ABSTRACT

BANINI, AKPENE ESI. Effects of Muscadine Grape Products on Healthy and Type 2 Diabetic Subjects: Blood Chemistry, Antioxidant Capacity and Membrane Lipids. (Under the direction of Dr Leon C Boyd and Dr Jonathan C. Allen)

Objective: Red wines and grape juices contain phenolic compounds with antioxidant properties believed to be protective against oxidative stress conditions that are associated with cardiovascular events including hypertension, insulin resistance and Type 2 Diabetes. This study was designed to evaluate the effects of muscadine grape juice, muscadine grape wine and dealcoholized muscadine grape wine on blood constituents, antioxidant capacity, and erythrocyte membrane lipids of Type 2 diabetics and control, non-diabetic subjects. Methodology: Control subjects were randomized into 2 groups: control group without supplementation (n = 15), and control group on muscadine juice (n = 8). Type 2 Diabetics were in the following categories: muscadine juice (n = 10), dealcoholized wine (n = 9) and muscadine wine (n = 10) groups. In addition to their regular diet, each group consumed 150 ml of the grape product. A 3-day diet record, anthropometric indices and blood pressure were recorded. Blood samples were obtained for the analyses of erythrocyte membrane lipid, plasma total polyphenols, oxygen radical absorbance capacity (ORAC), total reduced glutathione, glycated hemoglobin and insulin. A comprehensive metabolic panel, blood coagulating
indices, liver, renal and cardiac function tests were determined. All tests were carried out at baseline and 28 days post-treatment. **Results:** Glucose and glycated hemoglobin decreased post supplementation in all T2D groups, with the greatest decrease observed among the T2D– wine group. Dietary chromium and biotin levels were below 50% of recommended daily value for all subjects. Serum sodium and chloride levels significantly decreased in the T2D- wine group compared to their counterparts. Blood folate and vitamin $B_{12}$ increased significantly in the T2D taking dealcoholized wine compared to T2D who consumed wine. Trends of lowered triglyceride (TG), total cholesterol (TC) and low density lipoprotein cholesterol (LDL) were observed among T2D taking dealcoholized wine and T2D taking wine compared to T2D taking juice. Alanine aminotransferase (ALT) and aspartate transaminase (AST) were significantly lower in T2D taking wine compared to T2D who consumed dealcoholized wine. Increased ORAC values post supplementation compared to baseline were not significant. **Conclusion:** Decreased levels of blood glucose and glycated hemoglobin post supplementation indicated better glycemic control, especially in the T2D- wine group. Low dietary chromium and biotin indicates insulin inefficiency in stimulating glucose uptake. Reduced serum sodium and chloride levels among T2D- wine group after supplementation suggest reduced risk for hypertension. High folate and vitamin $B_{12}$ levels in T2D on dealcoholized wine indicate a favorable environment for homocysteine clearance and increased insulin sensitivity. Lowered TG, TC and LDL among T2D taking wine and DzW suggest improved
lipid metabolism. Decreased ALT and AST in T2D- wine group indicates better insulin sensitivity and reduced risk of impaired liver function. Finally, T2D- wine and DzW groups exhibited improved serum lipids, ALT, AST and glutathione compared to T2D- juice group. Also, improved serum electrolyte and cardiac function in T2D- wine group, and greater responses in insulin, folate and vitamin B_{12} in T2D- DzW group were observed. Moderate amounts of wine and dealcoholized wine intake may therefore improve diabetic metabolic functions.
EFFECTS OF MUSCADINE GRAPE PRODUCTS ON HEALTHY AND TYPE 2 DIABETIC SUBJECTS: BLOOD CHEMISTRY, ANTIOXIDANT CAPACITY AND MEMBRANE LIPIDS

By
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A dissertation submitted to the Graduate Faculty of North Carolina State University in partial fulfillment of the requirements for the Degree of Doctor of Philosophy

NUTRITION

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APPROVED BY

[Signatures]
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DEDICATION

I dedicate this dissertation to my mother, fondly called Daavi; to my brother Godsway; to my sister Bubu; to my husband Edem; and to the Lord God almighty.
BIOGRAPHY

Akpene Esi Banini was born on May 27th, 1973 to father Leonard Yao Banini (of blessed memory) and mother Fidelia Korsiwor Banini in Kumasi, Ghana. She spent her first eight years in Kumasi and then traveled to Nigeria with her parents and siblings where she attended secondary school and began her Bachelors program in University of Maiduguri, Borno State, Nigeria. She later went back to Ghana and completed her Bachelor of Science program in Biochemistry and Nutrition at University of Ghana, Legon, Ghana. After serving as a teaching assistant for a year within the Nutrition Department at the same university, she began her M.Phil program in Nutrition in 1998/99 academic year. During her graduate research year, she gained the opportunity in the Study Abroad program to travel to the United States of America and perform her M. Phil. research, investigating Type 2 diabetes, at North Carolina State University, Raleigh, North Carolina. She then went back to Ghana in December, 2000 to complete requirements for the M. Phil degree.

Akpene’s interest in Type 2 diabetes grew and so she applied to come back to continue research in that area. She joined North Carolina State University to pursue a Ph.D program in Nutrition in August, 2001 and obtained her Ph.D degree in August, 2004. Akpene is dedicated to studying the nutritional implications associated with Type 2 diabetes.
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CHAPTER ONE

LITERATURE REVIEW
1. **Pathophysiology and Prevalence of Type 2 Diabetes**

Type 2 Diabetes (T2D) or non-insulin-dependent diabetes mellitus (NIDDM) refers to multiple metabolic disorders characterized by increased glucose concentration in the blood due to insulin deficiencies or insulin resistance. In healthy individuals, blood glucose concentrations are found within a narrow range (between 80 and 100 mg/dl in the post-absorptive state).

Upon food intake, a rise in blood glucose levels triggers insulin release from the pancreatic beta cells. Insulin binds to its receptors in target peripheral tissues and enhances the transport of glucose into skeletal muscle and adipose tissue. Other effects are energy production and storage. Thus under normal conditions, insulin suppresses glycogenolysis and gluconeogenesis in the liver, and inhibits lipolysis in the adipose tissue. By decreasing hepatic and adipose glucose production, and by accelerating the uptake of glucose into peripheral tissues, the net effect of insulin’s action is to lower blood glucose concentration (Ruhe and McDonald, 2001).

In the T2D state, several events occur to hinder this process. Decreased insulin receptor sensitivity to insulin binding and reduced expression of glucose transporters decreases glucose transport into the liver, muscles and adipose tissues. Also, diminished response of the pancreatic beta cells to glucose stimulation may result in suboptimal insulin production to meet the
body needs. Synthesis of proteins, lipids, glycogen and gene expression is compromised. Glucose intolerance ensues, resulting in a condition known as insulin resistance. Eventually, the culmination of increased insulin resistance, inadequate insulin secretion in response to a glucose challenge and decreased expression of glucose transporters result in hyperglycemia, hyperinsulinaemia and other T2D complications (Ruhe and McDonald, 2001; Skrha, 2003).

Increased oxidative stress also leads to Type 2 Diabetes (Evans et al., 2002; Ceriello et al., 1998). In the presence of hyperglycemia and increased free fatty acids, mitochondrial reactive oxygen species increase and these trigger the activation of stress-sensitive signaling pathways including nuclear factor-kappa-B (NF-κB), p38- mitogen-activated protein kinase (MAPK), NH₂-terminal Jun kinases/stress-activated protein kinases (JNK/SAPK), advanced glycosylation end-products (AGE)/ receptor for AGE (RAGE), and diacylglycerol (DAG)/ protein kinase C (PKC). These activities result in mitochondrial dysfunction, inflammation, tumorigenesis and further aggravation of the T2D state. These proposed events are illustrated in Figure 1. This cascade of events can be prevented with dietary restrictions and weight loss (Dandona et al., 2004), healthy eating habits and at least a 30 – minute regular physical activity 3-5 times per week (CDC, 2004).
Type 2 Diabetes (T2D) continues to grow in epidemic proportions throughout the world. It is the 6th leading cause of death in the United States. More than 18 million Americans have diabetes, with another 16 million in the prediabetes state (elevated blood sugar level not to the threshold of being classified as diabetic). In addition, about 5.2 million Americans are unaware of their diabetic state. Diabetes costs the nation about $132 billion a year as medical cost and indirect costs due to lost productivity. The average annual health care cost for a diabetic person is $13,243 compared to $2,560 for a person without diabetes.
diabetes in 2002 (CDC, 2004). Incidences of overweight and obesity are also on the rise. Nationwide and in North Carolina, 3 out of every 5 people are overweight (NC Department of Health and Human Services, 2002). About 14% of the North Carolina population has T2D, with only two-thirds of this number diagnosed. Among the diagnosed population, about 20% exhibit diabetic complications upon diagnosis. Type 2 Diabetes results in a 2-4-fold increase in the risk of developing cardiovascular diseases. Other diabetic complications include stroke, blindness, kidney failure, pregnancy complications, lower-extremity amputations and deaths related to flu and pneumonia (CDC, 2004).

The increasing prevalence of diabetes in the United States is related to increasing aging, increased obesity (Sowers and Lester, 1999) and decreased physical activity due to reduced demand for physical labor and increased sedentary occupations. Almost a third of adult population in the US does not participate in exercise or other physical activity. Physical inactivity increases the risk of obesity and results in decreased insulin sensitivity and diminished glucose tolerance, each of which is associated with the development of NIDDM.

2. Free Radicals and Oxidative Stress in Type 2 Diabetes

Free radicals (or oxidants) are atoms or molecules that have one or more unpaired electrons in the outer shell. These are unstable and highly reactive
oxygen and nitrogen species (Mercuri et al., 2000). Free radicals are generated in biological systems through normal metabolic processes as well as exogenous sources such as food components, drugs, ultra-violet light, ionizing radiation and pollution (Prior, 1998; Abuja and Albertini, 2001). Several oxidants exist and these include hydroxyl - \( \cdot \text{OH} \), alkoxy - \( \cdot \text{L(R)O} \), hydroperoxyl - \( \cdot \text{HOO} \), peroxyl - \( \cdot \text{L(R)O} \), nitric oxide - \( \cdot \text{NO} \) and superoxide - \( \cdot \text{O}_2 \). Other highly reactive molecules derived from oxygen but are not free radicals are peroxynitrite - \( \cdot \text{ONOO} \), hypochlorite - \( \cdot \text{OCl} \), hydroperoxide - \( \cdot \text{L(R)OOH} \), singlet oxygen - \( \cdot \text{O}_2 \), and hydrogen peroxide - \( \text{H}_2\text{O}_2 \) (Abuja and Albertini, 2001). In healthy individuals, about 1-5% of these radicals remain in the blood. With these controlled amounts, they are beneficial to the body (Graier et al., 1996; Evans et al., 2002). Superoxide radicals and hydroxyl radicals trigger increased secretion of immune factors to fight against diseases. Nitric oxide functions as a regulator of vascular tone and a messenger in the central nervous system (Evans and Halliwell, 2001). Also, upon infection, free radicals in the effort to become stable ‘capture’ electrons from bacterial or viral active cells and inactivate the organism in the process. In the T2D state, however, this control mechanism breaks down, resulting in increased oxidative stress.

In T2D, chronic exposure to hyperglycemia and insulin resistance has been implicated in altered oxidative metabolism. Excessive plasma and tissue glucose can exert pathological effects through nonenzymatic glycosylation,
which lead to the production of superoxide and hydrogen peroxide (Mercuri et al., 2000). A reduced insulin action and hyperglycemia influence several oxido-reductive pathways including pentose, glycolytic and sorbitol pathways. The activities of two major insulin-induced enzymes in the hexose monophosphate shunt; glucose-6-phosphate-dehydrogenase (G-6PD) and 6-phosphogluconate dehydrogenase (6-PG) are impaired, leading to reduced NADPH availability. These negatively influence other enzymes and systems involved in defensive processes against oxidative agents, such as the glutathione system, thus increasing oxidative stress.

Type 2 Diabetes has also been associated with altered lipoprotein profile, coagulative parameters, endothelial and cell membrane activities (Caimi et al., 2003; Schaeffer et al., 1999). Increased free radicals and high glucose levels result in oxidation and glycation of low-density lipoprotein (LDL). These processes impair LDL-receptor recognition, resulting in increased circulating levels of LDL (Lipinski, 2001). In addition, myeloperoxidase secreted by neutrophils and monocytes causes aggregation of LDL and stimulates its uptake by macrophages (Lipinski, 2001). Oxidized LDL may also play a role in the recruitment and binding of monocytes to the endothelium thereby enhancing atherosclerotic progression (Berliner et al., 1990). The oxidized LDL can also promote chemotaxis, cytotoxicity, modulation of the expression of growth factors, and sequestration of macrophages (Aguirre et al., 1998).
Also, both radical and nonradical oxidants induce lipid peroxidation particularly of those lipoproteins that contain unsaturated fatty acids (Lipinski, 2001). Peroxynitrite, a product of the reaction between a superoxide anion and nitric oxide, is a strong oxidant of low-density lipoproteins (Violi et al., 1999). Aguirre et al., (1998) further reported that the oxidation of low-density lipoproteins increases erythrocyte membrane and endothelial cell rigidity thereby altering their role in cholesterol transport (Aguirre et al., 1998).

Oxidative stress accelerates hypercoagulable state, with decreased prostacyclin production and activation of thrombin (Asakawa et al., 2000; Yamada et al., 2000). Enhanced fibrinogen production in T2D has been associated with elevated levels of C-reactive protein (CRP) and increased white blood cell count (Held et al., 2000). These associations have also been observed as predictors of myocardial infarction and other cardiovascular events (Ceriello, 1993; Yarnell et al., 1991; Yamada et al., 2000).

The major abnormalities observed in cell membranes of T2D are decrease in membrane fluidity and lateral diffusion rate of protein molecules in the membrane (Kamada et al., 1992; Winocour et al., 1990). These abnormalities result from decreased unsaturated fatty acid content of the membrane due to decreased desaturase activity (Caimi et al., 1992). The loss of membrane fluidity increases the cell membrane susceptibility to free radical attack, subsequently altering the structure and function of lipids and proteins.
Furthermore, the membrane fluidity and membrane protein lateral mobility inversely correlate with the concentration of superoxide ions in the plasma and peripheral glucose utilization, and directly correlate with insulin levels, indicating the interdependence between oxidative stress and glucose metabolism (Paolisso et al., 1994).

Oxidative stress has been observed in people with obesity (Ruhe and McDonald, 2001). Obesity, a condition also prevalent in 81% of T2D (NC Department of Health and Human Services, 2002), promotes decreased insulin-receptor sensitivity and lowered glucose uptake and transport, leading to increased free radical production in the plasma (Ruhe and McDonald, 2001). Also, the presence of hypertriglyceridemia and/or hypercholesterolemia may enhance the generation of ROS. Studies have demonstrated that triglyceride-rich lipoproteins e.g. very low density lipoproteins, are more susceptible to oxidation than high density lipoproteins (Ceriello et al., 2001). Leighton et al., (1999) also found that a high fat diet was associated with oxidative stress. In addition, the observation that obese rats on a calorie-restricted diet have less oxidative stress than obese rats fed ad libitum indicates a relationship between obesity and increased levels of oxidative stress (Caimi et al., 2003).
3. Wine Phenolics and Antioxidants

The term phenolics comprise about 8000 naturally occurring compounds, all of which possess an aromatic ring bearing at least one hydroxyl substituent (Robbins, 2003). Phenolics are categorized into 2 major groups: simple phenols, which have a single aromatic ring containing one or more hydroxyl groups, and polyphenols, which have at least 2 phenol subunits. Examples of polyphenols include the flavonoids, and tannins (Waterhouse, 2002). Phenolics behave as antioxidants, due to the reactivity of the phenol moiety (OH substituent on the aromatic ring). The major mechanism of antioxidant activity is probably radical scavenging via hydrogen atom donation. Others are through electron donation and singlet oxygen quenching (Shahidi and Wanasundara, 1992).

Wine contains many phenolic substances, most of which originate in the grape berry. Phenols and tannins affect bitterness and astringency of red wine. They also impart color to red wine and enhance the preservative qualities as well as the aging process of wine. Phenolics oxidize easily when exposed to air. Wine phenolics include non-flavonoids: hydroxycinnamates (e.g. caffeic acid), hydroxybenzoates (e.g. gallic acid) and the stilbenes (e.g. resveratrol); and the flavonoids: flavones, flavanols, isoflavones, flavanones, flavonols and anthocyanins. Wines contain tannins as well, but their large molecular size probably hinder absorption (Waterhouse, 2002). The total phenol in a glass of red wine is about 200 mg while that in a glass of white wine is about 40 mg.
Red wine represents a concentrated source of anthocyanins, catechins and proanthocyanidins, and other phenolics (Mattivi, 2002).

Resveratrol, a phytoalexin that belongs to a group of compounds known as stilbenes, occurs in grapes and wine and are produced in plants as defense mechanisms in response to stress, such as fungal and microbial infections (Chan, 2002). Resveratrol has been reported to protect against coronary heart disease and exhibit cancer chemopreventive activity (Bertelli et al., 2002). Resveratrol also inhibits platelet aggregation (Bertelli et al., 1996) and reduces oxidative stress in PC12 cells (Chanvitayapongs et al., 1997).

Muscadine grapes (vitis rotundifolia, or alternatively, muscadiniana rotundifolia) are native to Southeastern United States, including North Carolina. The fruit is borne in small, loose clusters of 3 – 40 grapes. The round fruits about 1 – 11/2 inches in diameter, have thick, tough skin and contain up to 5 hard and oblong seeds. The color of muscadine fruits ranges from greenish bronze to dark colored varieties (NC Dept of Agric and consumer services, 2004). Muscadine grape products, especially muscadine red wine has been shown to contain high amounts of antioxidants, including contributions from resveratrol (Yilmaz and Toledo, 2004; Pastrana-Bonilla et al., 2003) as well as more capability to reduce collagen induced platelet aggregation (Pignatelli et al, 2002).
Bioavailability varies greatly among the various polyphenols, and the most abundant polyphenols in our diet are not necessarily more bioavailable (Manach et al., 2004). Most polyphenols are present in foods in their native form as glycosides, esters, or polymers, and in these forms they cannot be readily absorbed. Also, intestinal microflora may be involved in the absorption process. These microflora may reduce the efficiency of absorption since they degrade aglycones and produce simple aromatic acids in the process (Manach et al., 2004). In the course of absorption, polyphenols are conjugated through the process of sulfation, methylation and glucoronidation in the small intestine and liver. In circulation, these conjugated forms are bound to albumin.

Also other nutrient-nutrient interactions exist among the dietary components during absorption of polyphenols. For example quercetin is more readily and efficiently absorbed after ingestion of onions than after ingestion of apples (de Vries et al, 2001). This may be due to the composition of various glycosides present. Some researchers suggest that the alcohol content in red wine increase polyphenols and enhance absorption. Donovan et al., (1999), measured plasma catechin concentration in red wine as well as dealcoholized red wine and found a similar absorption profile between the two forms of wine. However, 20% more catechin metabolites (as sulfate conjugate and a conjugate containing both glucoronide and sulfate residues) were excreted in the urine after red wine intake than after dealcoholized red wine intake. The
excretion of more catechin metabolites in the alcohol fraction indicated a possible role of ethanol in enhancing the rate of elimination of certain metabolites, though this was not significant to cause a difference in the plasma antioxidant capacity of red wine compared to the dealcoholized wine.

The possibility that oxidative stress may be involved in hyperglycemia-induced coagulation activation was investigated by Ceriello et al., (1995). Prothrombin fragments were used as indices for thrombin activation, and the effect of oral glucose tolerance test and intravenous glutathione administration were investigated. The data suggested that hyperglycemia may induce thrombin activation, possibly inducing an oxidative stress, and that antioxidant glutathione may counterbalance this effect. In a similar work, Ceriello et al., (1997) explored the antioxidant property of plasma samples obtained from T2D. Total radical-trapping power, vitamin A, protein-bound thiol groups and uric acid levels were significantly reduced, whereas vitamin E concentration was significantly increased. Fibrinogen and prothrombin fragment 1 + 2 were increased in diabetic patients. This work concluded that total antioxidant capacity rather than any single plasma antioxidant is the most relevant marker of antioxidant status.

Antioxidants are oxidative agents that protect, prevent or reduce the extent of oxidative damage to biomolecules. These agents may be enzymatic, non-enzymatic or metal chelators. Enzymatic antioxidants include catalase,
glutathione peroxidase, and superoxide dismutase. Superoxide dismutase, a copper-zinc or manganese-containing enzyme, reacts with superoxide radical to form hydrogen peroxide, which is then converted to water by glutathione peroxidase (a glutathione-dependent selenoprotein), or catalase, a heme enzyme. Non-enzymatic antioxidants include alpha tocopherol, ascorbic acid, phenols and beta-carotene. Metal chelators that exhibit antioxidant properties include ceruloplasmin, transferrin and metallothionein. Metal chelators indirectly inhibit oxidation of polyunsaturated fatty acids (PUFA) and LDL by chelating metal ions which, when left circulating, act as prooxidants (Evans and Halliwell, 2001).

4. Dietary Fats and Lipid Alterations in T2D

Several studies have investigated the influence of a high fat diet and a fat restricted diet on insulin sensitivity. Petro et al., (2004) used rats to assess the effects of supplementation of isocaloric low fat diet and high fat diet, and ad libitum fed high fat diet. The objective was to separate the effects of fat intake from excess calorie intake. After 3 weeks of the 11 – week supplementation trial, weight and glucose levels were significantly increased in the isocaloric-fed high fat group compared to low fat group. Body composition showed that this increase was related to the percent fat consumed. This study indicated that a high fat diet is a risk factor for obesity.
A human study evaluating the influence of a Mediterranean diet, a high fat diet, and their supplementation with moderate amounts of red wine on the biochemical, physiological and clinical parameters related to chronic diseases found that oxidative stress was associated with the high fat diet, and that elevated plasma antioxidant capacity was associated with wine consumption and the Mediterranean diet (Leighton et al., 1999). Tsai et al., (2004), observed increased triglycerides and decreased plasma glutathione peroxidase after a high-fat meal, suggesting an association of a high fat meal to increased oxidative stress and depletion of serum antioxidant enzymes.

Olive oil, the principal fat source of the traditional Mediterranean diet, is associated with low incidence of coronary heart disease (Keys et al., 1986). Oleic acid is the major fat present in olive oil, and the phenolic compounds present include the nonpolar oleuropein- and ligstroside- aglycones; and the polar hydroxytyrosol and tyrosol. These compounds have favorable effects on cholesterol concentration as well as LDL and antioxidant activity (Caruso et al., 1999). Vissers et al., (2002), found that humans absorb a large portion of ingested olive oil phenols.

Mori et al., (2000) also reported that supplementation of fish oil (3.6g omega 3 fatty acid/day) for 8 weeks and 4g of purified EPA, DHA or olive oil for 6 weeks reduced oxidative stress (specifically urinary excretion of F2-isoprostanes) in T2D patients.
The type of dietary fat influences insulin sensitivity. Saturated fats (SAT) and trans fats worsen insulin sensitivity, whereas monounsaturated fats (MUFA) and polyunsaturated fats (PUFA) improve it (Vessby et al., 2001; Lichtenstein et al., 2003). Vessby et al., (2001) replaced SAT with MUFA in healthy subjects and observed improved insulin sensitivity. Summers et al., (2002) in a similar study replaced SAT with n-6 PUFA in obese and T2D individuals. Insulin sensitivity and plasma low density lipoprotein cholesterol concentrations improved with the PUFA diet. Other studies, however, did not record any change in insulin sensitivity after replacing SAT with PUFA among diabetic (Heine et al., 1989) and healthy subjects (Schwab et al., 1993; Rivellesse and Lilli, 2003).

In addition, the ratio of n-6 to n-3 PUFA is essential for proper functioning of the body. In the Western diets today, the ratio of n-6 to n-3 fatty acids ranges from about 30: 1 instead of the traditional range of about 2: 1. This high intake of n-6 has influenced the physiological state such that there is increased blood viscosity, vasoconstriction and decreased prothrombin time. By contrast, n-3 fatty acids have antiinflammatory, antithrombotic, antiarrhythmic, hypolipidemid and vasodilatory properties (Simopoulos, 1999). Also, changes in the fatty acid composition can easily modify membrane fluidity and structure (Buttar and Halpner, 2000). Essential n-3 and n-6 fatty acids must be supplied from the diet and these are alpha-linolenic acid (ALA; 18:3n-3) and linoleic
acid (LA; 18:2n-6). Food sources of ALA include soybean, fish oils, canola oils and green leafy vegetables and for LA: vegetable oils, safflower oil, primrose oil and corn oil.

Linoleic (LA) and linolenic (ALA) acids are important components of plant and animal cell membranes. When fish oils rich in n-3 foods are ingested, elongase and desaturase enzymes convert ALA to eicosapentaenoic acid (EPA) and docosahexaenoic acids (DHA), important in brain function. Alpha linolenic acid and LA compete for delta-6-desaturase enzyme in the desaturation chain elongation pathway. However, LA levels influence the incorporation of ALA into plasma and tissue lipids and its conversion to longer-chain n-3 fatty acids. Conversely, ALA and its metabolite, eicosapentaenoic acid, can decrease generation of thromboxane A2, a proaggregatory vasoconstrictor, through their inhibitory action on the conversion from LA to arachidonic acid and the activity of the enzyme cyclooxygenase (Kinsella et al., 1987).

In a study to assess the effect of low dose omega-3 fatty acid substitute (0.6 g omega-3 fatty acids as one capsule Maxigard, twice daily) in T2D, patients showed greater improvement in glycemic status, blood pressure and lipid profiles (Jain et al., 2002). Another non-absorbable, non-calorie fat substitute, Olestra, significantly reduced serum cholesterol and energy intake from fat when subjects consumed greater than 2g/d (Patterson et al., 2000). Other
studies indicate that replacing saturated or trans fats with LA rather than carbohydrate is more effective in reducing the risk of coronary heart disease (Hu et al., 2001)

Type 2 Diabetes is associated with increased lipid peroxidation. Free radicals react with PUFA to form peroxides, thus degrading lipids and releasing malondialdehyde as products. Kesavulu et al., (2001) investigated the relationship between serum lipids, lipoproteins, lipid peroxides [thiobarbituric acid reactive substances (TBARS)] and erythrocyte antioxidant enzymes (catalase, glutathione peroxidase and superoxide dismutase) in Type 2 diabetics with and without coronary heart disease. They observed hyperlipidemia, increased lipid peroxide concentrations and increased TBARS in both T2D groups compared to controls. Turk et al., (2002) also measured elevated TBARS in diabetics, though the increase was not significant. However, in another study, Oranje et al., (1999), after measuring lipid peroxidation assessed by (TBARS), production of copper-induced conjugated dienes (CD) from oxidation of LDL in vitro and levels of IgG and IgM autoantibodies by enzyme-linked immunosorbent assay, reported that lipid peroxidation indices remained unchanged. They also reported decreased glycated hemoglobin and reduced conjugated diene production.

There are numerous defects in lipoprotein metabolism in the T2D state. Low density lipoprotein (LDL) is the major lipoprotein that undergoes modification.
The oxidized lipoproteins are then rapidly engulfed (or internalized) by macrophages, which convert them to cholesterol-loaded foam cells with subsequent development of atherosclerotic lesions (Dandona et al., 2001). Treatment with antioxidants including vitamin C, vitamin E and probucol decrease the oxidation of lipoproteins (Jennings et al., 1987).

Some effective ways of improving lipoprotein profile include physical exercise, modest alcohol consumption, regular consumption of fish oils and avoidance of cigarettes (Krentz, 2003). Antidiabetic drugs, e.g. thiazolidinediones are effective in increasing HDL-C concentrations (Martens et al., 2002).

5. Type 2 Diabetes and Antioxidant Properties of Fruits and Wines

There have been several reports of high antioxidants present in fruits and vegetables. Proteggente et al., (2002) investigated the antioxidant activity of regularly consumed fruit and vegetables in the United Kingdom. Assays conducted included TEAC (Trolox Equivalent Antioxidant Capacity), the FRAP (Ferric Reducing Ability of Plasma) and ORAC (Oxygen Radical Absorbance Capacity). These assays comprise contributions from polyphenols, simple phenols and the ascorbate component. The results showed that the three assays correlated well with total phenolic and vitamin C contents. Among the 20 samples investigated, higher levels of antioxidants were found in
Strawberry, red plum, red cabbage, grapefruit, orange and lower levels in cauliflower, pear, tomato, peach, leek, banana and lettuce.

Block et al., (2001), investigated the plasma antioxidants that are most related to fruits and vegetables consumption. After subject recruitment and administration of food frequency questionnaire, blood samples were drawn from which ascorbic acid, beta-carotene, beta-cryptoxanthin, and alpha- and gamma-tocopherols were measured. A strong association of plasma ascorbic acid with fruits and vegetables consumption was observed.

Many researchers have lauded red wine as a beverage with a protective antioxidant capability (Whitehead et al., 1995; Perez et al., 2002). Maxwell et al., (1994) evaluated the plasma antioxidant activity in healthy volunteers after consuming a standard meal alone or with red wine as 5.7 ml/ kg Bordeaux. They found that the antioxidant activity was significantly higher in subjects whose meal was associated with red wine, and this elevated antioxidant activity remained for up to four hours after drinking. Kanner et al., (1994), recorded about twice the amount of phenolics in red wine as in grape juice.

A polyphenol extract from red wine, ethanol or a combination of both was administered to healthy control or streptozotocin-induced diabetic rats (Al-Awwadi et al., 2004). When plasma antioxidant capacity, biochemical parameters and histomorphomeric studies were carried out to determine the
effect of the supplement on the control and treatment groups, these parameters revealed that the polyphenol extract from red wine reduced glycemia and decreased food intake and body growth in diabetic and nondiabetic animals.

Another study by Rimm et al., (1995), found a reduced incidence of Type 2 diabetes in moderate drinkers as compared with abstainers, regardless of the beverage choice. Conigrave et al., (2001) carried out a 12-year prospective study on 46,892 healthy men. Their report stated that moderate (15-29 g/day) and regular (at least 5 days/week) alcohol consumption, regardless of the beverage type (beer, liquor, or wine), was associated with a significantly lower risk of diabetes onset. This was contrary to another study, which showed that only wine, and whisky, but not phenol-free spirit, induced a significant increase in plasma phenolic concentration and plasma antioxidant potential (Duthie et al., 1998). Gronbaek et al., (1995) reported a lower mortality rate from subjects with low to moderate wine intake. Higher mortality was reported for subjects consuming spirits and no effect on subjects consuming beer in a 12-year follow-up study.

Positive effects of moderate alcohol consumption include inhibition of gluconeogenesis (Siler et al., 1998), enhanced insulin-mediated glucose uptake (Facchini et al., 1994; Mayer et al., 1993; Kiechl et al., 1996) and increased HDL-cholesterol concentration (Facchini et al., 1994). Some
negative effects of alcohol consumption include headache and bronchoconstriction in patients suffering from histamine intolerance (Jarisch and Wantke, 1996), and gastric cancer development when consumed in large amounts (Falcao et al., 1994). Other studies report u-shaped curve relationship between high alcohol consumption and insulin resistance. Insulin resistance is minimal in individuals with regular to moderate alcohol consumption and increases in both heavy drinkers and subjects without any alcohol consumption (Magis et al., 2003; Van de Wiel, 1998).

Higher levels of free radicals are produced in diabetic patients after meal intake. Ceriello et al., 1998 showed that plasma glucose, but not insulin, rose significantly more in diabetic patients than in controls after meal intake, indicating a direct correlation between hyperglycemia and postprandial oxidative stress. In a similar study where volunteers consumed red wine in addition to their meals and the controls consumed water, red wine treatment significantly increased the plasma and erythrocyte antioxidant potential compared to the control group. Red wine may therefore prevent cellular peroxidation reactions and lessen atherosclerotic complications (Durak et al., 1999; Duthie et al., 1998).

A decline in the activation of thrombosis and subsequent decreased production of prothrombin fragments 1+2 and the activation of factor VII has been observed after red wine consumption (Caimi et al., 2001). The
antithrombotic action of wine may be attributed to the ethanol content, which is also known to decrease platelet aggregation induced by most agonists such as adenosine diphosphate, collagen, thrombin and epinephrine (Renaud and Ruf, 1996). In addition, Lacoste et al., (2001) found that a single alcohol drink inhibited platelet thrombus deposition and this effect persisted for 6 hours, long after the blood alcohol level had returned to baseline.

In another study, Day et al., (1997), examined whether the beneficial antioxidant effects of red wine can be reproduced using the red grape juice concentrate. Seven healthy subjects consuming 125 ml concentrated red grape juice had 50 umol/ L increase in serum Total Antioxidant Capacity (TAC), compared to baseline, after 8 days. The same study also recorded a reduced susceptibility of LDL to oxidation from this intake. Cao et al., (1998) investigated the effect of consuming strawberries, spinach, red wine or vitamin C in elderly women. Oxygen radical absorbance capacity, trolox equivalent antioxidant capacity and ferric reducing ability increased significantly by 7-25% during the 4 –hour period following consumption. In addition, there were increases in plasma vitamin C levels after the strawberry drink and serum urate levels after the strawberry and spinach treatments. However, these increased antioxidant levels could not be fully accounted for from the foods supplemented. This may imply that other components in the diet may increase antioxidant levels as well.
Some researchers examined the phenolics (or antioxidant) present in other foods. Chen et al., (2004) investigated the bioavailability of oat phenolics and vitamin C in hamsters. Using the copper-induced oxidation method, they recorded bioavailability of oat phenolics in hamsters and a synergistic effect of oat phenolics, including avenanthramides and vitamin C in protecting LDL from oxidation. Knekt et al., (2002) investigated the association between flavonoid intake and risk of several chronic diseases in Finland. Finnish food composition tables and databases for flavonoids were used to estimate the flavonoid intake from the diet history and other questionnaires administered. Persons with higher quercetin intakes had lower mortality from coronary heart disease and lower incidence of asthma. Also an association between higher quercetin and reduced risk of developing T2D was observed.

In addition to the antioxidant properties, cereal, fruit and vegetable fiber intake are associated with lower risk of ischemic heart disease and death, especially among the elderly (Mozaffarian et al., 2003). Among the milieu of polyphenols extensively investigated, tea (Ohnishi et al., 2001; Higdon and Frei, 2003; Anderson and Polansky, 2002), garlic (Ichikawa et al., 2002, Ou et al., 2003), and chocolate (Serafini et al., 2003; Steinberg et al., 2003; Mao et al., 2002) have received much attention.

6. Oxidative Stress, Supplementation Trials and T2D

Reactive oxygen species (ROS) induces oxidative damage to lipids, proteins, amino acids and DNA (Dandona et al., 1996). Dandona et al., (2001), reported
an increase in oxidative damage to lipids and proteins in obese subjects compared to controls, and reduced oxidative damage upon energy restriction in obese individuals. In addition, an earlier work discovered that plasma tumor necrosis factor –alpha (TNF-alpha) concentrations were elevated in obese subjects and falls after weight loss (Dandona et al., 2001). The inflammatory mediators, TNF-alpha and interleukin 6 are known to increase in insulin resistant states of obesity and T2D (Esposito et al., 2002; Dandona et al., 2004). In addition, hyperglycemia has been demonstrated to increase circulating cytokine concentrations, especially in the impaired glucose tolerance state. These suggest a role of inflammatory cytokines in the immune activation process in T2D (Esposito et al., 2002).

Generally, meal-induced oxidative stress has been observed and investigated in T2D by various researchers. Ceriello et al., (1999), evaluated the effect of two different standard meals on oxidative status and LDL oxidation on T2D patients. Their results showed that plasma glucose, insulin, triglycerides and malondialdehyde increased and non-esterified fatty acids and total trapping antioxidant power significantly decreased after consumption of the meal. Natella et al., (2001) investigated the effects of red wine on the postprandial upsurge of ROS in healthy subjects. They reported that red wine caused a significant increase in total plasma antioxidant capacity and higher plasma concentrations of alpha-tocopherol and thiol (SH-) groups. Postprandial LDL after the meal with red wine was more resistant to lipid peroxidation compared
to the group who consumed only alcohol with their meal. Ceriello et al., (2001), carried out a similar work among T2D patients and confirmed that red wine intake during a meal decreased absorptive phase free radicals, increased serum antioxidants and reduced LDL oxidation and thrombotic activation.

Some antioxidant systems may be micronutrients or depend on micronutrients for their function. Vitamin E and carotenoids stimulate cell-mediated and humoral immunity, and vitamin C scavenges free radicals (Neuhouser et al., 2001; Evans and Halliwell, 2001). Superoxide dismutase, glutathione peroxidase and catalase depend on manganese, selenium and heme respectively for their function (Evans and Halliwell, 2001). Synthesis of proteins and antioxidants such as albumin and glutathione require essential amino acids from the diet. The synergistic effects of various antioxidants may bring about a cumulative protective effect compared to any single antioxidant. In the healthy individual, the balance between reactive oxygen, nitrogen and chloride species on one hand, and antioxidants on the other, tips in favor of the reactive species such that these species are able to perform their biological functions without inducing risk factors associated with degenerative diseases such as insulin resistance, T2D and cardiovascular diseases (Evans and Halliwell, 2001). In the T2D state, this balance is lost such that excessive reactive species in the blood results in oxidative stress. Also, under obese conditions, decreased antioxidant defenses such as lower blood levels of carotenoids, alpha-tocopherol, serum retinol and other micronutrients result in
increased oxidative stress when compared to normal subjects (Neuhouser et al., 2001; Mohanty et al., 2000).

Darko et al., (2002), found that treatment with Vitamin C (1.5g daily) for 3 weeks did not significantly improve oxidative stress, blood pressure or endothelial function in T2D patients. However, Eriksson and Kohvakka, (1995) found beneficial effects of supplementing 2g of Vitamin C daily. Other supplementation studies with Vitamin E found significant improvements in serum glucose, total cholesterol and decreased low density lipoproteins (Paolisso et al., 1993) and improved beta-cell function, increased plasma insulin and C-peptide levels (Gokkusu et al., 2001).

Bertelli et al., (1996), measured cis- and trans-resveratrol in rats after administration of 6.5mg/L of total resveratrol for 15 days. Plasma, urine and tissue levels indicated low levels that were not pharmacologically significant. The authors attributed this finding to the short period of study. In another study, Van Velden et al., (2002) fed red and white wine (32g and 23g alcohol for males and females respectively) to healthy subjects. The researchers observed increased total antioxidant capacity during red wine consumption but not white wine.

Red wine has been extensively investigated due to the French paradox (Belleville, 2002). The French paradox relates to the evidence that the
mortality rate from heart disease was significantly lower in France than America despite consumptions of diets rich in comparable amounts of saturated fats (Zilkens and Puddey, 2003; Belleville, 2002). Some authors attributed this paradox to consumption of smaller portion sizes with fewer calories, and longer eating time of the French compared to the Americans (22.2 mins vs. 14.4 mins respectively) (Rozin et al, 2003). Others attributed the paradox to the intake of red wine, which contained higher amounts of polyphenols than white wine (Renaud and deLorgeril, 1992).

High polyphenolic content of red wines prepared from grapes other than muscadines has been shown to impart higher antioxidant and antiplatelet capability (Maxwell et al., 1994; Kanner et al., 1994; Bertelli et al; 2002) and reduce lipid peroxidation (Durak et al, 1999) in individuals consuming moderate amounts. However, muscadine red grape and wine supplementation trials have received little attention.

The current dissertation therefore investigated whether supplementation of muscadine grape products would have an effect on blood constituents, antioxidant capacity, and erythrocyte membrane lipids of Type 2 diabetic and healthy subjects.
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CHAPTER TWO

MUSCADINE GRAPE PRODUCTS INTAKE, DIET AND MEMBRANE LIPID COMPOSITION OF NON-DIABETIC AND TYPE 2 DIABETIC SUBJECTS
Introduction

Type 2 Diabetes is a condition characterized by a complex interplay of multiple metabolic disorders that include hyperglycemia, hyperinsulinemia, dyslipidemia and abnormalities of the coagulation system.

Hyperglycemia arises from increased glucose in the blood due to decreased glycolysis and impaired insulin sensitivity, altered pancreatic insulin production and release, and reduced expression of glucose transporters. These metabolic activities hinder transport of glucose into the cells, creating a ‘starved state’ condition in the cell. Hepatic and adipose glucose production is therefore accelerated in order to compensate for this condition. This further aggravates the hyperglycemic state (Ruhe and McDonald, 2001). Blood glucose concentration above 110 mg/dl is considered detrimental to health. However glucose concentrations below this cut-off have been observed to increase cardiovascular mortality. The Whitehall, Paris Prospective, and the Helsinki Policemen Studies show that nondiabetic subjects within the upper 2.5% of postprandial glucose levels had significant increases in cardiovascular mortality (Balkau et al., 1998).

High serum insulin concentrations, a characteristic feature of T2D individuals, may result from excessive pancreatic beta-cell insulin output in response to the high glucose levels in the blood. In the healthy person, insulin binding to its receptor facilitates glucose transport into cells. Also, synthesis of glycogen,
triglycerides and proteins from the transported glucose, fatty acids and amino acids respectively are enhanced. In T2D condition, however, the reduced glucose entry into cells and decreased insulin activity are detected by the pancreas as high glucose state (hyperglycemia) and this triggers increased insulin production (hyperinsulinemia) to compensate for the decreased insulin activity, or insulin resistance. Insulin resistance has been estimated to occur in about 25% of the 'non-diabetic' population in the US, almost the same rate as the occurrence of obesity (Cosford, 1999).

Dyslipidemia encompasses metabolic events including hypertriglyceridemia, low HDL-cholesterol and altered LDL-cholesterol composition. High triglyceride levels occur as a result of increased VLDL-cholesterol production that accompanies the insulin-resistant state (Krentz, 2003; Grundy et al., 2002). Also, with impaired insulin activity, adipose tissues increase the production and secretion of free fatty acids for energy production. Liver gluconeogenesis increases and lipoprotein lipase levels are reduced. These lead to impaired clearance of VLDL particles.

Low HDL-cholesterol observed in the dyslipidemic condition may result from decreased incorporation of apoproteins and phospholipids into HDL particles since they are entrapped in the VLDL particles. In addition, there is impaired synthesis of new HDL particles by the liver. Furthermore, increased hepatic
lipase activity enhances the removal of HDL particles from the blood stream resulting in low HDL-cholesterol levels in the blood (Krentz, 2003).

The characteristics of LDL particles in individuals with dyslipidemia are changed from its large, buoyant features to a small, dense particle containing less cholesterol esters and more apo-B particles. These changes increase the susceptibility of LDL to oxidation, nonenzymatic glycation, and increase adherence to and invasion of the arterial wall (Krentz, 2003).

Type 2 Diabetes has been associated with other disease conditions including obesity, hypertension, atherosclerosis and coronary artery diseases. In the United States, the National Center for Health Statistics estimates over 60 % of adults are overweight, and more than 30% are obese. Obesity occurs as a result of an imbalance between energy intake and expenditure, and this imbalance interferes with energy homeostasis, including hormones such as insulin and leptin; both of which regulate food intake and fat stores. Complications of obesity include dyslipidemia, sleep apnea, depression, hypertension, cardiovascular disease, cancer and myocardial infarction (Sowers, 2003).

A causal relationship has been established between obesity, particularly central or visceral obesity, in the presence of physical inactivity, and peripheral tissue insulin resistance. Central obesity is a predisposing factor in the
development of insulin resistance by increasing insulin demand and eventually leading to hyperinsulinemia (Allen et al., 2003). High-energy foods and diets rich in highly processed carbohydrates coupled with decreased physical activity also predispose individuals to obesity and insulin resistance. In addition, energy-rich carbohydrate diets increase plasma triglyceride levels by inhibiting the activity of lipoprotein lipase, compared to diets composed of higher percentage of dietary fat (Grundy et al., 2002). Regular, vigorous walking has been shown to result in a 36% decrease in insulin/glucose ratio with reduced endogenous insulin requirements and lowered fat stores (Cosford, 1999). Also, diets high in saturated fats and trans-fatty acids have been shown to decrease membrane fluidity and decrease insulin receptor binding, thus promoting insulin resistance (Cosford, 1999). Other studies have shown that with increased omega-6 to omega-3 ratio, there is substantial increase in cardiovascular diseases, hypertension, T2D and obesity (Simopoulos, 1999).

In addition to these energy and macronutrient factors, various micronutrients have been implicated in the development of cellular insulin resistance. Several micronutrients including calcium, magnesium, and sodium have been proposed as contributing factors. Some researchers also believe that most of the dietary and lifestyle factors that increase insulin resistance also induce a calcium/magnesium imbalance intracellularly. Magnesium is a vital mineral required for the proper functioning of over 300 enzymes and is involved in
glucose homeostasis by affecting insulin secretion and action. Low intracellular magnesium, often observed in diabetic patients, results in impairment of insulin action and a worsening of insulin resistance in hypertension and NIDDM. Supplementation of diets with magnesium, zinc, vitamin E and vitamin C has yielded positive benefits on the lipid profile (Favid et al., 2004) and glycemic indices (Song et al., 2004).

Chromium supplementation studies indicate inverse relationship with insulin sensitivity (Ghosh et al., 2002) and glycemic indices (Anderson et al., 1997) but its use is still controversial (Brown, 2003). Dietary fiber contained in processed foods such as guar gum, beet fiber and soy polysaccharides, and in unprocessed foods such as fruits, legumes and whole grains are known to lower blood cholesterol levels, slow down absorption of glucose to benefit postprandial glucose metabolism, and provide long term glucose control in T2D patients (ADA reports, 2002).

Increased energy intake and excess reactive oxygen species whose activities result in oxidative injury have been implicated in the development of cardiovascular diseases (Castelli, 1998; Kaplan and Aviram, 1999), diabetes (Vendemiale et al., 1999) and cancer (Ames et al., 1995; Willett, 2001). Increasing the serum antioxidant status has been proposed as a preventive measure to reduce the development of these diseases (Kay and Holub, 2002; Whitehead et al., 1995). Antioxidants are abundant in foods including whole
grains (e.g. wheat, oatmeal, and brown rice); legumes (e.g. lentils, beans, split peas); oils from corn, olive, soybean, safflower; dark orange, red, yellow and green vegetables, and fruits such as prunes, raisins, berries, and grapes.

Grape and grape products, especially red wine have been extensively studied for their antioxidant properties (Mattavi, 2002; Donovan et al., 1999; Ceriello et al., 1997). Also, the belief that polyphenols present in red wine may contribute to low mortality rate observed among the French (i.e. the French paradox) compared to Americans despite comparable intakes of high saturated fats increased research interest in this area (Belleville, 2002).

The use of muscadine grapes for fruits, juices and wines as part of daily food intake in order to harness their beneficial properties including antioxidant and antiplatelet abilities, as well as reduced lipid peroxidation have not received much attention. Therefore the objectives of this research were to investigate the effects of the phenolic properties in muscadine juice, wine and dealcoholized wine on the lipid profile, blood pressure, anthropometric indices and nutrient intakes of Type 2 Diabetic compared to non-diabetic individuals.

**Materials and Method**

**Subject recruitment and supplement administration**
The Institutional Review Board of North Carolina State University approved this research plan. All subjects voluntarily agreed to participate and informed consent was received from each participant. Subjects were recruited from local medical clinics and by word of mouth. Baseline information was collected and this included age, weight, height, blood pressure, list of medications, duration of diabetes and blood samples. The subjects were assigned to various groups. Type 2 diabetics (n=29) were assigned to consume 150 ml of Muscadine Juice (DJ, n=10), Muscadine Wine (DW, n=10), or Dealcoholized-wine (Dz-W, n=9) after dinner for a period of 28 days. Non-diabetic subjects were randomly assigned into the following categories: Muscadine Juice group (CJ, n = 8) and control group without supplementation (CS, n=15). Type 2 diabetic subjects on muscadine wine were regular wine consumers and were asked to substitute their wine intake with muscadine wine.

Muscadine juice and the wine were obtained from the Duplin Winery (Rose Hill, NC). The juice was filtered, pasteurized (180 °F for 1sec) and bottled under aseptic conditions. The wine was also filtered and bottled. Dealcoholized wine was prepared using the reverse osmosis system, which comprised a Venocon reverse membrane housing (JB Systems, LaGrange, GA) containing a Nanomax 95 reverse osmosis spiral module membrane (Millipore Corp, Bedford, MA). Separation was achieved based on the molecular size of the alcohol and the polyphenols. Alcohol had smaller molecular size compared to the polyphenols, and so the alcohol fraction, in
addition to some amount of water, passed through the filtration membrane and was collected separately. A similar volume of water extracted with the alcohol fraction was added back to the sample. This was then pasteurized and bottled.

Baseline and post-treatment data were collected on all subjects. Subjects were told to follow their daily dietary and physical activity habits throughout the study period.

**Anthropometric determination**

Body Mass Index (BMI) of subjects was computed from the ratio of their weight in kilograms to the square of their height in meters. Body Mass Index between 18.5 – 24.9 kg/m\(^2\) was classified as normal weight, 25 – 29.9 kg/m\(^2\) as overweight and over 30 kg/m\(^2\) as obese. Waist circumference was also measured for all the subjects. The distance around the abdomen at the level of the belly button (navel) was taken with a flexible tape while the subject was comfortably standing. The waist circumference gives an indication of cardiovascular risk. Waist circumference ≤ 102cm for men and ≤ 88cm for women are considered normal while values above these are considered overweight and increased cardiovascular risk.

**Diet Analysis**

A 3-day diet record consisting of 2 weekdays and a weekend day was obtained from each subject and the beginning, middle and end of the study.
Food models were used to enhance accurate quantification of the foods eaten. The diet information was analyzed using Nutritionist Pro (First Data Bank, Inc., San Bruno, CA). The analysis from the software included calorie intake, macronutrients (carbohydrates, proteins, fats – in addition to fatty acids and cholesterol), and comprehensive micronutrient data comprising vitamins and minerals.

**Blood sample collection and analysis**

**a) Erythrocyte membrane fatty acids**

Blood samples were collected in vacutainer tubes (Becton Dickinson, NJ) for various hematological analyses. Vacutainer tubes containing anticoagulant sodium heparin were used for collection of blood samples for erythrocyte membrane lipid determination. Blood samples were centrifuged and the plasma separated into aliquots. The packed cells were washed with 0.9% saline solution and brought up in an equal volume of the saline solution. A modified Folch procedure (Folch, 1956) was used for the lipid extraction. Briefly, 2ml aliquot of red blood cells were homogenized after addition of 20 ml of 2:1 chloroform: methanol prepared with 0.05% 3, 5 – di-tert-butyl-4, hydroxytoluene (BHT). This was filtered after which 0.08% KCL was added. The mixture was shaken thoroughly and allowed to separate for 2 hours. The supernatant was aspirated and the lower layer filtered with glass fiber filter (Fisher Scientific, Pittsburgh, PA) and dried under nitrogen. The dried sample was brought up to 5 ml with chloroform and 2 ml of this was passed through
the Sep – pak silica cartridges (Waters Associates, Inc., Milford, MA) for separation of the non-polar fraction (using hexane and acetone) from the polar fraction (using methanol).

The non-polar fraction was then saponified using sodium hydroxide dissolved in methanol at a temperature of 80°C. The saponified mixture was later esterified to form methyl esters, and cleaned up in preparation for Gas chromatography analysis. The Hewlett Packard 5890 GC (Avondale, PA, USA) attached to a computer (Dell, Round Rock TX, USA) using Chrom - Perfect Software (Justice Innovations, CA, USA) was used for initial data collection and storage subsequent to integration. The conditions of the Gas chromatography and column were the same as described by Boyd et al., (1999). The GC was set up to permit the detection of fatty acids ranging from 12 – 24 carbon chain saturated, monounsaturated and polyunsaturated fatty acids. Fatty acid standards (Nu check Prep, Inc. Elysian, MN) of known composition were prepared and run initially to establish retention times and response factors for all fatty acids identified. Total percentage fatty acid composition was used as an index of the amount of fatty acids present, and these fatty acids (Appendix 1) were grouped into saturated, mono- and polyunsaturated fatty acids.

b) Lipoproteins
Additional blood samples were collected into red-top vacutainers tubes (serum separator tubes). This was allowed to stand for 30 mins and centrifuged for 15 min using the Vanguard V6000 centrifuge (Becton Dickinson, Rutherford, NJ). The serum was then separated from the packed cells and delivered on ice (within one hour) to VA Medical Center, Durham, NC for various analyses including lipid profile comprising total cholesterol, triglycerides, low density lipoprotein cholesterol and high density lipoprotein cholesterol.

**Statistics**

Statistical analysis was carried out using SAS 8.2 software (SAS, Cary, NC, USA). Pooled analysis eliminating gender differences was carried out (except for waist circumference) since there were similarities in the measured parameters. Group means, standard deviation and standard errors were computed. Paired Student T-test was carried out to compare differences between initial (pre) values and post supplementation data for each group. Analysis of covariance using the general linear model procedure was applied to determine significance of preplanned comparisons between the main effects and the various treatments after adjusting for differences in baseline covariates, where the values prior to supplementation was the covariate. Probability (P) values less than 0.05 were considered statistically significant.
Results

All control subjects were overweight with average BMI range between 27.5 and 29.6 kg/m², and all the T2D subjects were obese with mean BMI range between 35.4 and 41.0 kg/m² (Table 1). Average waist circumference for T2D males and females, and Control females on Juice indicated overweight status, while control male subjects were within normal weight. As expected, systolic and diastolic blood pressures were similar for the control subjects but higher for the T2D subjects. Type 2 diabetic subjects taking juice recorded the greatest increase post supplementation compared the control subjects without supplementation, with a 26.6 mmHg increased systolic pressure and 11.2 mm Hg increase in diastolic blood pressure. These differences were however not statistically significant.

The average 3-day macronutrient diet data are presented in Table 2. A higher energy intake was observed for the control subjects compared to the T2D but this was not statistically significant. Type 2 Diabetic subjects taking muscadine juice reported the highest cholesterol intake compared to the other groups. Linoleic acid (PFA 18:2) intake was significantly lower for the T2D group taking muscadine wine (DW) compared to Dealcoholized wine (Dz-W) group. Dietary fiber intake was significantly lower for Diabetics taking Juice (DJ) and Diabetics taking Wine (DW) compared to the other groups. Carbohydrates, fats and proteins computed as percentages of total calorie intake showed 50% of calorie intake from carbohydrates for the control
subjects while T2D had 42% (Dz-W) and 44% (DJ and DW). Type 2 diabetics taking juice and dealcoholized wine also had higher percent fat calorie intakes compared to control subjects (Figure 1).

The average amount of minerals present in the 3-day dietary recall is presented in Table 3. Compared to Recommended Daily Intake (RDI) values, sodium, calcium, and selenium needs were met by the study population. About 50% of the RDI intakes were met for iron, zinc and manganese while molybdenum, chromium and fluoride were much lower than RDI. Also, significantly lower values were observed for magnesium and manganese between the DJ group (154 ±16 mg and 1.1 ± 4 mg) and the DW group (150 ± 14 mg and 1.2 ± 0.1 mg) compared to the Dz-W group (237 ± 46 mg and 2.4 ± 0.6 mg) respectively. Chromium intakes were significantly lower among the DJ group (15 ± 4 ug) and DW (18 ± 3 ug) compared to the Dz-W group (41 ± 10 ug).

Average dietary vitamin intake was significantly higher for α-carotene among the DW group compared to Dz-W subjects and the controls (Table 4). Trends indicate higher vitamin C and E for T2D taking Dz-W and MW compared to T2D on Juice. Vitamin C was nearly significantly different, with Control subjects taking Juice having higher intakes than T2D taking juice (P = 0.07). Total folate levels were also higher in T2D taking Dz-W as well as T2D taking
MW but not in diabetic Juice group. Compared to the recommended intakes, low biotin levels were recorded for all subjects.

Red blood cell membrane SATs were higher for T2D on Juice and Dz-W than their polyunsaturated fatty acids levels, though this trend was not significant. There was 13.6 % increase in polyunsaturated fatty acids for the diabetics taking wine after the supplementation period (Table 5). Monounsaturated fatty acids decreased for control subjects on Juice but increased for diabetic subjects on wine. The ratio of saturated to polyunsaturated fatty acid indicate lower ratios for control subjects taking juice and T2D subjects taking wine post supplementation (Figure 2).

There was a general trend towards decreased levels of triglycerides, total cholesterol (TC), low density lipoprotein (LDL) cholesterol and high density lipoprotein cholesterol (HDL) post supplementation. Type 2 diabetics taking wine observed the greatest decreases, with 18.0 mg/dl decrease in triglycerides, 18.6 mg/dl for total cholesterol and 12 mg/dl for low density lipoprotein cholesterol. The decrease in HDL-cholesterol was significant between DJ at baseline (41.0 ± 2.3 g/dl) and after the supplementation period (38.4 ± 1.9 g/dl) (Table 6). Total cholesterol to HDL-cholesterol ratio, an indicator of cardiovascular risk, was borderline risk for diabetic subjects compared to the controls (Figure 2).
Discussion

This work investigated the effect of supplementation with muscadine juice, wine and dealcoholized wine on blood pressure, anthropometric indices, nutrient intake, lipid profile and membrane fatty acid composition of T2D and control subjects. The number of subjects, age and duration of diabetes are presented in Table 1. Body mass index (BMI), coupled with waist circumference measurements, are known to predict obesity-related health risk better than BMI alone (Janssen et al, 2004; Kurpad et al, 2003). Type 2 diabetic subjects in this study were overweight. The BMI for the control subjects who volunteered for this study indicated overweight status. This is significant, considering the fact that increase in body weight precedes obesity, insulin resistance and other attendant adverse conditions. Though BMI is a good tool for obesity assessment, the distribution of body fat, specifically central or upper body fat has been more strongly associated with health risk (Hermansen, 2000). Waist circumference, an indicator of central obesity predicted greater health risk for T2D subjects as well as female control subjects on muscadine juice. Weight reduction and physical activity have proved beneficial in various ways. Physical activity can improve glucose tolerance, antioxidant capacity and reduce insulin resistance among obese individuals (Kelley and Goodpaster, 1999). In addition, insulin sensitivity in skeletal muscles and fat tissues are improved, thus reducing fasting glucose and insulin levels (Hughes et al., 1993).
In this study, increased blood pressure observed among the diabetic subjects compared to the control subjects without supplementation indicates a higher risk of hypertension. Weight reduction through physical activity improves blood pressure as well. Staessen et al., (1988) observed that each 1 kg decrease in body weight was associated with 1.2 and 1.0 mm Hg reduction in systolic and diastolic blood pressures respectively. Also, a combination of weight loss and sodium reduction has additive effects (Whelton et al., 1998).

Kirpichniko and Sowers, (2001) estimated that 35 – 75% of diabetic complications is related to hypertension. Also, the chance of developing hypertension is doubled in patients with diabetes or impaired glucose tolerance. Each 10 mm Hg rise in systolic blood pressure is associated with a 15% increase in the risk of death for diabetics. The rise in blood pressure among patients suffering from myocardial infarction, stroke and congestive heart failure increases their risk of death by 11%, 19% and 13% respectively.

Protein intake is required for normal development, growth and maintenance of body functions. In the United States, it is recommended that the average daily value of protein consumption should be 10% of energy requirement. However, US dietary protein intake ranges between 14 and 18% of total energy (NRC, 1989). This is consistent with the present study, where average range between 18 and 21% of the total energy intake (Figure 1) was observed. Although some authors suggest that low-protein diets for T2D may prevent or slow down the progression of renal failure, studies on the protective role of
low-protein diets on T2D patients without renal diseases have not yielded positive results (Horton and Napoli, 1996). Other researchers established that dietary protein restriction to the recommended level of daily requirement reduces postabsorptive and average daily blood glucose concentration without altering metabolism in T2D (Hoffer et al., 1998).

High carbohydrate diets (50 – 60% of energy) have been implicated in increasing postprandial glucose and triglycerides. Parillo et al., (1988) observed an accentuation of hyperglycemia and hyperinsulinemia after feeding a high-carbohydrate, low-fat meal to hypertensive subjects. A similar observation, in addition to increased postprandial accumulation in triglyceride-rich lipoproteins and postheparin lipoprotein lipase activity, was observed among T2D patients (Chen et al., 1995). Some authors proposed increased monounsaturated fats instead of carbohydrates to avoid adverse effects of high-carbohydrate diets. A study investigated this by assigning T2D subjects to 3 diets: a weight management diet, high-carbohydrate/ high fiber diet and a modified-lipid diet for 18 months. The study found that though there was reduced low density lipoprotein in the high-carbohydrate/ high fiber diet after 9 months, no lasting differences were found among the three diets (Milne et al., 1994).

Recommended intakes of fat not exceeding 30% of total energy, with 10% from saturated fats, have been proposed (Horton and Napoli, 1996). The
present study found moderate carbohydrate intake and high fat levels for T2D (Figure 1). Both control and diabetic subjects ingested over 30% of their energy from fat, with SAT constituting over 35% of the total fat intake. These data suggest that membrane fluidity and protein lateral mobility may be compromised since high levels of SAT enhance rigidity of the cell membranes and hinder free protein movement within the membrane (Kamada et al., 1992; Winocour et al., 1990). This condition would worsen the insulin resistance state and increase the risk of T2D for the control subjects and aggravate the T2D condition. High MUFA diets have, however, been beneficial to glycemic control. A study comparing high monounsaturated fat (MUFA) diet (50% of fat with 33% from MUFA) and high carbohydrate diet found that during the high fat supplementation, T2D subjects had lower plasma glucose and reduced insulin, though the total cholesterol, and LDL-cholesterol did not differ significantly (Garg et al., 1988). Considering the various individual differences in a complex metabolic syndrome such as T2D, individual monitoring and treatment plans designed towards specific nutritional and weight management goals are recommended.

The current study found low fiber intakes in all the subjects, especially the DJ and DW groups. Revillese et al., (1980) found that a high-fiber, normal-carbohydrate diet improved blood glucose control and decreased the concentration of atherogenic lipoproteins in diabetic patients. In another study, Salmeron et al., (1997) showed that cereal fiber was inversely associated with
risk of T2D, and the combination of a high glycemic load and a low cereal fiber intake further increased the risk of developing T2D.

Magnesium deficiency has been associated with insulin resistance and glucose homeostasis, as well as complications such as retinopathy, thrombosis, hypertension and T2D (Walti et al., 2002). Nadler et al., (1993), showed that dietary-induced magnesium deficiency increased thromboxane urinary concentration and enhanced angiotensin-induced aldosterone synthesis. Other studies indicated a relationship between elevated intracellular free calcium and reduced free magnesium in hypertension linked to obesity (Resnick, 1992). In the present study, magnesium levels were significantly higher for T2D taking Dz-W than T2D taking MW (Table 3). This higher value, in addition to those of the control subjects, was barely half the amount of recommended magnesium intake. These low values could worsen glucose tolerance and impair the activities of several magnesium-induced enzymes. Dietary magnesium supplements (400mg/day) were found to improve glucose tolerance in elderly individuals (Paolisso et al., 1992). The software used for diet analysis in this study estimated magnesium contributions of 16 mg, 16.4 mg, and 15 mg from red grape juice, wine and dealcoholized wine respectively (Appendix 2). Rich sources of magnesium include whole grain cereals, legumes and nuts.
Chromium is an essential trace element required for regulation of lipid and carbohydrate metabolism (Brown, 2003). Animal experimentation studies show that a deficiency in chromium can result in insulin resistance (Striffler et al., 1999). Evidence suggests that chromium supplementation improves glycemic control, enhances insulin sensitivity (Ghosh et al., 2002), and lowers triglyceride levels (Kim et al., 2002). Also, chromium supplementation promotes insulin-receptor binding, increases insulin receptor number and activates insulin receptor kinase activity (Shrivastava et al., 2002). These beneficial effects are observed at levels higher than the upper recommended limit. In our present study, chromium intake for control subjects was about one-third of RDI, with substantially lower values recorded for DJ and DW. Poor database for chromium intake from foods may contribute to the low values observed. Also, altered gastrointestinal function and polyuria may reduce absorption and enhance rapid excretion of chromium and other nutrients. With inadequate intake coupled with increased urinary excretion of nutrients, worsening the insulin resistance state in diabetic subjects and increased risk of developing insulin resistance in control subjects may ensue. Foods rich in chromium include foods from vegetables, fruits, meats and grain groups.

The current study found decreased levels of vitamin C in the DJ group, though all the other groups had higher vitamin C levels. Vitamin E intake was also lower in only the DJ group. Paolisso et al., (1994) demonstrated that vitamin E
improved insulin sensitivity in short-term supplementation trials. Vitamin E has also been shown to inhibit hyperglycemia-induced diacyl-glycerol protein kinase C pathway (Bursell et al., 1999). Another vitamin extensively studied, vitamin C, is known to act as an aldose reductase inhibitor of the hyperglycemia-induced polyol pathway (Millen et al., 2004). Vitamin C is required for neurotransmitter and collagen synthesis. Several prospective studies found a significant relationship between vitamin C intake and cardiovascular diseases (Carr and Frei, 1999). Also, vitamins C and E have been shown to reduce advanced glycosylated end products in normal rats, and reduced blood glucose and glycated hemoglobin in diabetic rats (Wan Nazaimoon and Khalid, 2002). As antioxidants, vitamins C and E defend against the adverse effects of reactive oxygen species thus alleviating oxidative stress and injury in conditions such as T2D (Millen et al., 2004). The reduced levels of vitamins C and E could indicate a compromised antioxidant capacity due to the diabetic state.

Biotin is required as a cofactor in gluconeogenesis and lipogenesis. Biotin deficiency results in impaired utilization of glucose (Zhang et al., 1997), decreased stimulation of glucokinase (McCarty, 1999) and decreased stimulation of insulin secretion (Romero-Navarro et al., 1999). High-biotin diet has been demonstrated to improve glucose tolerance in rats (Zhang et al., 1997) and T2D patients (Coggeshall et al., 1985). Dietary biotin below 50% of recommended intake observed in our study might probably aggravate the
insulin resistance state in T2D subjects as well as impair glucose homeostasis in healthy subjects. Rich sources of biotin include egg yolk, yeasts, organ meats and whole grains.

The fatty acid profile of the diet is known to influence the fatty acid composition of stored and structural lipids in different body compartments such as serum lipids and erythrocyte membranes (Anderrson et al., 2002). Research has shown that the fatty acid composition can provide significant correlates of insulin resistance (Felton et al., 2004). This is especially important where increased saturated fatty acid content and increased insulin resistance occur. The converse is observed for polyunsaturated fatty acid (PUFA); increased PUFA is associated with reduced level of insulin resistance. In our study, only the control group taking juice (CJ) and T2D taking wine (DW) had increased PUFA levels after the supplementation period (Table 5). There was generally higher percentage of fats as PUFA present in the erythrocyte membrane compared to that in the diet (Table 2). This suggests that desaturase and elongase enzymes are actively converting SAT and MUFA to PUFA in membranes such as the erythrocyte. The activities of these enzymes have been associated with variation in insulin sensitivity or with insulin itself (Felton et al., 2004). High amounts of erythrocyte membrane SAT especially among diabetic subjects are consistent with their dietary intake data and may be a reflection of poor membrane fluidity. Increased insulin resistance and decreased membrane fluidity have been reported by several investigators.
(Vessby et al., 2002; Felton et al., 2004). Olive oil, fish oil and canola oil are rich sources of unsaturated fatty acids.

Dyslipidemia, characterized by high triglyceride levels, increased small low-density lipoprotein particles and low HDL-cholesterol is a common feature in T2D individuals (Grundy et al., 2002). These features were observed in our study. In addition, there was decreased triglycerides and total cholesterol level after the supplementation period, though this was not significant. The greatest decrease was observed among Type 2 diabetics taking wine, with 18.0 mg/dl decrease in triglycerides, 18.6 mg/dl for total cholesterol and 12 mg/dl for low density lipoprotein cholesterol. These decreases suggest improved lipid profile among the wine group compared to the other groups. Pre and post supplementation differences in HDL level was not significant for almost all the groups except for the significant decrease in HDL level observed in T2D group taking juice (DJ) after the study period (Table 6). Elevated plasma triacylglycerol and reduced HDL-cholesterol are associated with insulin resistance (Zoratti et al., 2000) and increased risk of heart disease, a major cause of morbidity and mortality in T2D patients (Goh et al., 1997). Total cholesterol and HDL-cholesterol ratio is an indicator of cardiovascular disease risk (Figure 2). This ratio above 5 indicates increased risk. None of the subjects were at high risk, though, as expected, T2D had higher risk compared to control subjects.
In conclusion, all control and diabetic subjects were overweight or obese. Coupled with low reported chromium and biotin intake, insulin efficiency in stimulating glucose uptake may be impaired. Trends of elevated dietary vitamin C and E observed in the diabetic groups on dealcoholized wine may indicate improved antioxidant status. Also, trends of lowered triglycerides, total cholesterol and low density lipoprotein cholesterol observed in T2D taking dealcoholized wine and T2D taking muscadine wine post supplementation suggests improved lipid metabolism. Finally, indicators measured suggest that T2D taking MW and Dz-W responded better to the muscadine products compared to the T2D on juice.

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McCarty MF. 1999. High-dose biotin, an inducer of glucokinase expression, may synergize with chromium piconilate to enable a definitive nutritional therapy for type 2 diabetes. Medical Hypotheses. 52 (2): 401 - 406


Zoratti R, Godsland IF, Chaturvedi N, Crook D, Stevenson JC, McKeigue PM. 2000. Relation of plasma lipids to insulin resistance, nonesterified fatty acid levels, and body fat in men from three ethnic groups: Relevance to Variation in Risk of Diabetes and Coronary Disease. Metabolism. 49 (2): 245 - 252
Table 1: Average Body Mass Index (BMI), Waist Circumference and Blood Pressure of Control and Diabetic Subjects at Baseline and Post Supplementation

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects (CS)</th>
<th>Control Juice (CJ)</th>
<th>T2D Juice (DJ)</th>
<th>T2D Dz. Wine (Dz-W)</th>
<th>T2D Wine (DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Subjects (M/F)</td>
<td>15 (8/7)</td>
<td>8 (3/5)</td>
<td>10 (3/7)</td>
<td>9 (3/6)</td>
<td>10 (10/0)</td>
</tr>
<tr>
<td>Age (y)*</td>
<td>56 (7.5)</td>
<td>50 (13)</td>
<td>56 (8)</td>
<td>60 (12)</td>
<td>60 (15)</td>
</tr>
<tr>
<td>T2D duration (y)*</td>
<td>NA</td>
<td>NA</td>
<td>8.8 (7)</td>
<td>11.0 (10)</td>
<td>7.3 (6)</td>
</tr>
<tr>
<td>Waist Circ. (cm) Pre (M/F)</td>
<td>89.6/83.9</td>
<td>97.6/89.5</td>
<td>100.2/121.9</td>
<td>107.3/101.8</td>
<td>112.4/0</td>
</tr>
<tr>
<td>Post (M/F)</td>
<td>89.7/84.1</td>
<td>95.5/93.4</td>
<td>104.5/118.7</td>
<td>104.1/98.2</td>
<td>110.8/0</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>27.5 (1.4)</td>
<td>29.3 (1.4)</td>
<td>40.6 (3.5)</td>
<td>35.5 (4.0)</td>
<td>35.4 (1.7)</td>
</tr>
<tr>
<td>Post</td>
<td>27.9 (1.3)</td>
<td>29.6 (1.4)</td>
<td>41.0 (3.8)</td>
<td>35.9 (3.9)</td>
<td>35.7 (1.7)</td>
</tr>
<tr>
<td>Systole (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>123.1 (4.3)</td>
<td>127.8 (5.1)</td>
<td>153.4 (7.2)</td>
<td>140.0 (5.0)</td>
<td>141.7 (7.2)</td>
</tr>
<tr>
<td>Post</td>
<td>121.7 (4.6)</td>
<td>124.0 (4.4)</td>
<td>148.3 (8.8)</td>
<td>140.8 (8.1)</td>
<td>143.7 (4.8)</td>
</tr>
<tr>
<td>Diastole (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>71.1 (2.4)</td>
<td>76.8 (3.2)</td>
<td>85.9 (4.5)</td>
<td>79.4 (4.2)</td>
<td>75.7 (2.8)</td>
</tr>
<tr>
<td>Post</td>
<td>72.3 (2.5)</td>
<td>78.0 (4.8)</td>
<td>83.5 (4.8)</td>
<td>77.6 (2.5)</td>
<td>75.6 (2.0)</td>
</tr>
</tbody>
</table>

Values are means (standard error in parenthesis)

*Values are means (standard deviation in parenthesis)
Table 2: Average Macronutrient Intake Among Groups

<table>
<thead>
<tr>
<th></th>
<th>Daily Value</th>
<th>Control Subjects (CS)</th>
<th>Control Juice (CJ)</th>
<th>T2D Juice (DJ)</th>
<th>T2D Dz. Wine (Dz-W)</th>
<th>T2D Wine (DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (Kcal)</td>
<td>2000-2500</td>
<td>1601.3(96.7)</td>
<td>1743.4(161.1)</td>
<td>1448.0(110.9)</td>
<td>1541.2(195.6)</td>
<td>1407.1(154.1)</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>50 – 65</td>
<td>75.4(4.1)</td>
<td>74.7(5.5)</td>
<td>72.5(7.1)</td>
<td>71.2(10.2)</td>
<td>69.5(6.6)</td>
</tr>
<tr>
<td>CHO (g)</td>
<td>300 – 375</td>
<td>200.4(20.0)</td>
<td>217.6(25.9)</td>
<td>161.4(24.1)</td>
<td>163.3(22.4)</td>
<td>148.5(18.1)</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>65 – 80</td>
<td>54.6(4.1)</td>
<td>59.5(6.6)</td>
<td>57.8(4.5)</td>
<td>70.1(13.1)</td>
<td>52.4(9.4)</td>
</tr>
<tr>
<td>Chol (mg)</td>
<td>300</td>
<td>266.5(27.1)</td>
<td>284.7(63.6)</td>
<td>326.5(60.5)</td>
<td>286.1(52.2)</td>
<td>261.3(35.4)</td>
</tr>
<tr>
<td>SAT (g)</td>
<td>20 – 25</td>
<td>17.2(1.2)</td>
<td>19.4(2.2)</td>
<td>18.5(1.5)</td>
<td>19.6(3.1)</td>
<td>15.4(2.7)</td>
</tr>
<tr>
<td>MUFA (g)</td>
<td>20 – 25</td>
<td>16.7(1.8)</td>
<td>20.6(3.1)</td>
<td>19.2(1.6)</td>
<td>24.3(6.6)</td>
<td>12.1(4.2)</td>
</tr>
<tr>
<td>PUFA (g)</td>
<td>25 – 30</td>
<td>10.3(1.2)</td>
<td>10.9(2.1)</td>
<td>9.7(1.0)</td>
<td>14.5(4.0)</td>
<td>12.1(4.2)</td>
</tr>
<tr>
<td>PFA 18:2 (g)</td>
<td>NE</td>
<td>5.3(1.0)</td>
<td>6.0(1.2)</td>
<td>6.4(1.0)</td>
<td>10.2(4.0)</td>
<td>4.5(0.6) ¶</td>
</tr>
<tr>
<td>PFA 18:3 (g)</td>
<td>NE</td>
<td>0.43(0.1)</td>
<td>0.52(0.1)</td>
<td>0.62(0.1)</td>
<td>0.57(0.1)</td>
<td>0.43(0.1)</td>
</tr>
<tr>
<td>T. Diet. Fib (g)</td>
<td>20 – 25</td>
<td>14.2(1.5)</td>
<td>13.5(2.3)</td>
<td>9.4(1.3) ¥</td>
<td>14.7(2.1)</td>
<td>10.4(1.0) ¥</td>
</tr>
</tbody>
</table>

Values are means (standard error)

¶ Statistically significant difference: P < 0.05, Dz vs. DW.
¥ Statistically significant difference: P < 0.05, DJ and DW vs. CS, CJ, and DZ.

CHO = Carbohydrate, SAT = Saturated fatty acid, MUFA = Monounsaturated fatty acids, NE = None Existent, PFA 18:2 = Linoleic Acid, PFA 18:3 = Linolenic acid, T. Diet. Fib. = Total dietary fiber
Table 3: Average Dietary Mineral Intake of Control and Diabetic Groups

<table>
<thead>
<tr>
<th>Daily Value</th>
<th>Control Subjects (CS)</th>
<th>Control Juice (CJ)</th>
<th>T2D Juice (DJ)</th>
<th>T2D Dz. Wine (Dz-W)</th>
<th>T2D Wine (DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium (g)</td>
<td>2.4</td>
<td>2.2(0.2)</td>
<td>2.3(0.4)</td>
<td>2.5(0.3)</td>
<td>2.1(0.3)</td>
</tr>
<tr>
<td>Potassium (g)</td>
<td>3.5</td>
<td>2.0(0.1)</td>
<td>2.2(0.3)</td>
<td>1.7(0.2)</td>
<td>1.9(0.2)</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>1000</td>
<td>470(47)</td>
<td>410(76)</td>
<td>420(74)</td>
<td>530(79)</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>18</td>
<td>12(0.8)</td>
<td>12(0.9)</td>
<td>11(1.0)</td>
<td>11(1.0)</td>
</tr>
<tr>
<td>Phosphorus (mg)</td>
<td>10</td>
<td>876(62)</td>
<td>888(100)</td>
<td>829(80)</td>
<td>1008(130)</td>
</tr>
<tr>
<td>Magnesium (mg)</td>
<td>400</td>
<td>202(16)</td>
<td>217(25)</td>
<td>154(16)§</td>
<td>237(46)</td>
</tr>
<tr>
<td>Zinc (mg)</td>
<td>15</td>
<td>9.0(0.7)</td>
<td>8.6(0.9)</td>
<td>7.8(1.0)</td>
<td>7.8(1.3)</td>
</tr>
<tr>
<td>Copper (mg)</td>
<td>2</td>
<td>0.86(0.07)</td>
<td>0.99(0.14)</td>
<td>0.68(0.09)</td>
<td>1.00(0.19)</td>
</tr>
<tr>
<td>Manganese (mg)</td>
<td>3.5</td>
<td>1.9(0.3)</td>
<td>1.6(0.3)</td>
<td>1.1(0.2)§</td>
<td>2.4(0.6)</td>
</tr>
<tr>
<td>Selenium (mg)</td>
<td>55</td>
<td>64.0(5.0)</td>
<td>70.9(11.1)</td>
<td>66.1(7.1)</td>
<td>59.8(7.5)</td>
</tr>
<tr>
<td>Fluoride (mcg)</td>
<td>2750</td>
<td>224(63)</td>
<td>262(87)</td>
<td>173(64)</td>
<td>242(87)</td>
</tr>
<tr>
<td>Chromium (mcg)</td>
<td>120</td>
<td>36(7)</td>
<td>42(8)</td>
<td>15(4)§§</td>
<td>41(10)</td>
</tr>
<tr>
<td>Molybdenum(mcg)</td>
<td>16.2</td>
<td>9.8(2.3)</td>
<td>15.9(5.4)</td>
<td>10.1(4.1)</td>
<td>10.5(3.7)</td>
</tr>
</tbody>
</table>

Values are means (standard error)

¶ Statistically significant difference: P < 0.05, Dz vs DW.
§ Statistically significant difference: P < 0.05, DJ vs. CJ.
$ Statistically significant difference: P < 0.05, DJ vs. CS.
§§ Statistically significant difference: P < 0.05, DJ vs. DZ.
& Statistically significant difference: P < 0.05, DW vs. CS.
Table 4: Average Dietary Vitamin Intake of Control and Diabetic Groups

<table>
<thead>
<tr>
<th></th>
<th>Rec. Daily Intake (RDI)</th>
<th>Control Subjects (CS)</th>
<th>Control Juice (CJ)</th>
<th>T2D Juice (DJ)</th>
<th>T2D Dz. Wine (Dz-W)</th>
<th>T2D Wine (DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vit. A (RE)</td>
<td>800</td>
<td>616(110)</td>
<td>679(110)</td>
<td>556(120)</td>
<td>749(120)</td>
<td>1160(510)</td>
</tr>
<tr>
<td>β- Carot. (ug)</td>
<td>NE</td>
<td>791(260)</td>
<td>536(260)</td>
<td>1064(420)</td>
<td>1794(750)</td>
<td>1443(770)</td>
</tr>
<tr>
<td>α-Carot. (ug)</td>
<td>NE</td>
<td>78(28)</td>
<td>94(77)</td>
<td>311(185)</td>
<td>59(33)</td>
<td>566(350)¶</td>
</tr>
<tr>
<td>Vit. C (mg)</td>
<td>60</td>
<td>195(107)</td>
<td>95(29)</td>
<td>41(7)</td>
<td>71(14)</td>
<td>67(16)</td>
</tr>
<tr>
<td>Vit. D (ug)</td>
<td>5</td>
<td>1.2(0.4)</td>
<td>1.8(0.8)</td>
<td>1.0(0.2)</td>
<td>1.0(0.2)</td>
<td>1.3(0.3)</td>
</tr>
<tr>
<td>Vit. E (mg)</td>
<td>8</td>
<td>5.5(1.4)</td>
<td>5.9(1.1)</td>
<td>3.4(0.4)¶</td>
<td>8.2(2.1)</td>
<td>6.8(2.0)</td>
</tr>
<tr>
<td>Thiamin (mg)</td>
<td>1.5</td>
<td>1.2(0.1)</td>
<td>1.3(0.2)</td>
<td>1.0(0.1)</td>
<td>1.3(0.2)</td>
<td>1.1(0.2)</td>
</tr>
<tr>
<td>Riboflav. (mg)</td>
<td>1.7</td>
<td>1.3(0.1)</td>
<td>1.2(0.2)</td>
<td>1.2(0.1)</td>
<td>1.2(0.1)</td>
<td>1.5(0.3)</td>
</tr>
<tr>
<td>Niacin (mg)</td>
<td>20</td>
<td>18.2(2.0)</td>
<td>20.2(1.9)</td>
<td>17.1(1.9)</td>
<td>20.3(5.3)</td>
<td>17.6(1.8)</td>
</tr>
<tr>
<td>Pyridox. (mg)</td>
<td>2</td>
<td>1.4(0.1)</td>
<td>1.5(0.2)</td>
<td>1.2(0.1)</td>
<td>1.3(0.2)</td>
<td>1.2(0.1)</td>
</tr>
<tr>
<td>T. Folate (ug)</td>
<td>400</td>
<td>254(34)</td>
<td>265(44)</td>
<td>191(28)</td>
<td>241(35)</td>
<td>225(30)</td>
</tr>
<tr>
<td>Vit. B₁₂ (ug)</td>
<td>6</td>
<td>3.0(0.6)</td>
<td>2.9(0.5)</td>
<td>3.1(0.5)</td>
<td>2.7(0.4)</td>
<td>6.9(4.0)</td>
</tr>
<tr>
<td>Biotin (ug)</td>
<td>30</td>
<td>13.8(4.7)</td>
<td>12.1(5.7)</td>
<td>8.9(2.3)</td>
<td>9.6(3.1)</td>
<td>7.4(1.8)</td>
</tr>
<tr>
<td>Pant. Acid (ug)</td>
<td>10</td>
<td>3.2(0.5)</td>
<td>3.2(0.5)</td>
<td>2.5(0.3)</td>
<td>3.4(0.7)</td>
<td>3.3(0.9)</td>
</tr>
<tr>
<td>Vit. K (ug)</td>
<td>65</td>
<td>24.4(7.8)</td>
<td>30.3(10.0)</td>
<td>28.3(6.5)</td>
<td>28.0(6.5)</td>
<td>35.6(14.4)</td>
</tr>
</tbody>
</table>

Values are means (standard error)

† Statistically significant difference: P < 0.05, DJ vs. CJ.
& Statistically significant difference: P < 0.05, DW vs. CS.
¶ Statistically significant difference: P < 0.05, Dz vs. DW.

NE = None existent
Table 5: Erythrocyte Cell Membrane Fatty Acids of Control and Diabetic Subjects

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects (CS)</th>
<th>Control Juice (CJ)</th>
<th>T2D Juice (DJ)</th>
<th>T2D Dz. Wine (Dz-W)</th>
<th>T2D Wine (DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAT (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>38.4(3.2)</td>
<td>27.3(4.6)</td>
<td>38.1(5.1)</td>
<td>50.0(5.5)</td>
<td>49.3(5.5)</td>
</tr>
<tr>
<td>Post</td>
<td>46.7(4.6)</td>
<td>29.8(4.0)</td>
<td>40.0(5.1)</td>
<td>51.9(6.2)</td>
<td>30.5(3.6)</td>
</tr>
<tr>
<td>MUFA (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>27.5(2.5)</td>
<td>36.0(7.7)</td>
<td>29.0(3.8)</td>
<td>24.5(4.1)</td>
<td>24.8(2.9)</td>
</tr>
<tr>
<td>Post</td>
<td>31.0(2.8)</td>
<td>25.8(5.7)</td>
<td>29.2(3.3)</td>
<td>23.5(3.5)</td>
<td>30.1(3.3)</td>
</tr>
<tr>
<td>PUFA (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>34.1(4.8)*</td>
<td>36.6(7.8)</td>
<td>32.9(5.1)</td>
<td>25.6(3.3)</td>
<td>25.8(3.1)</td>
</tr>
<tr>
<td>Post</td>
<td>22.2(2.7)</td>
<td>44.4(8.2)</td>
<td>30.8(5.0)</td>
<td>24.5(2.5)</td>
<td>39.4(5.4)</td>
</tr>
</tbody>
</table>

Values are means (standard error)

* Statistically significant difference: P < 0.05, Pre (baseline) vs. Post supplementation

SAT = Saturated fatty acid, MUFA = Monounsaturated fatty acid, PUFA = Polyunsaturated fatty acid
Table 6: Plasma Lipid Profile (and standard error) of Control and Diabetic Subjects at Baseline and Post Supplementation

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects (CS)</th>
<th>Control Juice (CJ)</th>
<th>T2D Juice (DJ)</th>
<th>T2D Dz. Wine (Dz-W)</th>
<th>T2D Wine (DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trig. (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>79.3(14.6)</td>
<td>69.4(12.4)</td>
<td>123.0(28.7)</td>
<td>91.0(19.0)</td>
<td>94.0(9.8)</td>
</tr>
<tr>
<td>Post</td>
<td>78.3(15.4)</td>
<td>62.4(6.4) (¢)</td>
<td>125.5(20.8)</td>
<td>80.0 (14.4)</td>
<td>76.0(11.0)</td>
</tr>
<tr>
<td><strong>T. Chol. (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>172.4(10.9)</td>
<td>183.1(11.0)</td>
<td>194.2(29.0)</td>
<td>174.8(14.5)</td>
<td>187.8(13.0)</td>
</tr>
<tr>
<td>Post</td>
<td>164.6(9.7)</td>
<td>180.1(11.6)</td>
<td>189.6(25.4)</td>
<td>172.8(16.4)</td>
<td>169.2(15.1)</td>
</tr>
<tr>
<td><strong>LDL (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>112.8(8.0)</td>
<td>121.9(7.4)</td>
<td>128.5(23.3)</td>
<td>106.0(9.0)</td>
<td>131.0(10.7)</td>
</tr>
<tr>
<td>Post</td>
<td>106.7(6.2)</td>
<td>122.7(9.0)</td>
<td>128.7(24.4)</td>
<td>98.9 (12.4)</td>
<td>119.1(12.9)</td>
</tr>
<tr>
<td><strong>HDL (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>43.3(2.7)</td>
<td>47.8(5.8)</td>
<td>41.0(2.3)*</td>
<td>39.5(2.9)</td>
<td>36.8(3.8)</td>
</tr>
<tr>
<td>Post</td>
<td>41.8(3.3)</td>
<td>45.6(4.8)</td>
<td>38.4(1.9)</td>
<td>40.8(2.9)</td>
<td>34.2(4.1)</td>
</tr>
</tbody>
</table>

Values are means (standard error)

¢ Statistically significant difference: P < 0.05, Controls (CJ) vs. Diabetic (DJ)
* Statistically significant difference: P < 0.05, Diabetics, Pre (baseline) vs. Post supplementation

Trig. = Triglycerides, T. Chol. = Cholesterol, LDL = Low Density Lipoprotein Cholesterol, HDL = High Density Lipoprotein Cholesterol
Figure 1: Dietary Macronutrient Composition of Study Groups
Figure 2: Erythrocyte Membrane Saturated Fatty Acid to Polyunsaturated Fatty Acid Ratio
Figure 3: Total Cholesterol: HDL-cholesterol Ratio of Groups Before and After Supplementation.
CHAPTER THREE:

EFFECT OF MUSCADINE GRAPE PRODUCTS ON BLOOD CONSTITUENTS OF TYPE 2 DIABETIC AND NON-DIABETIC SUBJECTS
Introduction

Moderate red wine consumption is inversely associated with incidence of coronary ischemia and mortality from coronary heart disease (Freedman et al., 2001; Ceriello et al., 2000). Red wines and grape juices contain phenolic compounds with antioxidant and antiplatelet properties that may be protective against oxidative stress leading to hypertension, insulin resistance and Type 2 diabetes (De Lange et al., 2003). Uncontrolled oxidative stress is implicated in impaired insulin action and nitric oxide availability, altered membrane fluidity and lipoprotein profile, increased intracellular calcium content and modification in fibrinogen structure (Caimi et al., 2003; Lipinski, 2001; Diebolt and Andriantsitohaina, 2002). Though the mechanism of action is not known, hyperglycemia and hypertriglyceridemia may also increase reactive oxygen and nitrogen species, which result in oxidative stress, (Ruhe and McDonald, 2001).

Type 2 diabetes (T2D) continues to grow in epidemic proportions throughout the world, with over 135 million people diagnosed of this condition. In the United States, 18 million people have been diagnosed, with $132 billion spent annually as health care cost or lost of revenue due to disability and low productivity (CDC, 2004). While non-diabetics spend $2,560 on health care, T2D people spend $13,242 on health care (CDC, 2004). Longitudinal trends show increasing incidence of T2D and obesity as well (Wilson et al., 2001). Type 2 diabetes increases coronary heart disease (CHD) risk two to four fold
compared to healthy individuals (Coutinho et al., 1999). In North Carolina, 580,000 people (about 14% of population) are estimated to have T2D; one-third of which are undiagnosed (NC Department of Health and Human Services, 2002). About 20 percent of the T2D people exhibit diabetic complications upon diagnosis. These complications include hypertension, obesity, renal dysfunction and loss of vision. Few studies have examined the cardioprotective effect of wine on healthy subjects (Van Velden, 2002; Tedesco et al., 2000; Christiansen, 1993) but no studies were found that examined the effect of dark-skinned Muscadine grapes on blood constituents of Type 2 diabetics.

Muscadine grapes (*Vitis rotundifolia*) are native to Southeastern United States. The grapes grow in small clusters and are resistant to Pierce’s disease (*Xylella fastidiosa*). North Carolina harvests about 2000 tons of grapes annually, and three-quarters are used in wine production. Because of their availability and potential antioxidant benefit of grapes and grape products, this research explored the effect of muscadine juice (MJ), muscadine wine (MW) and dealcoholized muscadine wine (Dz-W) on blood constituents of healthy and T2D individuals.

**Methodology**

The Institutional Review Board of North Carolina State University approved this research plan. All subjects voluntarily agreed to participate and an
informed consent form was obtained from each participant. Subjects were recruited from local medical clinics and by word of mouth. Type 2 diabetics (n=29) were assigned to consume 150 ml of MJ (n=10), MW (n=10), or Dealcoholized- wine (n=9) after dinner for a period of 28 days. Non-diabetic subjects were randomly assigned into the following categories: Muscadine Juice group (n = 8) and control group without supplementation (n=15). T2D subjects on MW were regular wine consumers and were asked to substitute their wine intake for MW. The MJ and MW were obtained from the Duplin Winery, Rose Hill, North Carolina. The alcohol-free wine (Dz-W) was prepared using the reverse osmosis system consisting of a Venocon reverse membrane housing (JB Systems, LaGrange, GA) and a Nanomax 95 reverse osmosis spiral module membrane (Millipore Corp, Bedford, MA). Baseline and post-treatment data were collected on all subjects. Subjects were told to follow their daily dietary and physical activity habits.

A 3-day diet record, list of medications, blood pressure, body weight and abdominal girth were recorded (see previous chapter). All vacutainer tubes were obtained from Becton Dickinson, NJ. Blood samples were collected in vacutainer K₃ – EDTA (tri-potassium ethylenediaminetetraacetic acid) tubes for glycated hemoglobin (HbA₁C) analysis, serum separator vacutainer tubes for the determination of electrolytes (potassium, sodium, chloride, calcium, magnesium, phosphorus), fasting insulin level, some digestive enzymes (amylase, lipase) and kidney function tests (blood urea nitrogen, CO₂, alanine
aminotransferase, alkaline phosphatase, albumin). The laboratory tests above were performed at the VA Medical Center, Durham, North Carolina.

Statistical analysis was carried out using SAS 8.2 software (SAS, Cary, NC, USA). Group means, standard deviation and standard errors were computed. Paired Student T-test was carried out to compare differences between initial (pre) values and post supplementation data for each group. Analysis of covariance using the general linear model procedure was applied to determine significance of preplanned comparisons between the main effects and the various treatments after adjusting for differences in baseline covariates, where the values prior to supplementation was the covariate. Probability (P) values less than 0.05 were considered statistically significant.

**Results**

The diabetic population in this study had higher fasting blood glucose, insulin and glycated hemoglobin concentrations compared to nondiabetic, control subjects, as expected (Figure 1, Table 1). Decreases observed in fasting blood glucose and insulin indices were almost significant only for T2D taking MW (P = 0.06). Higher insulin levels observed in the diabetics were reduced post supplementation but this reduction was significant only among the T2D taking Dz-W (pre: 14.8 ± 3.0 uU/ml, post: 9.5 ± 1.6 uU/ml). Lowered fasting glucose and glycated hemoglobin were significant for the T2D taking wine, comparing baseline and post supplementation values.
Serum sodium concentration post supplementation was significantly lower for T2D taking MW than T2D taking MJ and Dz-W (Table 2). This lowered sodium level post supplementation among the T2D taking MW was also significant compared to the control subjects. Chloride levels followed a similar pattern. Changes observed in potassium and carbon dioxide concentrations were not statistically significant.

Alkaline phosphatase levels of T2D on MW compared favorably with the control subjects post supplementation, and were significantly lower than T2D on MJ and Dz-W (Table 3). Calcium significantly decreased among the T2D taking MW, comparing baseline and post supplementation data. Higher calcium concentrations were recorded among the T2D on MJ, Dz-W and Control subjects compared to T2D taking MW. Phosphorus levels significantly increased in the T2D group taking juice after the supplementation period (pre, 3.1 ± 0.1; post, 3.8 ± 0.1, p < 0.05). Type 2 diabetics taking MJ also recorded significantly higher change in phosphorus levels compared to their diabetic counterparts.

The liver function indices studied were alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transpeptidase (GGT) and lactate dehydrogenase (LD), total protein, bilirubin and albumen. The results are presented in Table 4. Control subjects recorded significantly lower LD concentration compared to the diabetics. Type 2 diabetics taking MW had
significantly lower LD values (comparable to controls) than T2D taking MJ and Dz-W. Though decreased ALT and AST were observed in most groups after the supplementation, significant increase in ALT and AST occurred in T2D taking Dz-W than T2D taking MW.

Renal function tests measured included uric acid, blood urea nitrogen, creatinine and anion gap. A significant decrease in anion gap was observed with T2D taking wine compared to T2D taking MJ. Changes observed in uric acid, blood urea nitrogen and creatinine were not significant.

**Discussion**

Several studies have demonstrated that slight increases in fasting and postprandial blood glucose concentration can contribute to macrovascular injury (Kawamori, 1998). Also, in a metaregression analysis of 20 studies, a progressive relationship between glucose levels and cardiovascular risk extends below the diabetic threshold (Coutinho et al., 1999). This study measured high glucose concentrations in all the diabetic population, as expected (Table 1). A similar trend was observed with the glycated hemoglobin levels. These high concentrations of fasting glucose and glycated hemoglobin were greatly reduced post supplementation among the T2D subjects taking MW by factors of 21.6 mg/dl for glucose and 0.6% for glycated hemoglobin. This improved glycemic control may be a result of improved
insulin action. Insulin concentrations, however, only significantly decreased post supplementation for the T2D taking Dz-W (Table 1), though there was 4.2 uU/ml decrease among the T2D taking juice and 2.0 uU/ml decrease among T2D subjects taking wine. Fasting glucose to insulin ratio below 7 predicts the risk of insulin resistance (Figure 1). Type 2 diabetics taking juice and diabetics taking wine recorded a worsened insulin resistant state compared to T2D taking dealcoholized wine and the control groups.

Sodium and potassium ions are involved in the regulation of blood pressure and the onset of hypertension. In this study, sodium levels post supplementation was significantly lower for T2D taking MW than T2D on MJ and Dz-W, as well as control subjects. Chloride levels followed a similar pattern (Table 2). The lowering of sodium and chloride in the T2D group taking MW indicates a better control of hypertension, which usually exists with diabetes. Campese et al., (1998) determined the mechanism responsible for hypertension in T2D patients. They found that high salt intake increased blood pressure and decreased heart rate in the study group. High salt intake also increased the vascular response to norepinephrine in T2D hypertensive subjects. Also, increased salt levels due to high salt sensitivity and retention may alter sodium and water balance and cause vascular reactivity in the angiotensin-rennin-aldosterone system, increasing the risk of kidney damage (Tuck et al., 1990; Brewster and Perazella, 2004). In another study looking at the effect of salt loading (1% NaCl solution) on blood indices, the noticeable
change was increased urinary sodium excretion in the salt loaded group compared to the control group (Iwase et al., 1992). In agreement with our findings, research conducted to assess potassium balance in essential hypertension and diabetic patients reported insignificant changes in potassium levels (Michea et al., 1997).

Amylase and lipase trends showed decreases after the supplementation period in all the diabetic groups. Kawamori et al., (1995) discovered in a similar study that baseline amylase activity was significantly lower in diabetic subjects compared to non-diabetic controls. Also, they noticed negligible changes in the lipase activity and protein concentration. These changes, observed after the subjects had adhered to a 3-month strict glycemic control regimen, led these authors to conclude that glycemic control could partially improve amylase activity and endogenous insulin secretion in T2D (Kawamori et al., 1989).

A balance between membrane influx and efflux mainly regulates calcium homeostasis: the influx is due both to voltage-dependent and receptor-operated channels, while the main mechanism of efflux is the active transport by the calcium -ATPase. Altered sodium and calcium transport has been described in some pathological conditions (Mazzanti and Mutus, 1997) including T2D (Schaeffer et al., 1999). In our study, calcium significantly decreased among the T2D taking MW post supplementation and higher
calcium concentrations were recorded among the T2D on MJ and Dz-W as well as control subjects (Table 3). Calcium is required for bone health and normal cell growth. The decrease observed in the T2D on MW however, was not low enough to compromise calcium homeostasis since recommended levels in the blood are above 8.6 mg/dl.

Phosphorus metabolism is closely related to calcium. Phosphorus is widely distributed in the body as phosphate, a component of nucleic acids, phospholipids, carbohydrates and small molecules such as ATP and creatine phosphate. Phosphorus levels significantly increased in the T2D group taking juice after the supplementation period.

Magnesium (Mg) deficiency interrupts insulin secretion in the pancreas, increases insulin resistance in the body’s tissues and causes cardiovascular injury (Barbagallo et al., 2003; Haffner et al., 1990). Working with T2D patients, Barbagallo et al., (1998) demonstrated that Mg improves cellular uptake of glucose by improving insulin action. Also, suppressed intracellular magnesium and high calcium levels have been implicated as risk factors in the development of T2D (Vinik et al., 2001). Contrary to studies, however, all the T2D participating in this study had similar magnesium levels to the control subjects.

The liver function indices studied were alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transpeptidase (GGT)
lactate dehydrogenase (LD), total protein, total bilirubin and albumin (Table 4). In our study, decreased ALT and AST were observed in most groups after the supplementation, though significant increase in ALT and AST occurred in T2D taking Dz-W than T2D taking MW. Studies indicate elevated ALT is associated with insulin insensitivity. Vozarova et al., (2002) examined whether the elevated hepatic enzymes, ALT, AST and GGT were associated with changes in liver or insulin sensitivity and whether these elevated enzymes predict the development of type 2 diabetes in Pima Indians. They found that only higher ALT concentrations were associated with obesity and a decline in insulin sensitivity. ALT was therefore recommended as a predictor of the development of T2D.

One of the complications of T2D is renal function impairment. To assess this, data were obtained on uric acid, blood urea nitrogen, creatinine, and anion gap (Table 5). Changes observed in uric acid, blood urea nitrogen and creatinine were not significant. Significant decreases in anion gap were observed with T2D taking wine compared to T2D taking MJ. Anion gap measures the difference between the concentration of unmeasured anions and unmeasured cations and is used to classify and identify acid-base disorders such as ketoacidosis, uremia and hypoalbuminemia. All the subjects recorded comparable levels to the control groups.
In conclusion, lower levels of blood glucose and glycated hemoglobin post supplementation indicated better glycemic control especially with T2D taking MW. Lower sodium and chloride values for the T2D taking MW post supplementation suggest lower risk to developing hypertension. Type 2 diabetics taking MJ and DzW responded with higher serum phosphorus and calcium concentrations compared to T2D taking MW. Decreased hepatic function indicators, ALT and AST among T2D taking MW compared to T2D taking Dz-W suggest better insulin sensitivity and reduced tendency of impaired liver.

References


Figure 1. Glucose/Insulin Ratio of Diabetic and Healthy Subjects Pre and Post Supplementation
Table 1: Fasting Blood Glucose, Insulin and Glycated Hemoglobin Concentrations of Control and Diabetic Subjects at Baseline and Post Supplementation

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects (CS)</th>
<th>Control Juice (CJ)</th>
<th>T2D Juice (DJ)</th>
<th>T2D Dz. Wine (Dz-W)</th>
<th>T2D Wine (DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Glucose (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>87.7(3.8)</td>
<td>90.4 (2.7)</td>
<td>150.0(21.0)</td>
<td>125.4 (19.3)</td>
<td>140.5(21.0)</td>
</tr>
<tr>
<td>Post</td>
<td>88.2(2.6)</td>
<td>93.8 (2.2)</td>
<td>133.6(29.6)</td>
<td>124.3 (13.0)</td>
<td>118.9(18.1)</td>
</tr>
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<td><strong>Insulin (uU/ml)</strong></td>
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<td></td>
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</tr>
<tr>
<td>Pre</td>
<td>10.9(1.6)</td>
<td>12.5(4.5)</td>
<td>33.2(13.5)</td>
<td>14.8(3.0)*</td>
<td>26.4(7.7)</td>
</tr>
<tr>
<td>Post</td>
<td>10.9(1.3)</td>
<td>11.8(2.3)</td>
<td>28.6(13.4)</td>
<td>9.5 (1.6)</td>
<td>24.4(6.5)</td>
</tr>
<tr>
<td><strong>Gly. Hemoglobin (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>5.5 (0.3)</td>
<td>5.5 (0.1)</td>
<td>8.4 (0.7)</td>
<td>7.6 (0.5)</td>
<td>7.4 (0.5)</td>
</tr>
<tr>
<td>Post</td>
<td>5.2 (0.2)</td>
<td>5.8 (0.3)</td>
<td>8.3 (0.7)</td>
<td>7.5 (0.4)</td>
<td>6.8 (0.8)</td>
</tr>
</tbody>
</table>

Values are means (standard error)

* Statistically Significant difference: P < 0.05, Pre vs. Post
<table>
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<tr>
<th></th>
<th>Control Subjects</th>
<th>Control Juice</th>
<th>T2D Juice</th>
<th>T2D Dz. Wine</th>
<th>T2D Wine</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control (CS)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Juice (CJ)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pre</strong></td>
<td>4.2 (0.2)</td>
<td>4.1 (0.1)</td>
<td>4.2 (0.1)</td>
<td>4.0 (0.2)</td>
<td>4.5 (0.1)</td>
</tr>
<tr>
<td><strong>Post</strong></td>
<td>4.1 (0.1)</td>
<td>4.0 (0.1)</td>
<td>4.1 (0.1)</td>
<td>4.1 (0.2)</td>
<td>4.3 (0.2)</td>
</tr>
<tr>
<td><strong>Sodium (mmol/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pre</strong></td>
<td>138.6 (0.7)</td>
<td>139.9 (0.7)</td>
<td>138.9 (1.0)</td>
<td>136.9 (1.5)</td>
<td>139.1 (0.9)*</td>
</tr>
<tr>
<td><strong>Post</strong></td>
<td>136.1 (1.8)&amp;</td>
<td>139.5 (0.8)</td>
<td>140.0 (0.8)#</td>
<td>137.3 (2.1)¶</td>
<td>135.1 (1.9)</td>
</tr>
<tr>
<td><strong>Chloride (mmol/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pre</strong></td>
<td>101.9 (0.6)</td>
<td>103.9 (0.9)</td>
<td>103.1 (0.9)</td>
<td>99.9 (1.3)</td>
<td>104.3 (1.3)*</td>
</tr>
<tr>
<td><strong>Post</strong></td>
<td>100.6 (1.7)&amp;</td>
<td>103.6 (0.6)</td>
<td>102.9 (0.7)#</td>
<td>101.1 (1.5)</td>
<td>101.5 (1.6)</td>
</tr>
<tr>
<td><strong>Carbon diox.(mmol/L)</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td><strong>Pre</strong></td>
<td>26.3 (0.9)</td>
<td>25.6 (0.8)</td>
<td>25.9 (0.6)</td>
<td>26.8 (0.9)</td>
<td>25.5 (0.7)</td>
</tr>
<tr>
<td><strong>Post</strong></td>
<td>26.1 (0.8)</td>
<td>26.5 (0.8)</td>
<td>26.1 (0.8)</td>
<td>26.5 (0.7)</td>
<td>24.5 (0.6)</td>
</tr>
<tr>
<td><strong>Amylase (U/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pre</strong></td>
<td>79.8 (11.6)</td>
<td>102.9 (9.1)</td>
<td>89.5 (14.0)</td>
<td>100.3 (26.7)</td>
<td>95.3 (17.4)</td>
</tr>
<tr>
<td><strong>Post</strong></td>
<td>84.3 (10.2)</td>
<td>115.5 (14.3)</td>
<td>87.6 (13.8)</td>
<td>89.9 (16.4)</td>
<td>92.0 (19.8)</td>
</tr>
<tr>
<td><strong>Lipase (U/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pre</strong></td>
<td>31.3 (3.0)</td>
<td>21.8 (1.7)</td>
<td>27.8 (3.6)</td>
<td>43.1 (7.5)</td>
<td>24.9 (1.8)</td>
</tr>
<tr>
<td><strong>Post</strong></td>
<td>32.9 (3.1)</td>
<td>23.3 (2.1)</td>
<td>26.3 (1.9)</td>
<td>25.4 (5.2)</td>
<td>22.0 (2.4)</td>
</tr>
</tbody>
</table>

Values are means (standard error)

* Statistically Significant difference: $P < 0.05$, Pre vs. Post
& Statistically Significant difference: $P < 0.05$, Control Subjects vs. T2D taking MW
# Statistically Significant difference: $P < 0.05$, T2D taking MJ vs. T2D taking MW
¶ Statistically Significant difference: $P < 0.03$, T2D taking Dz-W vs. T2D taking MW
<table>
<thead>
<tr>
<th></th>
<th>Control Subjects (CS)</th>
<th>Control Juice (CJ)</th>
<th>T2D Juice (DJ)</th>
<th>T2D Dz. Wine (Dz-W)</th>
<th>T2D Wine (DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alk Phos (IU)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>44.8 (2.7)</td>
<td>60.8 (7.3)</td>
<td>65.2 (6.8)</td>
<td>57.0 (4.6)</td>
<td>65.2 (5.1)</td>
</tr>
<tr>
<td>Post</td>
<td>49.9 (3.3)</td>
<td>59.4 (7.7)</td>
<td>66.2 (7.3)#</td>
<td>67.8 (6.0)¶</td>
<td>47.9 (7.1)</td>
</tr>
<tr>
<td>Calcium (mg/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>9.4 (0.1)</td>
<td>9.7 (0.1)</td>
<td>9.4 (0.1)</td>
<td>9.4 (0.2)</td>
<td>9.2 (0.1)*</td>
</tr>
<tr>
<td>Post</td>
<td>9.1 (0.1)&amp;</td>
<td>9.6 (0.1)</td>
<td>9.5 (0.1)#</td>
<td>9.4 (0.2)¶</td>
<td>8.8 (0.2)</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>3.7 (0.1)</td>
<td>3.6 (0.1)</td>
<td>3.1 (0.1)*</td>
<td>3.8 (0.3)</td>
<td>3.3 (0.2)</td>
</tr>
<tr>
<td>Post</td>
<td>3.7 (0.1)§</td>
<td>3.7 (0.1)€</td>
<td>3.8 (0.1)#</td>
<td>3.6 (0.2)</td>
<td>3.2 (0.2)</td>
</tr>
<tr>
<td>Magnesium (mg/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>2.0 (0.1)</td>
<td>2.1 (0.1)</td>
<td>1.9 (0.1)</td>
<td>2.0 (0.1)</td>
<td>2.0 (0.1)</td>
</tr>
<tr>
<td>Post</td>
<td>2.0 (0.1)</td>
<td>2.0 (0.1)</td>
<td>1.9 (0.1)</td>
<td>1.9 (0.1)</td>
<td>2.1 (0.1)</td>
</tr>
</tbody>
</table>

Values are means (standard error)

* Statistically Significant difference: P < 0.05, Pre vs. Post
& Statistically Significant difference: P < 0.05, Control Subjects vs. T2D taking MW
§ Statistically Significant difference: P < 0.05, Control Subjects vs. T2D taking MJ
€ Statistically Significant difference: P < 0.05, Control Juice vs. T2D taking MJ
# Statistically Significant difference: P < 0.05, T2D taking MJ vs. T2D taking MW
¶ Statistically Significant difference: P < 0.03, T2D taking Dz-W vs. T2D taking MW
Table 4: Liver Function Tests for Control and Diabetic Subjects at Baseline and Post Supplementation

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects (CS)</th>
<th>Control Juice (CJ)</th>
<th>T2D Juice (DJ)</th>
<th>T2D Dz. Wine (Dz-W)</th>
<th>T2D Wine (DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD (IU/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>125.3(7.9)</td>
<td>122.6(11.2)</td>
<td>169.0(13.3)</td>
<td>159.1(15.1)</td>
<td>154.0(9.2)</td>
</tr>
<tr>
<td>Post</td>
<td>122.8(5.2)$^†$</td>
<td>129.5(12.1)</td>
<td>163.0(13.0)#</td>
<td>161.6(15.0)$^¶$</td>
<td>151.5(7.3)</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>27.4(4.4)</td>
<td>28.0(3.7)</td>
<td>26.2(5.1)</td>
<td>29.6(4.0)</td>
<td>36.7(10.4)</td>
</tr>
<tr>
<td>Post</td>
<td>25.8(3.2)</td>
<td>27.1(5.4)</td>
<td>24.6(3.9)</td>
<td>30.5(3.8)$^¶$</td>
<td>23.8(1.8)</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>19.5(2.9)</td>
<td>15.5(1.7)</td>
<td>14.8(2.2)</td>
<td>20.9(3.2)</td>
<td>20.3(4.1)</td>
</tr>
<tr>
<td>Post</td>
<td>16.9(1.8)$^†$</td>
<td>17.1(3.5)</td>
<td>16.6(3.2)</td>
<td>25.0(5.6)$^¶$</td>
<td>15.0(1.3)</td>
</tr>
<tr>
<td>GGT (IU/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>24.3(4.3)</td>
<td>24.9(4.5)</td>
<td>32.9(10.4)</td>
<td>33.9(6.0)</td>
<td>28.5(5.4)</td>
</tr>
<tr>
<td>Post</td>
<td>23.3(3.4)</td>
<td>22.4(5.7)</td>
<td>28.0(5.9)</td>
<td>32.3(5.6)</td>
<td>34.4(8.9)</td>
</tr>
<tr>
<td>T. Prot (g/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>6.9(0.1)</td>
<td>7.1(0.1)</td>
<td>6.9(0.2)</td>
<td>6.4(0.5)</td>
<td>7.0(0.1)</td>
</tr>
<tr>
<td>Post</td>
<td>6.7(0.1)</td>
<td>7.0(0.1)</td>
<td>6.9(0.2)</td>
<td>6.8(0.1)</td>
<td>6.7(0.1)</td>
</tr>
<tr>
<td>T. Bilirubin (mg/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>0.70(0.10)$^$</td>
<td>0.54(0.06)</td>
<td>0.43(0.05)$^*$</td>
<td>0.74(0.06)</td>
<td>0.64(0.05)</td>
</tr>
<tr>
<td>Post</td>
<td>0.65(0.06)</td>
<td>0.53(0.06)</td>
<td>0.35(0.05)</td>
<td>0.71(0.04)</td>
<td>0.59(0.06)</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>4.00(0.10)</td>
<td>4.02(0.04)</td>
<td>3.49(0.16)</td>
<td>3.82(0.12)</td>
<td>3.74(0.08)</td>
</tr>
<tr>
<td>Post</td>
<td>3.86(0.12)</td>
<td>3.95(0.05)</td>
<td>3.48(0.17)</td>
<td>3.69(0.09)</td>
<td>3.62(0.07)</td>
</tr>
</tbody>
</table>

Values are means (standard error)

* Statistically Significant difference: P < 0.05, Pre vs. corresponding Post supplementation
† Statistically Significant difference: P < 0.05, Control Subjects vs. T2D taking Dz-W
$ Statistically Significant difference: P < 0.04, Control Subjects vs. T2D taking MJ
¶ Statistically Significant difference: P < 0.05, T2D taking Dz-W vs. T2D taking MW
# Statistically Significant difference: P < 0.05, T2D taking MJ vs. T2D taking MW

LD = Lactate Dehydrogenase, ALT = Alanine Aminotransferase, AST = Aspartate Transaminase, GGT = Gamma Glutamyl transferase, T. Prot = Total Protein.
### Table 5: Renal Function Tests for Control and Diabetic Subjects at Baseline and Post Supplementation

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects (CS)</th>
<th>Control Juice (CJ)</th>
<th>T2D Juice (DJ)</th>
<th>T2D Dz. Wine (Dz. W)</th>
<th>T2D Wine (DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Uric Acid (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pre</strong></td>
<td>5.1(0.4)</td>
<td>5.0(0.4)</td>
<td>5.6(0.7)</td>
<td>6.0(0.8)</td>
<td>6.1(0.8)</td>
</tr>
<tr>
<td><strong>Post</strong></td>
<td>5.2(0.3)</td>
<td>5.1(0.4)</td>
<td>5.7(0.7)</td>
<td>5.5(0.7)</td>
<td>5.7(0.6)</td>
</tr>
<tr>
<td><strong>BUN (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pre</strong></td>
<td>12.2(0.9)</td>
<td>10.8(1.0)</td>
<td>15.2(1.8)</td>
<td>24.2(6.8)</td>
<td>15.0(2.4)</td>
</tr>
<tr>
<td><strong>Post</strong></td>
<td>12.3(0.7)</td>
<td>12.0(1.1)</td>
<td>16.0(1.5)</td>
<td>17.1(2.4)</td>
<td>16.3(2.0)</td>
</tr>
<tr>
<td><strong>Creat. (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pre</strong></td>
<td>0.8(0.1)</td>
<td>1.0(0.1)</td>
<td>1.0(0.1)</td>
<td>1.1(0.2)</td>
<td>1.1(0.1)</td>
</tr>
<tr>
<td><strong>Post</strong></td>
<td>0.9(0.1)</td>
<td>1.0(0.1)</td>
<td>1.0(0.1)</td>
<td>1.0(0.1)</td>
<td>1.1(0.1)</td>
</tr>
<tr>
<td><strong>An. Gap (mol/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pre</strong></td>
<td>10.4(0.4)*</td>
<td>10.3(0.6)</td>
<td>9.9(0.4)</td>
<td>10.2(0.8)</td>
<td>9.3(0.7)</td>
</tr>
<tr>
<td><strong>Post</strong></td>
<td>9.0(1.4)$</td>
<td>9.9(0.5)</td>
<td>11.1(0.6)#</td>
<td>9.5(0.5)</td>
<td>9.1(0.5)</td>
</tr>
</tbody>
</table>

Values are means (standard error)

* Statistically Significant difference: P< 0.005, Control Subjects vs. T2D taking MW
# Statistically Significant difference: P <0.03, T2D taking MJ vs. T2D taking MW
$ Statistically Significant difference: P < 0.02, Control Subjects vs. T2D taking MJ

CHAPTER FOUR

ANTIOXIDANT CAPACITY AND COAGULATION INDICES OF HEALTHY AND TYPE 2 DIABETIC SUBJECTS AFTER SUPPLEMENTATION OF MUSCADINE GRAPE PRODUCTS
Introduction

Oxidative stress refers to an interplay of intracellular and extracellular factors that leads to chemical or metabolic generation of reactive nitrogen, oxygen or chloride species (Mercuri et al., 2000). These highly reactive species, also known as free radicals, are produced in excessive amounts in disease conditions such as cardiovascular diseases, atherosclerosis, cancer, Alzheimer’s disease, arthritis and type 2 diabetes (Evans et al., 2002; Leighton et al., 1999). The damage caused by these reactive species to biomolecules are reduced or prevented by antioxidants. Several categories of antioxidants exist. These include: enzymatic antioxidants such as superoxide dismutase, glutathione peroxidase and catalase; and non-enzymatic antioxidants such as vitamin C, vitamin E, beta-carotene and phenols (Mercuri et al., 2000; Abuja and Albertini, 2001).

Several methods have been developed to measure the antioxidant capacity of food and biological fluids. These include Electron Spin Resonance (ESR) spectroscopy (Sonta et al., 2004), Chemiluminescence (Maxwell et al., 1994), Conjugated Diene (DC) assay (Gavella et al., 2002; Masella et al., 2001), Thiobarbituric Acid Reactive Substance (TBARS) (Cheng et al., 2004), Ferric Reducing Antioxidant Potential (FRAP) (Cao et al., 1998a; Sugherini et al., 2000), Total Radical-trapping Potential (TRAP) (Mercuri et al., 2000), Trolox Equivalent Antioxidant Capacity (TEAC) (Cao et al., 1998b), and Oxygen Radical Absorbance Capacity (ORAC) (Cao et al., 1998b; Huang et al., 2002).
The ORAC assay is an in vitro method for determining the antioxidant scavenging activity against peroxyl radical induced by AAPH (2,2', azobis {2-amidinopropane} dihydrochloride) at 37°C. The assay depends on the detection of chemical damage to a fluorescent probe, fluorescein, through the decrease in fluorescence emission. The loss of fluorescence in the presence of reactive species is an index of oxidative damage to proteins (Huang et al., 2002). In the presence of antioxidants, free radical damage is inhibited. This is observed in the protection against the change of probe fluorescence in the ORAC assay, thus giving a measure of the antioxidants’ free radical scavenging ability (Huang et al., 2002).

The ORAC assay measures free radical scavenging capacity of all known nonenzymatic water- or lipid-soluble antioxidants. Examples of these antioxidants include ascorbic acid, alpha-tocopherol, beta-carotene, glutathione, methionine, uric acid, bilirubin, phenolic acids, flavanols, flavonols, flavones, isoflavones, flavanones, and anthocyanins (Cao et al., 1998b).

The assay is, to date, the only method that takes a free radical reaction to completion and uses an area under the curve (AUC) technique for quantifying antioxidant capacity. The assay combines both the inhibition time and the degree of inhibition of the free radical action by antioxidants into a single quantity (Cao et al., 1998b). The ORAC assay is used to determine the
antioxidant capacity of various samples including foods such as fruits, vegetables, cereals, tea, chocolate; and animal tissues including plasma and erythrocytes. (Cao et al., 1998b; Manach et al., 1998; Day et al., 1997)

Another index of antioxidant ability is the measurement of the phenolic compounds. Phenols act as antioxidants due to the reactivity of the hydroxyl substitute on the aromatic ring (Robins, 2003). Phenols present in fruits, vegetables, tea and wines have been extensively studied (Cao et al., 1998; Day et al., 1997; Negishi et al., 2004). Polyphenolic substances most common in wine, one of the products of interest in this research, include flavonoids, which are subdivided into flavonols (quercetin, kaempferol, and myrecetin), flavan-3-ols (catechin, epicatechin, tannins), and anthocyanins.

Several researchers investigated the effect of antioxidants present in foods on healthy subjects. Leighton et al., (1999) evaluated the antioxidant status of subjects after supplementation of two diets with red wine, namely, a Mediterranean diet and a high fat diet. There was 13.5% increase in plasma vitamin C observed in the group on Mediterranean diet supplemented with red wine. Also, total antioxidant capacity increased 28% above basal levels in the Mediterranean diet group, but not in the high fat diet group.

Cao et al., (1998b), investigated the responses in serum antioxidant capacity of subjects following consumption of strawberries, spinach, red wine or vitamin
C. They found that the total antioxidant capacity significantly increased by 7 – 25% during the 4-hour period following the consumption of these food products.

In another study the subjects consumed red and white wine, an equivalent of 32g and 23g of alcohol/ d for males and females respectively. The authors observed increased total antioxidant capacity during red wine consumption (Van Velden et al., 2002) but not with white wine. Donovan et al., (1999) measured one of the abundant flavonoids, (+)-catechin, and its metabolites in the plasma of healthy subjects after they consumed 120ml of red wine and de-alcoholized red wine. Plasma levels of catechin, 3’methylcatechin and all conjugates at baseline was < 2 nmol/ L. After 1 hour average levels increased to 91± 14 nmol/ L in red wine and 81 ± 11 nmol/ L in dealcoholized red wine. Other studies, however, reported insignificant or no changes in antioxidant status after supplementation (Bertelli et al., 1996; Darko et al., 2002).

Increased oxidative stress in Type 2 diabetes is enhanced by free radical generation during hyperglycemia, hyperinsulinemia and insulin resistance state. Hyperglycemia-induced oxidative stress results in a reduced number of glucose transporters, impaired insulin signaling (Wolff et al., 1991), and reduced pancreatic beta-cell insulin secretion (Aguirre et al., 1998). Type 2 diabetics have also been observed to have low levels of alpha-tocopherol and vitamin C in plasma, decreased total glutathione, increased serum
malonaldehyde and increased urinary F2-isoprostanes. In addition, increased lipid peroxidation impairs membrane permeability and membrane fluidity (Cabarello, 1993).

Reactive species are also implicated in other complications associated cardiovascular events and T2D (Baynes, 1991). These complications include vasoconstriction, decreased nitric oxide production (Schaefer et al., 2004), increased expression of adhesion molecules, and activation of the coagulation cascade (Abuja and Albertini, 2001). Coagulation parameters exhibit a pattern that promotes thrombosis (Ceriello et al., 2001), increased fibrinogen and plasminogen activator inhibitor (PAI)-1 levels (Collier et al., 1992), and accentuate abnormalities in platelet function (Vinik et al., 2001; Carr, 2001).

Insulin resistance state coexist with coagulation abnormalities such as platelet aggregability, platelet adhesion, and increased levels of thromboxane, von Willebrand factor, factor VII, tissue plasminogen activator (TPA) and increased fibrinogen (Ceriello, 1997). Also, decreased fibrinolytic activity, increased glucose concentration and modified lipoproteins all increase levels of PAI-1, which ultimately promotes thrombosis (Vinik et al., 2001; Carr, 2001). In addition, microalbuminuria (Asakawa et al., 2000), and hyperhomocysteinemia (Baliga et al., 2000; Sowers et al., 2001) have been observed among T2D. Polyphenolic compounds present in red wine, rather than the alcohol, are
known to have an inhibitory effect on activation of coagulation factors (De Lange et al., 2003; Ceriello et al., 2001).

The implications of supplementation of various antioxidant foods on the parameters mentioned above have been investigated mainly in healthy subjects (Serafini et al., 1998; Cao et al., 1998). Researchers investigating antioxidant capacity through supplementation studies among T2D mostly adopted a single nutrient approach, such as the effect of vitamin E (Sharma et al., 2000; Paolisso et al., 1993), vitamin C and magnesium (Eriksson and Kohvakka, 1995) and reduced glutathione activity (De Mattia et al., 1998). However, in vivo, antioxidants act synergistically to bring about maximum protection against several types of reactive species (Aguirre et al., 1998). We therefore investigated the effect of muscadine juice, wine and dealcoholized wine supplementation on the antioxidant capacity and coagulation indices of T2D subjects. The muscadine products were investigated because of their antioxidant properties, availability in North Carolina and the lack of data on biological properties of muscadines.

Materials and Methods

Subject recruitment and sample preparation

The Institutional Review Board of North Carolina State University approved this research plan. All subjects voluntarily agreed to participate and an
informed consent form was received from each participant. Subjects were recruited from local medical clinics and by word of mouth. Type 2 diabetics (n=29) were assigned to consume 150ml of MJ (n=10), MW (n=10), or Dealcoholized- wine (n=9) after dinner for a period of 28 days. Non-diabetic subjects were randomly assigned into the following categories: Muscadine Juice group (n = 8) and control group without supplementation (n=15). T2D subjects on MW were regular wine consumers and were asked to substitute their wine intake for MW.

The muscadine products were obtained from the Duplin Winery, Rose Hill, North Carolina. The juice and wine samples were filtered, pasteurized and bottled under aseptic conditions. Dealcoholized wine was prepared by passing the wine sample through a reverse osmosis unit consisting of a Venocon reverse membrane housing (JB Systems, LaGrange, GA) containing a Nanomax 95 reverse osmosis spiral module membrane (Millipore Corp, Bedford, MA). This separated the alcohol from the other polyphenols based on their molecular size. The alcohol content was intermittently measured using an ebulliometer with a percent alcohol calculator (Dujardin-Salleron, Paris) and procedure outlined in Amerine and Ough, 1980. A 0.7 % alcohol content was achieved. The dealcoholized wine was then filtered, pasteurized and bottled.
**Polyphenol Determination**

Total phenolic content in the muscadine products and plasma were measured using the Folin-Ciocalteau method. The procedure outlined in Amerine and Ough, (1980) was used. Briefly, 200 g anhydrous sodium carbonate was dissolved in deionized water in a 1L volumetric flask and warmed to enhance solubility. This was left to stand at room temperature for 24 hrs and then filtered before use. A 0.5 g standard, gallic acid (GA) (Sigma-Aldrich Co, Louis, MO), was dissolved first in 10ml ethanol and then made up to 100ml with deionized water. Appropriate dilutions (0.5-25 mg/L GA) were prepared for the standard curve plot and the regression equation used to calculate the phenol content of samples. Samples (100ul) were added to 7.9 ml deionized water, 0.5ml Folin reagent, and 1.5 ml sodium carbonate mixture and allowed to incubate in the dark at room temperature for 2 hours. The Spectronic Genesys 2 spectrophotometer (Milton Roy, Rochester, NY) set at 765 nm was used for the absorbance readings.

**Oxygen Radical Absorbance Capacity (ORAC)**

A modified procedure from Cao et al., (1993) and Huang et al., (2002) was used. The final reaction mixture contained in a 300ul well for blank, standard, ((R)-(+)6-hydroxy-2,5, 7, 8-tetramethylchroman-2-carboxylic acid, abbreviated as Trolox) and sample is shown in Table 1. A 96-well plate (Nalge Nunc International, Rochester, NY) was used for the assay.
Table 1: ORAC Assay Final Reaction Mixture for Blank, Standard (Trolox) and Plasma Sample

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Blank (ul)</th>
<th>Trolox (ul)</th>
<th>Sample (ul)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphate Buffer, 0.75M, pH 7.4</td>
<td>130</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>Trolox, 0.05mM</td>
<td>-</td>
<td>60</td>
<td>-</td>
</tr>
<tr>
<td>Plasma (1:400 in Phosphate buffer)</td>
<td>-</td>
<td>-</td>
<td>60</td>
</tr>
<tr>
<td>Fluorescein, 4uM</td>
<td>60</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>AAPH (0.7g in 10ml Phosphate buffer)</td>
<td>60</td>
<td>60</td>
<td>60</td>
</tr>
</tbody>
</table>

Wines and juices were diluted 1:500 with phosphate buffer (ph 7.4). The cytofluor II fluorescence multiwell plate reader (PerSeptive Biosystems, Framingham, MA) was used with filters for an excitation wavelength of 485 ± 20 nm and an emission wavelength of 530 ± 25 nm. The cyttofluor temperature ranged between 37 – 40 °C, with a gain of 57. Readings were taken every 5 mins for 70 mins. Fluorescent readings are acceptable if the reading at time 70 mins was less than 5% of the reading at time 0 min. The reaction was begun after addition of AAPH to the reaction mixture prior to separate incubation of the AAPH and reaction mixture for 15mins.
A Microsoft Excel program (Microsoft, Roselle, IL) was used for the final relative ORAC value calculation. The net area for the Trolox, blank and samples were calculated. The area under the curve was calculated as:

\[ AUC = 5 \times (0.5 + f_1/f_0 + \ldots + f_{65}/f_0 + f_{70}/f_0) \] ..........................

Where: \( f_0 \) = fluorescence reading at time 0, from 0 – 70mins,
and \( f_i \) = Time point for fluorescence readings, from 0-70 mins.

The net area under the curve (N) for sample is calculated as:

\[ N = \left[ \frac{(AUC_{sample} - AUC_{blank})}{AUC_{Trolox} - AUC_{blank}} \right] \] ..........................

The final ORAC value was expressed in mmol Trolox equivalent/ L and this was calculated as:

\[ \text{ORAC value} = \frac{(N \times \text{dilution factor})}{(\text{Sample amount} \times \text{Trolox concentration})} \] ..........................

All samples, blank and Trolox were analyzed in triplicate and the averages used for the calculation of ORAC values.
**Glutathione, Bilirubin and Albumin Determination**

Total reduced glutathione was determined using Bioxytech GSH 420 assay kit (Oxis International, Inc. Portland, OR). The procedure was modified to determine total glutathione using the microplate reader (Molecular Devices Corporation, Sunnyvale, CA).

Total bilirubin and albumin were measured at the VA Medical Center, Durham North Carolina.

**Measurement of Coagulation and Cardiac Indices**

Fibrinogen levels, platelet count, prothrombin time (PT), activated partial thromboplastin time (APTT), homocysteine, folate and vitamin B₁₂, troponin, creatine phosphokinase (CPK) and Creatine Kinase –MB isozyme were determined at the VA Medical Center, Durham, North Carolina.

**Statistical Analysis**

Statistical analysis was carried out using SAS 8.2 software (SAS, Cary, NC. USA). Group means, standard deviations and standard errors were computed. A paired Student T-test was carried out to compare differences between baseline and post supplementation indices for each group. Analysis of covariance using the general linear model procedure was applied to determine significance of preplanned comparisons between the main effects and the various treatments after adjusting for differences in baseline covariates, where
the values prior to supplementation was the covariate. Probability (P) values less than 0.05 were considered statistically significant.

Results
Total phenols present in the Muscadine products were highest for wine, followed by the juice and then the dealcoholized wine (2120 ± 50 mg/L GAE, 1770 ± 110 mg/L GAE, and 1120 ± 140 mg/L GAE) respectively (Table 2). For ORAC, the wine also contained the highest antioxidant activity (24.69 ± 1.09 mmol TE/L), but the dealcoholized wine contained higher amounts of antioxidants than the juice (21.48 ± 1.29 mmol TE/L and 16.04 ± 0.18 mmol TE/L) respectively.

A trend towards increased phenols was observed for all the subjects (Table 3). However, T2D on dealcoholized wine recorded decreased polyphenol concentration after the supplementation period (257.5 vs. 171.3 mg/L GAE, pre vs. post). ORAC values increased post supplementation compared to baseline but these changes were not significant.

Total bilirubin was statistically lower for diabetics on juice compared to control subjects and dealcoholized wine groups. Changes in serum albumin and total reduced erythrocyte glutathione levels among groups were not significant.
There was increased platelet reading post supplementation for T2D on MW (DW) group compared to baseline. Type 2 diabetics on Juice recorded significantly higher platelet readings compared to DW. Type 2 diabetics on juice had similar prothrombin time reading compared to CS and CJ but significantly lower values than Dz-W and DW.

Fibrinogen and homocysteine levels were higher among diabetic subjects compared to the control subjects without supplementation. These high levels of fibrinogen increased post supplementation, though the increases were not statistically significant. Folate levels were higher for DJ and Dz-W comparing baseline and post values but this was significant only for the DJ group. Folate increase was significant in the Dz-W compared to DW. Vitamin B\textsubscript{12} levels were significantly higher for the Dz-W group post supplementation than the DW group.

Creatine phosphokinase (CPK) was significantly higher in T2D taking Dz-W than T2D taking MW post supplementation. Creatinine kinase - MB (CKMB) levels were almost significantly higher among T2D taking Dz-W comparing baseline to post supplementation data (pre: 3.2 ± 1.3 pg/ml, post: 4.6 ± 1.8 pg/ml; p = 0.06). No significant differences were observed among the groups as regards troponin concentrations.
Discussion
This study investigated the effect of supplementation of Muscadine Juice, wine and dealcoholized wine on antioxidant indices and coagulation parameters. Total phenols and oxygen radical absorbance capacity (ORAC) of MJ, MW and dealcoholized muscadine wine are presented in Table 2. Total phenols present in dealcoholized wine were 53% compared to the amount in the wine, using Folin-Ciocaltaeu’s method, and 87% using ORAC. The heat generated during the dealcoholization process may have leached out some simple phenols. Oxygen radical absorbance capacity determines the antioxidant contributions from polyphenols, simple phenols and ascorbate. This index may therefore have better sensitivity to the antioxidant components compared to the determination of total phenols.

Total plasma polyphenols increased in T2D on juice (DJ) and wine (DW) but not in dealcoholized wine group (Dz- W) (Table 3). Serafini et al., (1998), carried out a time-course study to monitor the polyphenol concentration in alcohol-free wine (3636 mg quercetin eq/ L) in plasma. A significant increase in phenol was observed 50 mins after ingestion. However in the same study, plasma polyphenol concentration was not significant 30 mins or 120 mins after ingestion of the wine. This could explain the increase in plasma total phenols observed in DJ and DW groups and the decrease in the Dz-W group in our study: plasma polyphenols are optimum within a certain time period, after which the presence of enzymes or transporters may facilitate transport into other tissues thereby reducing the concentration.
Oxygen Radical Absorbance Capacity (ORAC) for juice and wines were similar to those reported by Ou et al., (2001), Huang et al., (2002) and Maxwell et al., (1994), though the plasma readings in this study were higher than those reported. A trend toward higher ORAC was observed for all the study population, though these were not significant. Cao et al., (1998b), reported increased serum ORAC after 4 hours supplementation of red wine, though urine ORAC did not increase appreciably. Maxwell et al., (1994) reported increased serum antioxidant capacity 4 hours after subjects consumed red wine (5.7 ml Bordeaux/ kg) with a meal compared to subjects who consumed the same meal without red wine. Both of these studies were, however, short-term. Bertelli et al., (1996) fed 4 ml of red wine containing 6.5 mg/L of total resveratrol to rats. They however detected resveratrol levels lower than required for pharmacological activity. Chopra et al., (2000) reported that supplementation of non-alcoholic red wine extract or quercetin did not affect plasma vitamin C and E, retinol and carotenoid concentrations. Limitations to this study include the fact that the T2D volunteers presented various complications such as obesity and hypertension and these may mask the response variables measured.

Bilirubin, a bile pigment and metabolite of hemoglobin, also plays a role as an antioxidant by scavenging peroxyl radicals (Minetti et al., 1998). Bilirubin and albumin are also indicators of liver function. The protective role of bilirubin
includes inhibition of oxidative modification of plasma proteins and formation of protein carbonyl groups (Stocker et al., 1987). Bilirubin in circulation is mainly found bound to albumin (Minetti et al., 1998). Localization of bilirubin on albumin protects albumin from oxidation as well as the albumin-bound linoleic acid from peroxyl radical induced oxidation (Stocker et al., 1987; Wu, 1991). This albumin-sparing function of bilirubin was observed in our study, since significant changes only occurred in bilirubin concentrations.

Platelets are the initial defense mechanism upon loss of vascular integrity. Interaction of platelet membrane receptors with injury-related factors such as collagen, microfibrils and von Willebrand factor, induce adhesion to other platelets and to the vessel wall. The adhered platelets rapidly change their shape, activating the release of aggregating agents such as ATP and serotonin from their storage granules (Mazzanti and Mutus, 1997).

Platelets from diabetic patients show hyperactivity to several physiological stimuli including thrombin, ADP and collagen. This may be due to alteration in calcium transport, as calcium plays a central role in platelet activation in response to stimulation (Mazzanti and Mutus, 1997). In support of this, Ardlie et al., (1986) demonstrated in T2D patients that there was increased intraplatelet calcium concentration in the resting state. In our study, there was a significant difference between the platelet counts for T2D taking juice and T2D taking wine after adjusting for baseline values. This was not
accompanied by high calcium concentrations, which would otherwise cause high aggregability.

Fibrinogen is a precursor to fibrin, a protein that binds platelets to form blood clots. Other blood clotting indices measured were prothrombin time (PT) and activated partial thromboplastin time (APTT). Fibrinogen concentrations are associated with atherosclerosis and CVD (Lipinski 2001). A high blood fibrinogen level is believed to contribute to thrombosis. Results of the present study did not indicate any significant differences among the study groups.

Homocysteine, a sulphur-containing amino acid, is an intermediary product in methionine metabolism. Hyperhomocysteinemia is known to be associated with atherosclerosis, and this association is stronger in individuals with Type 2 diabetes than in nondiabetic subjects. In this study, higher homocysteine levels were observed among the diabetics compared to the control subjects but these differences were not significant. Homocysteine is transformed biochemically into methionine and cysteine in cells with adequate folate, Vitamin B12 and Vitamin B6. Insufficient amounts of folate and B12 will therefore result in accumulation of homocysteine in the blood. The present study recorded significantly higher folate and vitamin B12 values for Dz-W group than the T2D-Juice and wine groups. This suggests that there is enhanced homocysteine disposal as well as 1-carbon metabolism among the T2D taking Dz-W.
Total CPK, along with CK-MB and troponin levels, are used to determine damage to heart muscle cells. Our data showed that CPK values did not significantly differ from the control subjects, though a significant difference was obtained between the higher CPK levels of T2D groups taking Dz-W and the T2D group on MW (Table 5). All our subjects (except the T2D taking Juice) had a better CPK profile post supplementation, including the control subjects without supplementation. This effect may be due to other dietary components other than our wine products. Dietary analysis was carried out on all the subjects studied (data shown in previous chapter). Lazarov et al., (1990) examined serum levels of CPK and its isozyme, creatine kinase – MB (CKMB) in addition to other enzymes in 72 diabetic patients. They found increased CPK and CKMB levels. Lesions of the myocardium, thyroid gland or the striated muscles, which could explain this rise, were not observed.

In conclusion, a significant increase in total polyphenols and a decrease in total bilirubin recorded post supplementation for T2D taking juice may indicate a compensation effect by the polyphenols. Higher blood folate and vitamin B\textsubscript{12} for T2D taking dealcoholized wine indicate a favorable environment for insulin action and homocysteine clearance from the blood. Type 2 diabetics taking juice had lower prothrombin time compared to their diabetic counterparts taking wine and dealcoholized wine suggesting reduced risk of thrombosis. Elevated trends of homocysteine and fibrinogen observed among diabetics indicate insulin resistance tendencies. Overall, T2D taking wine and
dealcoholized wine exhibited significant responses to the indicators measured compared to the muscadine juice group. Moderate amounts of wine and dealcoholized wine may improve diabetic state.

A follow-up controlled diet study with a larger diabetic population on the same medication and consuming the muscadine samples used in this research is recommended. Also, a database for antioxidant content of various foods would enable easier quantitation of antioxidant contributions from the foods eaten.

References


Cao G, Russel RM, Lischner N, Prior RL. 1998b. Serum antioxidant capacity is increased by consumption of strawberries, spinach, red wine or vitamin C in elderly women. J Nutr. 128: 2383 - 2390


multichannel liquid handling system coupled with a microplate fluorescence reader in 96-well format. J Agric. Food Chem. 50: 4437 - 4444


not insulin secretion in elderly type 2 diabetes patients. Diabetes Care. 16 (11): 1433 - 1437


Table 2: Total Phenols and Oxygen Radical Absorbance Capacity of Muscadine Juice, Wine and Dealcoholized wine

<table>
<thead>
<tr>
<th></th>
<th>Muscadine Juice (MJ)</th>
<th>Dealcoholized Wine (Dz-W)</th>
<th>Muscadine Wine (MW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. Phenol (mg/L GAE)</td>
<td>1770(110)</td>
<td>1120 (140)</td>
<td>2120(150)</td>
</tr>
<tr>
<td>ORAC (mmol TE/L)</td>
<td>16.04(0.18)</td>
<td>21.48 (1.29)</td>
<td>24.69(1.09)</td>
</tr>
</tbody>
</table>

Values are means (Standard error)

GAE = Gallic acid equivalence, TE = Trolox equivalence
Table 3: Plasma Total Phenols and Oxygen Radical Absorbance Capacity (ORAC) for Control and Diabetic Subjects at Baseline (Pre) and after supplementation (Post)

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects (CS)</th>
<th>Control Juice (CJ)</th>
<th>T2D Juice (DJ)</th>
<th>T2D Dz. Wine (Dz. W)</th>
<th>T2D Wine (DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. Phenols (mg/L GAE)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>198.9(13.3)</td>
<td>188.0(6.7)</td>
<td>178.7(7.3)</td>
<td>257.5(47.2)</td>
<td>156.3(20.0)</td>
</tr>
<tr>
<td>Post</td>
<td>233.4(19.9)</td>
<td>189.8(13.7)</td>
<td>191.2(6.5)</td>
<td>171.3(41.6)</td>
<td>200.0(28.6)</td>
</tr>
<tr>
<td>ORAC (mmol TE/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>21.10(1.32)</td>
<td>16.11(1.38)</td>
<td>18.76(1.37)</td>
<td>20.48(1.02)</td>
<td>20.89(1.40)</td>
</tr>
<tr>
<td>Post</td>
<td>22.14(1.56)</td>
<td>21.80(2.73)</td>
<td>19.57(0.85)</td>
<td>23.01(1.53)</td>
<td>21.13(1.38)</td>
</tr>
</tbody>
</table>

Values are means (Standard Error)

GAE = Gallic acid equivalence, TE = Trolox equivalence
Table 4: Serum Total Bilirubin, Albumin and Erythrocyte Glutathione Quantitation and Baseline and 28-day Post Supplementation

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects (CS)</th>
<th>Control Juice (CJ)</th>
<th>T2D Juice (DJ)</th>
<th>T2D Dz. Wine (Dz. W)</th>
<th>T2D Wine (DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. Bilirubin (mg/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>0.70(0.1.)</td>
<td>0.54(0.06)</td>
<td>0.43(0.05)*†</td>
<td>0.74 (0.06)</td>
<td>0.64(0.05)</td>
</tr>
<tr>
<td>Post</td>
<td>0.65(0.06)$</td>
<td>0.53(0.06)</td>
<td>0.35(0.05)</td>
<td>0.71 (0.04)</td>
<td>0.59(0.06)</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>4.00(0.10)</td>
<td>4.02(0.04)</td>
<td>3.49(0.16)</td>
<td>3.82(0.12)</td>
<td>3.74 (0.08)</td>
</tr>
<tr>
<td>Post</td>
<td>3.86(0.12)</td>
<td>3.95(0.05)</td>
<td>3.48(0.17)</td>
<td>3.69(0.09)</td>
<td>3.62 (0.07)</td>
</tr>
<tr>
<td>Glutathione (uM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>108.6(19.5)</td>
<td>86.7(25.0)</td>
<td>272.0(68.2)</td>
<td>156.6(31.9)</td>
<td>142.7(30.0)</td>
</tr>
<tr>
<td>Post</td>
<td>94.9 (26.1)</td>
<td>104.0(29.5)</td>
<td>232.4(65.0)</td>
<td>174.9(29.2)</td>
<td>165.2(37.0)</td>
</tr>
</tbody>
</table>

Values are means (standard error)

* Statistically significant different: P < 0.05, T2D on Juice, pre and post supplementation
$ Statistically significant different: P < 0.05, Control Subjects vs. T2D taking MJ
† statistically significant different: P < 0.05, Controls taking MJ vs. T2D taking Dz-W
Table 5: Cardiac Function and Coagulation Tendency for Control and Diabetic Subjects at Baseline and Post Supplementation

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects (CS)</th>
<th>Control Juice (CJ)</th>
<th>T2D Juice (DJ)</th>
<th>T2D Dz. Wine (Dz. W)</th>
<th>T2D Wine (DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Platelet</strong> ($10^3$/mm$^3$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>270.2 (21.2)</td>
<td>231.9(38.9)</td>
<td>232.1(33.6)</td>
<td>252.1 (25.6)</td>
<td>248.2(18.8)*</td>
</tr>
<tr>
<td>Post</td>
<td>237.4(22.0)</td>
<td>276.9(23.9)</td>
<td>274.1(32.8)</td>
<td>258.6 (23.2)</td>
<td>267.1(24.2)</td>
</tr>
<tr>
<td><strong>PT (Seconds)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>11.9(0.1)</td>
<td>11.9 (0.2)</td>
<td>11.4 (0.2)</td>
<td>12.0 (0.3)</td>
<td>12.9 (1.0)</td>
</tr>
<tr>
<td>Post</td>
<td>12.0(0.2)</td>
<td>12.0 (0.2)</td>
<td>11.5 (0.2) #§</td>
<td>12.3 (0.4)</td>
<td>12.9 (0.3)</td>
</tr>
<tr>
<td><strong>APTT (Seconds)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>27.2 (0.8)</td>
<td>29.4(1.3)</td>
<td>26.2(1.2)</td>
<td>25.9(1.3)</td>
<td>26.6(1.1)</td>
</tr>
<tr>
<td>Post</td>
<td>29.0(1.6)</td>
<td>29.0(1.3)</td>
<td>26.1(1.1)</td>
<td>26.6(1.4)</td>
<td>30.0(0.6)</td>
</tr>
<tr>
<td><strong>Fibrinogen (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>285.0(14.8)</td>
<td>351.0(35.1)</td>
<td>367.7(32.0)</td>
<td>366.1(22.7)</td>
<td>388.3(27.7)</td>
</tr>
<tr>
<td>Post</td>
<td>282.4(24.0)</td>
<td>360.7(35.9)</td>
<td>379.7(41.2)</td>
<td>417.2(31.1)</td>
<td>401.7(23.6)</td>
</tr>
<tr>
<td><strong>Homocysteine (umol/ L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>9.6(1.0)</td>
<td>8.8(0.5)*</td>
<td>12.2(1.7)</td>
<td>12.3(2.0)</td>
<td>13.9(1.5)</td>
</tr>
<tr>
<td>Post</td>
<td>9.1(0.8)</td>
<td>9.2(0.5)</td>
<td>11.2(0.9)</td>
<td>12.3(1.7)</td>
<td>13.7(2.0)</td>
</tr>
<tr>
<td><strong>Folate (ng/ ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>12.9(1.2)</td>
<td>11.0(1.6)</td>
<td>10.0(1.3)*</td>
<td>12.4(1.9)</td>
<td>10.0(1.3)</td>
</tr>
<tr>
<td>Post</td>
<td>12.3(1.0) †</td>
<td>9.6 (1.3)</td>
<td>11.1(1.5)</td>
<td>15.4(1.7) ¶</td>
<td>9.8(1.4)</td>
</tr>
<tr>
<td><strong>Vitamin B$_{12}$ (pg/ ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>465.9(40.8)*</td>
<td>580.0(72.6)</td>
<td>459.5(49.8)</td>
<td>528.4(52.5)</td>
<td>543.6(68.9)*</td>
</tr>
<tr>
<td>Post</td>
<td>422.8(38.9)</td>
<td>530.5(66.8)</td>
<td>460.4(50.9)</td>
<td>597.3(92.5) ¶</td>
<td>489.0(74.1)</td>
</tr>
<tr>
<td><strong>CPK (IU/ L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>239.8(83.7)</td>
<td>207.1(39.9)</td>
<td>185.9(55.4)</td>
<td>253.7(82.6)</td>
<td>227.7(61.2)</td>
</tr>
<tr>
<td>Post</td>
<td>165.6(35.6)</td>
<td>197.7(34.3)</td>
<td>220.4(73.2)</td>
<td>201.8(56.8) ¶</td>
<td>163.9(32.5)</td>
</tr>
<tr>
<td><strong>CK-MB (pg/ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>1.4(0.4)</td>
<td>1.7(0.6)</td>
<td>2.2(0.6)</td>
<td>3.2(1.3)</td>
<td>4.0(1.2)</td>
</tr>
<tr>
<td>Post</td>
<td>2.1(0.5)</td>
<td>1.7(0.6)</td>
<td>2.1(0.8)</td>
<td>4.6(1.8)</td>
<td>3.0(0.6)</td>
</tr>
<tr>
<td><strong>Troponin (ng/ ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>0.01(0.002)</td>
<td>0.02(0.003)</td>
<td>0.03(0.006)</td>
<td>0.02(0.01)</td>
<td>0.02(0.004)</td>
</tr>
<tr>
<td>Post</td>
<td>0.01(0.003)</td>
<td>0.01(0.002)</td>
<td>0.02(0.007)</td>
<td>0.03(0.02)</td>
<td>0.02(0.007)</td>
</tr>
</tbody>
</table>

Values are means (standard error)

* Statistically significantly different: P < 0.05, Pre vs. corresponding Post supplementation
† Statistically significantly different: P < 0.05, Control Subjects vs. T2D taking Dz-W
# Statistically significantly different: P < 0.05, T2D taking MJ vs. T2D taking MW
§ Statistically significantly different: P < 0.03, T2D taking MJ vs. T2D taking Dz-W
¶ Statistically significantly different: P < 0.05, T2D taking Dz-W vs. T2D taking MW
CPK = Creatine phosphokinase, CK –MB = Creatine Kinase – MB.
### Fatty Acids Constituting Saturated (SAT), Monounsaturated (MUFA) and Polyunsaturated (PUFA) Fatty Acids

**SAT**  
C12:0; C13:0; C14:0; C15:0; C16:0; C17:0; C18:0; C19:0; C20:0; C22:0; C23:0; C24:0

**MUFA**  
C12:1; C13:1; C14:1; C15:1; C16:1c; C16:1t; C17:1; C18:1n-9; C18:1n11; C19:1; C20:1; C22:1; C23:1; C24:1

**PUFA**  
C18:2; C18:2tt; C18:3n-6; C18:3n-3; C19:2; C20:2; C20:3n-3; C20:3n-6; C20:4; C20:5; C22:2; C22:3; C22:4; C22:5; C22:6
### Appendix 2

* Nutrient Composition of Red Grape Juice, Wine and Dealcoholized (Dz) Wine

<table>
<thead>
<tr>
<th></th>
<th>Juice</th>
<th>Wine</th>
<th>Dz. Wine</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Macronutrients</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy (Kcal)</td>
<td>96.0</td>
<td>126.8</td>
<td>8.83</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>0.88</td>
<td>0.31</td>
<td>0.74</td>
</tr>
<tr>
<td>CHO (g)</td>
<td>23.5</td>
<td>3.8</td>
<td>1.62</td>
</tr>
<tr>
<td>Fat (total) (g)</td>
<td>0.13</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Alcohol (g)</td>
<td>0.00</td>
<td>12.9</td>
<td>0.00</td>
</tr>
<tr>
<td>Saturated Fat (g)</td>
<td>0.39</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Monounsaturated Fat (g)</td>
<td>0.01</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Polyunsaturated Fat (g)</td>
<td>0.035</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Dietary Fiber (g)</td>
<td>0.16</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td><strong>Vitamins</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Vit A (RE)</td>
<td>1.57</td>
<td>0.00</td>
<td>0.00</td>
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<tr>
<td>Vit. C (mg)</td>
<td>0.16</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Vit. E (mg)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Thiamin (mg)</td>
<td>0.04</td>
<td>0.02</td>
<td>0.00</td>
</tr>
<tr>
<td>Riboflavin (mg)</td>
<td>0.06</td>
<td>0.04</td>
<td>0.02</td>
</tr>
<tr>
<td>Niacin (mg)</td>
<td>0.41</td>
<td>0.12</td>
<td>0.15</td>
</tr>
<tr>
<td>Pyridoxine (mg)</td>
<td>0.10</td>
<td>0.05</td>
<td>0.03</td>
</tr>
<tr>
<td>Folate (ug)</td>
<td>4.12</td>
<td>1.49</td>
<td>1.47</td>
</tr>
<tr>
<td>Vit. B12 (ug)</td>
<td>0.00</td>
<td>0.02</td>
<td>0.00</td>
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<tr>
<td>Biotin (ug)</td>
<td>ND</td>
<td>0.90</td>
<td>ND</td>
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<tr>
<td><strong>Minerals</strong></td>
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<tr>
<td>Sodium (mg)</td>
<td>4.72</td>
<td>15.22</td>
<td>10.30</td>
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<tr>
<td>Potassium (mg)</td>
<td>207.6</td>
<td>173.0</td>
<td>129.44</td>
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<tr>
<td>Calcium (mg)</td>
<td>14.2</td>
<td>11.94</td>
<td>13.24</td>
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<tr>
<td>Iron (mg)</td>
<td>0.38</td>
<td>1.40</td>
<td>0.59</td>
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<tr>
<td>Phosphorus (mg)</td>
<td>17.3</td>
<td>19.39</td>
<td>22.06</td>
</tr>
<tr>
<td>Magnesium (mg)</td>
<td>15.7</td>
<td>16.41</td>
<td>14.71</td>
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<tr>
<td>Zinc (mg)</td>
<td>0.08</td>
<td>0.15</td>
<td>0.12</td>
</tr>
<tr>
<td>Copper (mg)</td>
<td>0.04</td>
<td>0.04</td>
<td>0.02</td>
</tr>
<tr>
<td>Manganese (mg)</td>
<td>ND</td>
<td>0.26</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND = Not determined
* 150 ml of each product analyzed using Nutritionist Pro (First Data Bank, Inc., San Bruno, CA)