of other vulnerable populations.)*

I have read and understand the Informed Consent and conditions of this project. I have had all my questions answered. I hereby acknowledge the above and give my voluntary consent:

_____Date_____
Subject signature

_____ Date _____
Witness (Optional except for certain classes of subjects)

Should I have any questions about this research or its conduct, I may contact:

Tim Radak	**828-262-2631**	**radaktl@appstate.edu**
Primary Investigator	Office Phone	e-mail
Jay Cranston	**828-262-2692**	**johnsonrl@appstate.edu**
Administrator, IRB	Telephone	e-mail

Graduate Studies and Research
Appalachian State University
Boone, NC 26608

Subjects must be given a complete copy (or duplicate original) of the signed Informed Consent.

APPENDIX B

Recruitment Flier of the Wine and Grape Juice Study

Spring 2009

RED WINE STUDY

OVERWEIGHT/OBESE MALES/FEMALES NEEDED

We are looking for overweight or obese, non-smoking volunteers (males or females, aged 40 or over, no prescription medications).

Benefits of participation:

Free! Diet analysis, bone density and body composition via DEXA scan ($250 value) and results of all blood tests.

Study summary:

- Wine and grape juice has been shown to exert many health benefits. This study will investigate the effects of red wine and grape juice consumption on weight loss, body fat, and other indicators of health.

Your obligations:

- Provide two 3-day diet records and a total of six blood samples (far less than that given in blood donation).
- Drink 10oz of wine daily for 2 weeks and 10oz of grape juice daily for 2 additional weeks.
- Four visits to ASU campus for 2-3 hours each visit.
- Avoid all dietary supplements during study.

This study has been approved by the Institutional Review Board at Appalachian State University and is being conducted by Dr. Tim Radak, Dr. Steve McAnulty, and Dr. Lisa McAnulty in the Departments of Family and Consumer Sciences and Health, Leisure, and Exercise Science.

(Please response no later then 9am, Friday, April 3rd, 2009

If interested, please remove tag from bottom:

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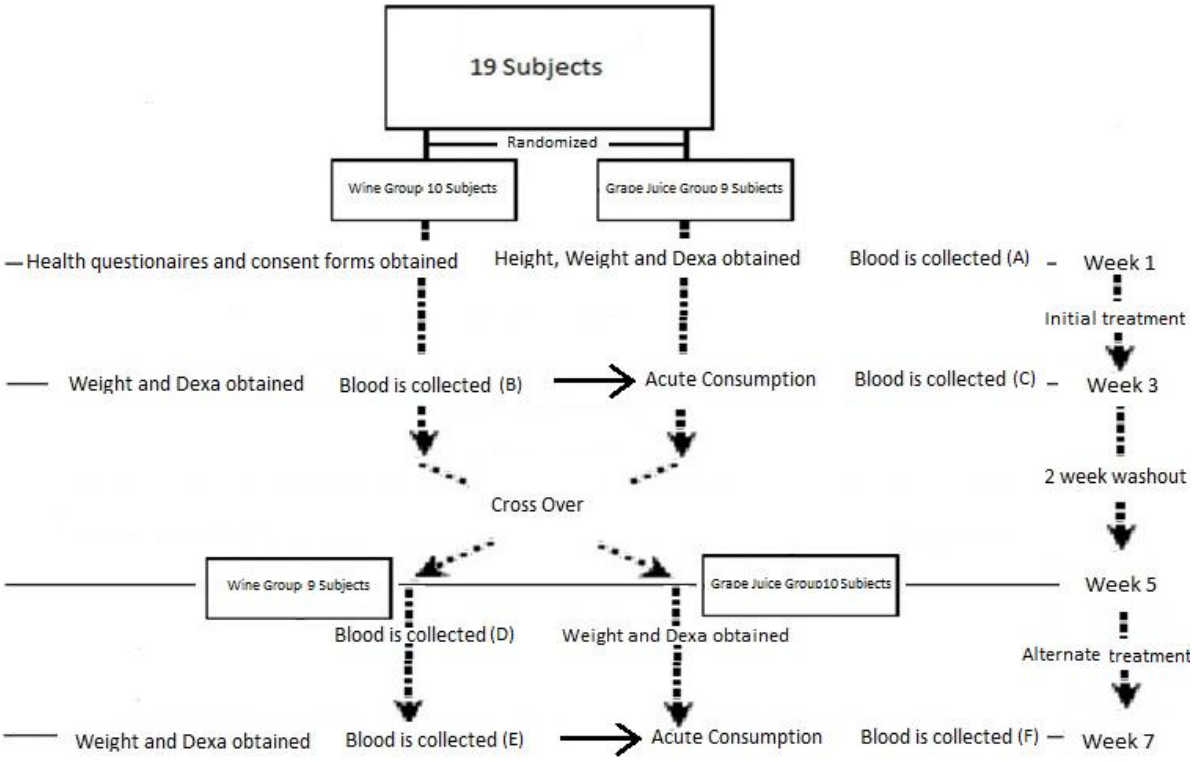
APPENDIX C

Randomized Crossover Design


19 Subjects
Randomized
Wine Group 10 Subjects
Grape Juice Group 9 Subjects
Health questionaires and consent forms obtained
Height, Weight and Dexa obtained
Blood is collected (A)
Week 1
Initial treatment
Weight and Dexa obtained
Blood is collected (B)
Acute Consumption
Blood is collected (C)
Week 3
2 week washout
Cross Over
Wine Group 9 Subjects
Grape Juice Group10 Subjects
Week 5
Blood is collected (D)
Weight and Dexa obtained
Alternate treatment
Weight and Dexa obtained
Blood is collected (E)
Acute Consumption
Blood is collected (F)
Week 7

APPENDIX D

Health Questionnaire

Subject ID: _____ Interviewer's Name:_____
Subject's Name:_____ Date (mm/dd/yy):_____

HEALTH AND MEDICAL HISTORY QUESTIONNAIRE

BACKGROUND

1. What is your highest level of education?
   __Elementary __Jr High School __High School __College __Post College

2. What is your ethnic background?
   __Hispanic or Latino (Cuban, Mexican, Puerto Rican, South or Central American, or other Spanish origin)

   __Not Hispanic or Latino

3. What is your race? __White (Europe, the Middle East, or North Africa) __ African American __ Asian
   __ Native Hawaiian/Pacific Islander __ American Indian/Alaska Native

OVERALL HEALTH

4. *How would you rate your present health condition?*
   __Poor __Fair __Good __Excellent

5. Typically, how many days/year are you sick enough to stay in bed?_____

WEIGHT HISTORY

6. Has your weight changed more than 10 lbs in the last 12 months? __Yes __No

   If yes, why:

   __change in diet __change in physical activity __illness __depression/stress __other

7. Do you have a history of an eating disorder, such as anorexia or bulimia?__No __Yes

8. Have you ever smoked?

   __Never __Not now, but more than 12 months ago __Not now, but within the past 12 months

   __Yes, currently smoking

MEDICAL HISTORY

9. Please check which of the following conditions you have had or now have. Also check medical conditions in your family (father, mother, brother(s), or sister(s)). Check as many as apply

Personal	Family	Medical History
□	□	Coronary heart disease, heart attack
□	□	Surgery
□	□	Angina
□	□	High blood pressure
□	□	Peripheral vascular disease
□	□	Phlebitis or emboli

HEALTH AND MEDICAL HISTORY QUESTIONNAIRE

Personal	Family	Medical History
□	□	Other heart problems (specify:_____)
□	□	Lung cancer
□	□	Breast cancer
□	□	Prostate cancer
□	□	Colorectal cancer
□	□	Skin cancer
□	□	Other cancer (specify:_____)
□	□	Stroke
□	□	Chronic obstructive pulmonary disease (emphysema)
□	□	Pneumonia
□	□	Asthma
□	□	Bronchitis
□	□	Diabetes mellitus
□	□	Thyroid problems
□	□	Kidney disease
□	□	Liver disease (cirrhosis of the liver)
□	□	Hepatitis (A,B,C,D, or E)
□	□	Gallstones/gallbladder disease
□	□	Osteoporosis
□	□	Arthritis
□	□	Gout
□	□	Anemia (low iron)
□	□	Stomach/duodenal ulcer
□	□	Rectal growth or bleeding
□	□	Cataracts
□	□	Glaucoma
□	□	Depression
□	□	Substance abuse problems (alcohol, drugs etc)

HEALTH AND MEDICAL HISTORY QUESTIONNAIRE

10. Please indicate the approximate number of alcoholic beverages **per every two weeks**. (Beer:

    one drink = one 12-ounce beer; Liquor: One drink = 1.5 ounces of liquor; Wine: One drink = 5 ounces)

    □ 0 Drinks

    □ 1-2 Drinks

    □ 3 or more Drinks

11. Please check any of the following **medications** (prescription and/or over the counter) you currently take regularly. Also give the name of the medication.

	Medication	**Name of Medication**
□	Heart Medicine	_____
□	Blood Pressure Medicine	_____
□	Blood cholesterol Medicine	_____
□	Hormones	_____
□	Birth Control pills	_____
□	Medicine for breathing/lungs	_____
□	Insulin	_____
□	Other medicine for diabetes	_____
□	Arthritis Medicine	_____
□	Medicine for depression	_____
□	Medicine for anxiety	_____
□	Thyroid Medicine	_____
□	Medicine for Ulcers	_____
□	Pain killer Medicine	_____
□	Allergy Medicine	_____
□	HIV/AIDS Medicine	_____
□	Hepatitis Medicine	_____
□	Other (please specifiy)	_____

HEALTH AND MEDICAL HISTORY QUESTIONNAIRE

Supplement Use

12. Are you presently using or have you used within the last 12 months the following supplements at least three times/week:

Type of Dietary Supplement Note: If calcium and vitamin D are taken as one supplement, separate into two categories under "single vitamin" and "single mineral". If a supplement contains more than 3 components, enter as either "multivitamin", "multimineral", or "multivitamin/mineral".	Provide Brand Name or Type (i.e., vitamin E, calcium, iron, etc.) Add important comments	Use			Dosage/Tab (single substances) & Units Cups for herbal teas Spoons or scoops for some
		No. Tabs per time point	No. Times: Per day or Per week	Last Used (mm/yyyy)	
Multivitamin					NA
Multimineral					NA
Multivitamin/mineral					NA
Single vitamin(s)					
Single mineral(s)					
Herbal dietary supplement(s)					
Herbal tea*		NA			
Other over-the-counter supplement(s)					
Fiber Supplement (i.e., Metamucil, Fibercon)					

HEALTH AND MEDICAL HISTORY QUESTIONNAIRE

Physical Fitness, Physical Activity/Exercise

13. In general, compared to other persons your age, rate how physically fit you are:

1 □ 2 □ 3 □ 4 □ 5 □ 6 □ 7 □ 8 □ 9 □ 10 □

Not at all	Somewhat	Extremely
Physically active	physically active	physically fit

14. Outside of your normal work or daily responsibilities, how often do you engage in exercise that at least moderately increases your breathing and heart rate, and makes you sweat, for at least 20 minutes (such as brisk walking, cycling, swimming, jogging, aerobic dance, stair climbing, rowing, basketball, racquetball, vigorous yard work, etc.)

□ 5 or more times per week □ 3 to 4 times per week □ 1 to 2 times per week

□ Less than 1 time per week □ Seldom or never

15. How much hard physical work is required on your job?

□ A great deal □ A moderate amount □ None

16. How long have you exercised or played sports regularly?

□ I do not exercise regularly □ less than 1 year □ 1 to 2 years

□ 2-5 years □ 5-10 years □ more than 10 years

Name of personal physician:_____ Phone #:_____

Address:_____

APPENDIX E

Instructions for Subjects

RED WINE STUDY INSTRUCTIONS

ORIENTATION DATE: Saturday April 4, 2009

TIME: 8 a.m.

LOCATION:

- Holmes Convocation Center (Department of Health, Leisure and Exercise Science) on Rivers St. You may enter the double doors on Rivers St and turn left at the information desk and the lab will be on the right.
- If you need further directions, please email or call me at 423-361-2239.

DURING ORIENTATION: Subjects will sign consent forms, answer health questionnaires, undergo an initial screening (height, weight, etc), blood pressure, diet instruction for 3 day food records, DEXA scan, and blood draws.

WHAT TO WEAR:

- Please wear loose fitting clothes such as sweatpants, lounge pants, shorts, t-shirts, etc.
- Also, minimal jewelry is recommended. Jewelry cannot be worn during the DEXA scan.
- Be consistent in what you wear each time you come to the lab.

HOW TO PREPARE: Each time you come to the lab for testing and for orientation you need to be fasted. You will need to fast after midnight the night before you come to lab. The orientation session should last about 2 hours.

WHAT TO STOP:

- You will need to stop any mega-dose supplements (>100% RDA) beyond what is in a multivitamin.
- Do not drink any additional wine, white or grape juice during the study other than what you are given.
- Do not consume fruits or fruit juices 3 days before coming in for testing or orientation. Vegetables are ok.

ALLERGY: Please let me know if you have a sulfite allergy. Wine is known to possibly contain sulfites.

You will be given during orientation the other dates and times you will need to come to the lab.

We look forward to seeing you!

If you have any questions, please contact Dr Lisa McAnulty at 828-773-0251 or mcanultyl@appstate.edu. Thank you.

Appalachian State University Wine and Grape Juice Study

Departments of Family and Consumer Sciences and Health Leisure and Exercise Science

What To Do When Home

- ✓ Be consistent with each day when you consume wine (i.e. after evening meal)
- ✓ You may consume two 150-mL portions together or separately
- ✓ Wine and grape juice is best if served chilled
- ✓ Do not store in direct sunlight or heat – keep wine bottles cold. Once opened, put in refrigerator
- ✓ You will need a wine opener
- ✓ You have been given enough wine/juice to provide two 150-mL servings per day; however, bottle six will roughly be half full. Please return all six bottles at next testing visit
- ✓ At your next visit, don't forget to bring your Food Records Sheet and to arrive 'fasted'.
- ✓ If you have any questions or any doubt about what to do call us anytime at: Dr. Lisa McAnulty 828-773-0251.

Food Recording Form
(Please fill in and bring with you on April 19th at 8:00 am)

Name _____ Age _____

Mailing Address _____

_____

Height _____ Weight _____ Gender _____

Current e-mail address: _____

Directions for Using the Food Diary

1. Please record ALL food, beverage, and supplements eaten/ingested for three days: Monday, Wednesday, and Saturday (April 13, 15, 18). These three days should be as typical as possible.

2. To reduce error, please follow these directions:
    a. Keep your food diary current (list foods immediately after they are eaten).
    b. Whenever possible, MEASURE the volume consumed by using cups and tablespoons. Record amounts in household measures---ounces, tablespoons, cups, and slices.
    c. Be specific when describing the foot item eaten, and the method that was used to prepare the food. Remember to include condiments, sugar, oils, butter, and other visible fats For example:

Apple, raw, fresh, with peel	*1 medium*
Bread, whole wheat, fresh	*2 slices*
Margarine, soft from tub	*1 tablespoon*
Cereal, corn flakes	*1.5 cups*
Sugar, white	*1 teaspoon*
Milk, non fat	*2 cups*
Fish, salmon, baked	*10 ounces*

    d. This food record will be analyzed with a computerized dietary analysis program, and the report will be sent to you with an explanation of the results.

Time Food/Beverage Consumed	**Food Item and Method of Preparation**	**Amount Eaten** (in cups, tablespoons, ounces, etc.)

APPENDIX F

Collected Data

Subject Number	Initial treatment	Gender (F/M)	Age (years)	Height (m)	Weight (kg) A	Weight (kg) B	Weight (kg) D	Weight (kg) E
1	Wine Group	M	40	1.73	85.88	86.27	86.64	86.91
4	Wine Group	F	44	1.73	92.17	92.62	92.17	92.71
6	Wine Group	F	50	1.68	100.97	102.87	103.42	104.14
10	Wine Group	M	58	1.75	104.51	106.32	105.32	105.41
16	Wine Group	M	49	1.73	102.06	104.05	104.6	103.78
17	Wine Group	M	53	1.83	97.89	100.03	101.06	102.24
26	Wine Group	M	46	1.78	114.85	115.21	116.66	115.67
30	Wine Group	F	49	1.6	71.3	73.57	74.3	73.84
38	Wine Group	F	50	1.65	87	88.18	87.27	89.09
54	Wine Group	M	53	1.75	108.77	107.5	107.95	108.68
37	Grape Juice Group	F	40	1.6	68.49	68.31	68.67	69.31
40	Grape Juice Group	F	47	1.6	78.29	79.02	79.29	78.93
41	Grape Juice Group	F	49	1.63	66.04	65.86	65.41	65.68
53	Grape Juice Group	F	47	1.57	97.16	96.89	98.34	99.43
57	Grape Juice Group	F	47	1.5	69.85	68.67	70.13	68.95
59	Grape Juice Group	M	51	1.85	105.87	105.69	105.14	105.05
60	Grape Juice Group	F	57	1.68	106.87	108.68	106.87	106.59
62	Grape Juice Group	M	41	1.7	86	86.45	85.64	85.73
63	Grape Juice Group	F	56	1.6	83.37	83.64	83.55	83.37

Subject Number	Initial treatment	Total Fat Mass Percentage (%) A	Total Fat Mass Percentage (%) B	Total Fat Mass Percentage (%) D	Total Fat Mass Percentage (%) E
1	Wine Group	28.60	28.10	27.10	28.50
4	Wine Group	36.40	38.20	37.10	38.70
6	Wine Group	45.20	44.10	43.10	44.60
10	Wine Group	40.00	41.00	39.60	40.00
16	Wine Group	28.90	27.60	27.20	28.80
17	Wine Group	29.20	28.00	28.90	28.90
26	Wine Group	28.10	29.40	28.40	29.80
30	Wine Group	36.30	37.30	35.40	36.30
38	Wine Group	38.50	38.40	40.00	38.80
54	Wine Group	37.40	38.60	39.00	37.30
37	Grape Juice Group	35.50	34.40	35.80	36.60
40	Grape Juice Group	40.30	40.90	42.00	40.70
41	Grape Juice Group	43.30	42.00	44.50	41.20
53	Grape Juice Group	47.90	47.90	49.30	46.00
57	Grape Juice Group	35.60	34.40	34.80	33.90
59	Grape Juice Group	27.80	28.80	27.90	26.50
60	Grape Juice Group	41.00	41.80	40.50	41.10
62	Grape Juice Group	29.90	30.90	28.70	29.40
63	Grape Juice Group	46.60	46.70	46.80	44.20

Subject Number	Initial treatment	CRP (mg/L) A	CRP (mg/L) B	CRP (mg/L) D	CRP (mg/L) E
1	Wine Group	1.96	3.92	2.92	3.16
4	Wine Group	4.50	4.13	3.29	5.42
6	Wine Group	5.02	7.31	10.04	9.27
10	Wine Group	2.67	0.85	1.23	0.96
16	Wine Group	1.68	1.15	1.82	0.56
17	Wine Group	2.54	2.93	3.05	2.17
26	Wine Group	0.45	1.17	1.60	0.84
30	Wine Group	1.42	2.13	1.95	2.92
38	Wine Group	2.56	--	5.89	3.64
54	Wine Group	6.92	4.42	4.89	7.13
37	Grape Juice Group	7.17	8.95	8.92	9.09
40	Grape Juice Group	4.26	6.00	5.37	8.47
41	Grape Juice Group	21.58	19.20	14.07	23.88
53	Grape Juice Group	10.20	51.20	14.01	10.00
57	Grape Juice Group	4.73	4.19	--	4.74
59	Grape Juice Group	2.56	1.72	--	2.66
60	Grape Juice Group	2.17	11.58	--	2.40
62	Grape Juice Group	1.57	3.67	--	2.03
63	Grape Juice Group	4.23	5.22	--	6.56

Subject Number	Initial treatment	Cholesterol (mg/dL) A	Cholesterol (mg/dL) B	Cholesterol (mg/dL) D	Cholesterol (mg/dL) E
1	Wine Group	258.00	232.00	235.00	253.00
4	Wine Group	205.00	224.00	210.00	220.00
6	Wine Group	171.00	168.00	167.00	152.00
10	Wine Group	227.00	233.00	224.00	226.00
16	Wine Group	254.00	215.00	207.00	194.00
17	Wine Group	162.00	171.00	161.00	179.00
26	Wine Group	185.00	173.00	163.00	174.00
30	Wine Group	224.00	245.00	215.00	208.00
38	Wine Group	178.00	--	177.00	177.00
54	Wine Group	215.00	257.00	204.00	212.00
37	Grape Juice Group	293.00	289.00	277.00	265.00
40	Grape Juice Group	236.00	204.00	217.00	191.00
41	Grape Juice Group	237.00	222.00	235.00	225.00
53	Grape Juice Group	193.00	189.00	185.00	200.00
57	Grape Juice Group	251.00	217.00	--	228.00
59	Grape Juice Group	194.00	169.00	--	183.00
60	Grape Juice Group	260.00	226.00	--	243.00
62	Grape Juice Group	235.00	230.00	--	255.00
63	Grape Juice Group	165.00	137.00	--	161.00

Subject Number	Initial treatment	HDL (mg/dL) A	HDL (mg/dL) B	HDL (mg/dL) D	HDL (mg/dL) E
1	Wine Group	45.00	38.00	44.00	43.00
4	Wine Group	53.00	51.00	51.00	54.00
6	Wine Group	39.00	39.00	37.00	38.00
10	Wine Group	40.00	39.00	40.00	35.00
16	Wine Group	43.00	41.00	43.00	41.00
17	Wine Group	30.00	34.00	30.00	26.00
26	Wine Group	34.00	31.00	32.00	30.00
30	Wine Group	70.00	72.00	68.00	70.00
38	Wine Group	56.00	–	57.00	60.00
54	Wine Group	35.00	29.00	34.00	33.00
37	Grape Juice Group	43.00	47.00	39.00	45.00
40	Grape Juice Group	64.00	52.00	56.00	47.00
41	Grape Juice Group	73.00	65.00	77.00	74.00
53	Grape Juice Group	59.00	60.00	54.00	71.00
57	Grape Juice Group	66.00	50.00	--	55.00
59	Grape Juice Group	58.00	46.00	--	53.00
60	Grape Juice Group	59.00	48.00	--	54.00
62	Grape Juice Group	39.00	40.00	--	38.00
63	Grape Juice Group	61.00	52.00	--	59.00

Subject Number	Initial treatment	LDL (mg/dL) A	LDL (mg/dL) B	LDL (mg/dL) D	LDL (mg/dL) E
1	Wine Group	183.00	162.00	165.00	178.00
4	Wine Group	119.00	136.00	139.00	149.00
6	Wine Group	107.00	111.00	112.00	89.00
10	Wine Group	165.00	164.00	160.00	159.00
16	Wine Group	178.00	149.00	142.00	131.00
17	Wine Group	109.00	111.00	110.00	--
26	Wine Group	116.00	92.00	86.00	115.00
30	Wine Group	131.00	148.00	123.00	114.00
38	Wine Group	109.00	--	93.00	93.00
54	Wine Group	134.00	87.00	128.00	--
37	Grape Juice Group	200.00	191.00	180.00	157.00
40	Grape Juice Group	147.00	124.00	129.00	112.00
41	Grape Juice Group	137.00	138.00	135.00	124.00
53	Grape Juice Group	122.00	116.00	117.00	109.00
57	Grape Juice Group	158.00	133.00	--	138.00
59	Grape Juice Group	122.00	109.00	--	121.00
60	Grape Juice Group	177.00	149.00	--	164.00
62	Grape Juice Group	138.00	134.00	--	--
63	Grape Juice Group	85.00	67.00	--	83.00

Subject Number	Initial treatment	Triglyceride (mg/dL) A	Triglyceride (mg/dL) B	Triglyceride (mg/dL) D	Triglyceride (mg/dL) E
1	Wine Group	152.00	159.00	130.00	162.00
4	Wine Group	166.00	183.00	102.00	87.00
6	Wine Group	127.00	92.00	91.00	123.00
10	Wine Group	112.00	150.00	118.00	159.00
16	Wine Group	163.00	126.00	108.00	112.00
17	Wine Group	117.00	131.00	106.00	614.00
26	Wine Group	174.00	250.00	225.00	147.00
30	Wine Group	114.00	123.00	122.00	118.00
38	Wine Group	65.00	--	134.00	119.00
54	Wine Group	229.00	1060.00	208.00	526.00
37	Grape Juice Group	250.00	253.00	289.00	316.00
40	Grape Juice Group	123.00	142.00	162.00	162.00
41	Grape Juice Group	134.00	93.00	114.00	136.00
53	Grape Juice Group	58.00	67.00	72.00	98.00
57	Grape Juice Group	134.00	169.00	--	176.00
59	Grape Juice Group	68.00	71.00	--	43.00
60	Grape Juice Group	120.00	143.00	--	124.00
62	Grape Juice Group	291.00	282.00	--	506.00
63	Grape Juice Group	96.00	62.00	--	93.00

Subject Number	Initial treatment	FRAP (µmol Ascorbate/L) A	FRAP (µmol Ascorbate/L) B	FRAP (µmol Ascorbate/L) C	FRAP (µmol Ascorbate/L) D	FRAP (µmol Ascorbate/L) E	FRAP (µmol Ascorbate/L) F
1	Wine Group	513.08	502.51	565.19	486.32	530.33	537.02
4	Wine Group	488.78	550.05	653.57	564.49	570.12	596.53
6	Wine Group	468.01	474.70	606.04	448.29	465.19	462.73
10	Wine Group	573.29	643.36	707.09	563.43	596.18	606.39
16	Wine Group	1249.70	604.63	647.94	621.88	599.35	603.22
17	Wine Group	607.09	560.26	650.75	635.61	691.25	669.77
26	Wine Group	698.29	667.65	744.06	735.96	709.56	722.23
30	Wine Group	565.79	605.79	721.86	657.21	573.29	548.64
38	Wine Group	477.93	492.57	533.64	538.64	516.50	519.71
54	Wine Group	753.69	1178.20	1181.80	730.49	778.20	726.57
37	Grape Juice Group	541.50	497.21	460.07	455.07	502.93	565.43
40	Grape Juice Group	–	538.33	653.37	510.88	541.93	542.91
41	Grape Juice Group	418.07	422.65	370.42	412.40	456.63	535.72
53	Grape Juice Group	558.27	439.64	478.53	466.44	499.44	549.44
57	Grape Juice Group	405.60	414.86	499.74	425.06	443.26	538.94
59	Grape Juice Group	506.84	434.62	439.56	407.77	492.64	531.84
60	Grape Juice Group	453.44	438.94	466.41	484.93	420.11	405.30
62	Grape Juice Group	542.95	524.43	561.47	519.80	626.28	714.25
63	Grape Juice Group	371.65	384.62	367.95	424.74	449.74	481.22

Subject Number	Initial treatment	ORAC (µmol TE/L) A	ORAC (µmol TE/L) B	ORAC (µmol TE/L) C	ORAC (µmol TE/L) D	ORAC (µmol TE/L) E
1	Wine Group	41.93	42.89	51.85	42.20	47.56
4	Wine Group	38.80	49.28	41.79	54.18	50.50
6	Wine Group	32.12	32.70	34.28	23.69	32.48
10	Wine Group	–	19.76	25.25	21.53	21.01
16	Wine Group	29.13	21.93	33.73	33.79	30.16
17	Wine Group	34.64	15.94	20.56	30.08	16.11
26	Wine Group	29.04	28.54	22.97	29.87	21.13
30	Wine Group	26.38	27.24	28.89	23.81	29.28
38	Wine Group	42.64	19.94	23.43	22.78	18.95
54	Wine Group	7.87	15.31	18.03	15.75	16.57
37	Grape Juice Group	33.81	30.01	32.24	28.41	31.60
40	Grape Juice Group	–	20.35	19.18	21.42	23.44
41	Grape Juice Group	31.69	40.04	37.78	35.00	36.49
53	Grape Juice Group	37.66	30.30	39.62	39.15	35.45
57	Grape Juice Group	19.06	19.58	15.36	23.69	24.59
59	Grape Juice Group	19.36	22.63	26.48	23.59	25.65
60	Grape Juice Group	15.71	18.58	15.43	17.13	14.17
62	Grape Juice Group	11.24	16.09	25.15	23.92	14.40
63	Grape Juice Group	29.13	13.50	33.73	33.79	30.16

APPENDIX G

Nutrition Information of the Red Wine and the Grape Juice Used

Nutrition Information of the Red Wine and the Grape Juice Used

Hatteras Red - Duplin Winery (Rose Hill, NC)

INGREDIENTS: 100% Muscadine grapes

Serving Size: 4 fl oz (118 mL)
Amount in one bottle: 750 mL

Amount per serving:
Calories: 76.8
Total Fat: 0g
Total Carbohydrate: 19.2 g
Sugars
Sugar Alcohols
Protein: 0g
Acid level: 0.67%
Resveratrol content: 73.39 parts per million (this is an estimate)

100% Muscadine Grape Juice - D'Vine Foods (Elizabethtown, NC)

INGREDIENTS: 100% Muscadine grape juice, may contain sulfites

Serving size: 4 fl oz (118 mL)
Servings per container: 6.25

Amount per serving:
Calories: 80
Calories from Fat: 5
Total Fat: 0 g
Saturated Fat: 0 g
Trans Fat: 0 g
Total Carbohydrate: 22 g (7% daily value)
Dietary Fiber: 1 g (4% daily value)
Sugars: 20 g
Protein: 1 g
Cholesterol: 0 mg
Sodium: 0 mg

Vitamin A: 2% Vitamin C: 8%
Calcium: 2% Iron: 2%

Percent daily values are based on a 2,000 calorie diet.
*Resveratrol content not available from company

VITA

Weng On Ho was born in Macao, China on December 8, 1985. Her parents are Chio Seng Ho and Wai Man Tang. She has one elder brother. She attended primary and secondary school at Chan Sui Ki Perpetual Help College, Macao and graduated in June 2003. The following August she moved to the United States at the age of seventeen on her own and entered El Camino College in Torrance, California. She received an Associate's Degree in June 2005. Weng On continued her education at the University of California at Davis and was awarded the Bachelor of Science in Clinical Nutrition in December 2007. In the fall of 2008, she accepted the North Carolina Tuition Scholarship and graduate assistantship at Appalachian State University and began her study towards a Master's Degree in Foods and Nutrition. This degree was awarded in December 2010. Upon successfully passing the Registration Examination for Dietitians credentialed by the CDR of the American Dietetic Association, Ms. Ho will be recognized as a Registered Dietitian.

The author is a member of Phi Kappa Phi and Kappa Omicron Nu Honor Societies.