CRANBERRY FLAVONOID SUPPLEMENTATION AND BIOMARKERS OF OXIDATIVE STRESS IN SUBJECTS WITH METABOLIC SYNDROME

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CHAPTER I

INTRODUCTION

Diet and lifestyle remain principal factors in the prevention and treatment of chronic disease. Epidemiologic evidence has consistently indicated fruit and vegetable consumption in the prevention of cardiovascular disease as well as other chronic diseases (1, 2). The proposed mechanism underlying this correlation includes the high phytochemical content of fruits and vegetables which have been shown to exhibit antioxidant and immune regulatory activities in the body (3). Phytochemicals are non-nutritive, plant compounds that, due to their biological activity, are related to a reduced risk of chronic health conditions including cancer as well as cardiovascular disease (4).

A specific chronic health condition that plagues the United States is obesity which, according to data collected from NHANES, affects 33.8% of Americans (5). Obesity is characterized by excess adipose tissue and is associated with an increased risk of cardiovascular disease (CVD), osteoarthritis, type 2 diabetes mellitus (T2D) and certain cancers (6). Although obesity is a risk factor for chronic disease development, it is not a strong predictor of CVD development alone. However, obesity related comorbidities clustered together are more accurate predictors of CVD and T2D development. This

clustering of co-morbitities is collectively termed metabolic syndrome and while its etiology is multifactoral, oxidative stress has been suggested as a contributor (7).

Oxidative stress occurs when production of free radicals exceeds the antioxidant capacity of the body (8). Free radicals are highly reactive and can induce damage to a variety of cells and tissues. Dietary antioxidants such as vitamins A, C and E as well as phytochemicals have been investigated for their role in combating oxidative stress and subsequent biological damage.

Cranberries are a good source of phytochemicals including polyphenols. Due to their high phytochemical content, cranberries have been the focus of research regarding chronic disease prevention and reduction of oxidative stress. In fact, certain studies have observed improvements to certain aspects of metabolic syndrome including blood pressure and cholesterol with various cranberry interventions (9, 10). However, no studies have examined the effects of cranberry juice supplementation in individuals with metabolic syndrome which was the focus of our study.

The purpose of our study was to investigate the effects of low calorie cranberry juice supplementation on biomarkers of oxidative stress (oxLDL and MPO) and clinical features of metabolic syndrome (blood pressure, waist circumference, triglycerides, glucose and HDL cholesterol) in obese adults.

The null hypotheses were:

1. Low calorie cranberry juice supplementation will have no effects on features of metabolic syndrome (waist circumference, BP, HDL, blood glucose, TG)

2. Low calorie cranberry juice supplementation will have no effects on biomarkers of oxidative stress associated with metabolic syndrome.

CHAPTER II

REVIEW OF THE LITERATURE

Obesity has become a growing concern worldwide as the prevalence has increased dramatically in the last few decades (11). The World Health Organization (WHO) has defined a body mass index (BMI) over 25 kg/m^2 as overweight and > 30 kg/m^2 as obese (12). Gradual but substantial shifts in American culture and lifestyle have contributed to progressive incidence of obesity. Factors such as decreased physical activity and overconsumption of energy dense, nutrient poor foods have all been implicated in the growing epidemic (13). Obesity can have significant effects on homeostasis by altering blood pressure, glucose metabolism and serum lipids. Consequently, several comorbidities are associated with obesity including: T2D and CVD (14). Research has indicated a positive correlation between BMI and risk of developing T2D or dyslipidemia, a precursor to CVD (13, 15). These related diseases can significantly reduce life expectancy and represent a significant challenge to public health and wellness.

Metabolic Syndrome

The obesity related comorbidities manifest themselves in what is commonly termed "metabolic syndrome". BMI or obesity alone are not a strong enough predictors for CVD risk when compared to a collection of features that characteristically alter normal

metabolic function. Stated simply, metabolic syndrome is a clustering of risk factors when present in an individual can promote the development of T2D and CVD. In 2009, a joint scientific statement was released by the International Diabetes Federation Task Force on Epidemiology and Prevention, National Heart, Lung, and Blood Institute, American Heart Association, World Heart Federation, International Atherosclerosis Society and International Association for the Study of Obesity (16). The statement reviewed the existing criteria for clinical diagnosis of metabolic syndrome as recommended by various organizations and proposed common criteria for clinical diagnosis. The new recommendations advise that three or more of the following constitute a diagnosis of metabolic syndrome: waist circumference varies depending upon population and country but for individuals of European origin: $\geq$35 inches in women, 40 in men, triglyceride level $\geq$ 150 mg/dL, HDL level $\leq$ 50 mg/dL in women, 40 mg/dL in men, blood pressure $\geq$ 130/85 mm Hg and fasting blood glucose $\geq$ 100 mg/dL. Aside from the diagnostic criteria, metabolic syndrome is characterized by altered lipid and carbohydrate metabolism, inflammation, endothelial dysfunction and abnormal oxidative stress (17-20). A diagnosis of metabolic syndrome increases individual risk for developing T2D fivefold and doubles the risk of CVD development (21). The collection of individual risk factors that were previously thought to be independent of one another is now revealing a more involved metabolic relationship that can result in substantial health problems.

In regards to the basis of the diagnostic criteria, visceral obesity as measured by waist circumference provides a better assessment of metabolic risk as opposed to subcutaneous

adiposity. Visceral obesity has been indicated in the development of insulin resistance and the elevation of inflammatory markers that have significant vascular consequences (14). Insulin resistance has been suggested as the primary connection between each of the diagnostic criteria of metabolic syndrome (22). It has been suggested that the effect of insulin resistance on the normal metabolic state is substantial and can negatively impact blood pressure, cholesterol and triglyceride levels significantly enough to elevate CVD risk.

Metabolic Syndrome and Oxidative Stress

While many factors have been implied in the etiology of metabolic syndrome, research has indicated a strong association between oxidative stress and the aforementioned comorbidities related to metabolic syndrome (23). Oxidative damage is most easily defined as the imbalance in the reactive species including reactive oxygen species (ROS) produced from normal metabolic activity, namely aerobic respiration, and the antioxidants that eliminate or neutralize the highly reactive free radicals. An impaired balance between antioxidants and prooxidant radicals such as ROS allows for uncontrolled reactions between the ROS and proteins and lipids thereby inducing tissue damage. Damage can include impaired endothelial cell function from lipid peroxidation, damage to DNA, degradation of structural proteins and inactivation of enzymes (23).

The endogenous antioxidant defense system employs various mechanisms to reduce the concentration of circulating free radicals including superoxide anions, peroxides and hydroxyl radicals (3). These mechanisms protect the body from free radical attack and subsequent damage. Mechanisms include enzymatic and non-enzymatic free radical

scavengers. Enzymatic scavengers consist of: superoxide dismutase (SOD) which scavenges superoxide radicals, catalase (CAT) which neutralizes hydrogen peroxide thereby finishing the detoxification started by SOD and glutathione peroxidase (GPx) which neutralizes hydrogen peroxide and other organic peroxides (3, 24). Non-enzymatic scavengers include: vitamin C which reduces a variety of radicals, vitamin E that protects cell membranes from damage by trapping radicals, glutathione (GSH) and dietary phytonutrients among others. If these antioxidant mechanisms become overwhelmed or down regulated resulting in oxidative stress, certain biomarkers present in the blood can be identified. These biomarkers can include myeloperoxidase (MPO), oxidized LDL cholesterol and malondialdehyde (MDA).

Markers of Oxidative Stress

Myeloperoxidase is an enzyme secreted by activated monocytes and neutrophils that, once secreted, can form free radicals including ROS and RNS (25). Furthermore, MPO can oxidatively modify lipids as it acts as an enzymatic catalyst in the oxidation of LDL-C. The formation of free radicals and the oxidation of lipids promote oxidative stress in the body. Oxidized LDL-C is an important biomarker as its uptake into the endothelium is required for the development of atherosclerosis (26). LDL-C must be modified by free radical or enzymatic oxidation in order for the atherosclerotic process to initiate. Because LDL-C must be oxidized by products which promote oxidative stress, Ox-LDL-C provides a reasonable measure of oxidative stress in the body. MDA is an end product of lipid peroxidation that has been shown to have carcinogenic and mutagenic activities (27).

In regards to the role of oxidative stress in metabolic syndrome, several studies have highlighted the various mechanisms at play in the onset of this condition. For example, when examining the biomarkers of oxidative stress in obese individuals, Furukawa et al found that TBARS, a marker of lipid peroxidation, was positively correlated with both BMI and waist circumference (28). Additionally, they noted an inverse correlation between adiponectin, a protective anti-inflammatory adipokine, and both BMI and waist circumference. Furukawa et al then hypothesized that fat accumulation alone without the influence of hyperglycemia was enough to elevate oxidative stress ultimately leading to the dysregulation of adipokine production. These factors, oxidative stress, adipokine dysregulation and obesity can be the beginning of a complex change in the metabolic state. More recently, Girona et al reported data indicating that diabetic patients have elevated oxidative stress as quantified by markers of lipid peroxidation (29). Furthermore, oxidized LDL was positively correlated with metabolic syndrome and the development of atherosclerosis in diabetic patients. These findings illustrate the health risks associated with both oxidative stress and metabolic syndrome.

Fruit and Vegetable Intake and CVD

Epidemiologic studies have frequently shown a reduced risk of CVD development with increasing consumption of fruits and vegetables (1). In fact, data from the Health Professionals Follow-up study and Nurses Health Study with a follow up duration of 14 years indicated that 8 servings per day of fruits and vegetables can reduce the risk of coronary heart disease by nearly 20% (30). Additionally, results from meta-analysis examining the relationship between prevalence of ischemic heart disease and fruit and vegetable intake showed a reduced risk of ischemic heart disease in individuals

consuming roughly 5 servings of fruits and vegetables daily (31). Among the proposed mechanisms underlying this correlation, growing attention has been placed on the role of phytochemicals in the prevention of CVD.

Phytochemicals

According to the USDA, plant chemicals commonly referred to as phytochemicals are plant components shown to promote human health by scavenging free radicals, up regulating immune responses and repairing injured DNA (32). Extensive research has been conducted to further understand the possible mechanisms that result in positive health benefits from foods containing phytochemicals. Phytochemicals can be categorized into various classes including carotenoids, alkaloids, phenolics, nitrogen containing compounds and organosulfur compounds and of the multiple classes of phytochemicals, phenolics including flavanoids and polyphenols have been particularly of interest in research regarding cardiovascular disease (33). Phenolic compounds are identified by their aromatic rings with attached hydroxyl groups which provide the free radical scavenging potential (34).

Flavonoid consumption is inversely associated with risk of heart disease (35). Research has indicated that flavonoids and polyphenols have potent free radical scavenging abilities and may reduce inflammation, modify lipid profiles, reduce platelet aggregation and reduce blood pressure (36-39). Foods containing polyphenols include: wine, tea, soybeans, vegetables and citrus fruits. Fruits such as strawberries, raspberries, blueberries and cranberries are particularly rich in the subclass of flavonoids called anthocyanidins. Anthocyanidin components contribute to the deep red, purple, blue or

black colors in berries and possess both anti-inflammatory and antioxidant activities that may positively benefit cardiovascular health (40, 41).

Cranberries

Cranberries are a good source of phenolic phytochemicals like anthocyanidins and have recently been under investigation regarding their effects on cardiovascular disease and inflammation (37). According to the USDA, the free radical scavenging ability of cranberries as measured by oxygen radical absorbance capacity (ORAC) is among the highest of other fruits and vegetables (42). However, as seen in Table 1, cranberries and cranberry products are not naturally high in antioxidant vitamins C or E. Therefore, it can be assumed that the antioxidant ability observed in cranberries is due to their high polyphenolic concentration, as noted in Table 2.

Many of the flavonoids present in cranberries can reduce oxidative stress by inhibiting prooxidant enzymes, directly scavenging free radicals and activating antioxidant enzymes like SOD, CAT and peroxidase in the body (41). It is important to note, however, that the antioxidant activity depends greatly upon the cultivation and environmental circumstances and conditions that can affect the berry's phytochemical content (40). These factors include but are not limited to: temperature, water and nutrient availability, exposure to sunlight, soil content or treatment with heavy metals and maturity of plant at time of harvest (43). Although these factors can potentially alter the phytochemical content of cranberries, Viskelis et al found similar anthocyanin content between berries of four separate cultivars (40). Furthermore, there was no significant difference in the average radical scavenging activity of all the studied cranberry cultivars.

Table 1: Nutritional Profile of Cranberry Products per 1 cup

Nutrient	Whole Raw	Dried sweetened	Juice unsweetened	Juice cocktail
Energy (kcal)	46.00	308.00	46.00	137.00
Water (g)	87.13	16.00	87.13	218.00
Protein (g)	0.39	0.07	0.39	0.00
Carbohydrate (g)	12.20	82.39	12.20	34.21
Fat (g)	0.13	1.37	0.13	0.25
Fiber (g)	4.60	5.70	0.10	0.00
Potassium (mg)	85.00	40.00	77.00	35.00
Sodium (mg)	2.00	3.00	2.00	5.00
Vitamin C (mg)	13.30	0.20	9.30	107.00
Vitamin E (mg)	1.20	1.07	1.20	0.56
β-Carotene (mg)	36.00	52.00	27.00	13.00

Source: USDA National Nutrient Database

Table 2: Flavonoid Profile of Cranberry Products

Food	Class	Flavonoid	Mean
Raw			
	Anthocyanidins		
		Cyanidin	41.81
		Delphinidin	7.66
		Peonidin	42.10
	Flavonols		
		Myricetin	6.78
		Quercetin	15.09
Dried, sweetened			
	Anthocyanidins		
		Cyanidin	0.60
		Delphinidin	0.10
	Flavonols		
		Myricetin	2.40
		Quercetin	4.50
Juice cocktail			
	Anthocyanidins		
		Cyanidin	0.38
		Delphinidin	0.03
	Flavonols		
		Myricetin	.51
		Quercetin	1.27
Juice, unsweetened			
	Flavonols		
		Myricetin	4.41
		Quercetin	16.41

Units: mg/ 100 g edible portion
Source: USDA Flavonoid Database 2007

Cranberry Intervention: Human Studies

Research examining the effects of phenolics and cranberries on markers of oxidative stress including LDL oxidation has varied and is considerably limited. Ruel et al (2008) supplemented 31 healthy men with increasing doses of cranberry juice every 4 weeks for 12 weeks following a run in period in which participants consumed a low calorie cranberry juice placebo (44). Blood pressure and total cholesterol (TC), triglycerides (TG), very low density lipoproteins (VLDL), high density lipoprotein (HDL), low density lipoprotein (LDL-C) and Ox-LDL-C were measured. Significant reductions in Ox-LDL-C and significant increases in plasma HDL were reported although changes in LDL-C failed to reach significance. Also, a reduction in plasma triglycerides that did not reach significance was noted. Lee et al (2008) examined the effects of cranberry extract supplementation on lipid profiles of individuals with T2D (9). In this randomized, placebo-controlled, double-blind study, 30 type 2 diabetic patients taking oral glucose lowering medication were enrolled and instructed to take 3 capsules of cranberry extract or placebo daily for 12 weeks. Blood pressure was recorded and fasting blood was drawn at baseline and 12 weeks. HDL, LDL-C, TC, TG, Ox-LDL-C, fasting glucose, hemoglobin A_{1c} (HbA_{1c}) and C-reactive protein were assessed. This study revealed a significant reduction in TC and LDL-C following cranberry extract supplementation daily for 12 weeks. However, no significant changes in oxidized LDL, HDL, TG or C-reactive protein levels were reported. Pedersen et al conducted a postprandial study investigating the effects of blueberry and cranberry juice consumption on plasma antioxidant status (45). Nine healthy female volunteers consumed 500 ml of either blueberry, cranberry or placebo juice after an overnight fast on three separate occasions one week apart. On each

occasion, the participant would consume a different beverage. Blood was drawn 5 minutes before and 30 minutes, 1, 2 and 4 hours postprandial and analyzed for antioxidant capacity by two different methods. The data indicated that only the cranberry juice caused a significant increase in plasma antioxidant capacity although the FRAP value of the blueberry juice was twice that of the cranberry juice. The increase in total plasma antioxidant activity was still evident at 4 hours postprandial. While these results point to the free radical scavenging ability of the cranberry, it is difficult to ascertain if the elevation in plasma antioxidant activity can be attributed to cranberry phenolics, to the vitamin C with which the juice was fortified or to the combined effects of the antioxidants present in the cranberry juice.

While previous studies have reported significant results regarding cranberry's effects on LDL oxidation and other lipid markers for metabolic syndrome, other studies have failed to show significant data supporting the theory of cranberry's phenolic cardioprotective ability. Duthie et al (2006) sought to examine the effects of a 2 week cranberry juice supplementation on oxidative stress markers including lipid values in 20 healthy females (46). Participants were asked to consume either cranberry juice or a placebo drink daily for two weeks. Fasting plasma was drawn at baseline, 0, 1, 2 and 3 weeks and analyzed for lipid profile, MDA, CAT, SOD, homocysteine and phase II enzymes: GSH and GPx. No changes in TC, TG, HDL or LDL were detected. Additionally, phase II enzymes, homocysteine and MDA were not significantly different from baseline or compared to placebo.

The apparent variability of findings between these studies may be explained by differences in population groups being investigated. The human intervention studies that

reported improvement in blood lipid profiles examined participants with average BMIs above 26 and with elevated waist circumference and the study by Lee et al (9) included individuals with T2D who were on glucose lowering medication. The human intervention studies that reported no significant changes in lipid profiles involved healthy individuals. The disparity among the conclusions of these studies indicates a possible link between health status and potential lipid lowering benefits from cranberry phytochemicals. Another explanation may be the design of the studies which could have contributed to these effects. Possible differences including the length of the intervention, analytical differences, type of cranberry product employed and the dose administered during the intervention may have contributed to the conflicting conclusions. For example, the two intervention studies that showed positive effects of cranberry supplementation on lipid levels were of 12 week duration in contrast to the two interventions showing no effects which were either a 2 week intervention or a postprandial study.

In Vitro Studies: Cranberry Juice and Extracts

In vitro studies can provide valuable insight into the mechanisms of action underlying the hypothesized antioxidant activity of cranberries. Cell culture studies have produced more consistent findings in regard to the hypolipidemic and free radical scavenging activity of cranberry phenolics compared to human studies. One such study by Wilson et al reported inhibition of LDL oxidation in vivo when exposed to diluted cranberry extract (47). This study obtained blood samples from 5 healthy male volunteers followed by isolation of LDL-C. The LDL particles were oxidized following incubation with 0, 0.10, 0.05, 0.01 or 0.005% of diluted cranberry juice and then analyzed for TBARS. LDL oxidation was

inhibited significantly in the presence of 0.10% diluted cranberry juice. This study shows effects of cranberries to favorably modify oxidative stress; however, it must be noted that digestion and absorption can modify phenolic compounds and the bioavailability of other compounds may be limited. Similar findings were established in a two part study conducted by Chu and Liu (48). Again, blood samples were collected from healthy males, LDL was isolated and oxidized in the presence of varying concentrations of fresh cranberry extract and analyzed for oxidation. Additionally, human liver cells were procured and incubated with varying concentrations of cranberry extract to investigate effects on LDL receptor expression and intracellular cholesterol levels. Data revealed that LDL oxidation was completely inhibited with 10 mg/ml cranberry extract and LDL receptor expression in the hepatocyte increased over 5 fold. Moreover, intracellular cholesterol increased nearly 3 fold, indicating an elevation in cholesterol uptake and efficiency. This study dramatically attests to the potent lipid modulating potential of cranberries.

Not all in vitro studies have found such notable findings. For example, Porter et al sought to determine the classes of cranberry flavonoids strongly associated with preventing LDL-C oxidation (49). This study treated LDL-C isolated from human plasma with 6 individual phenol fractions isolated from concentrated cranberry powder. The plasma mixtures and control mixture were then oxidized using Cu^{2+} and assessed for oxidation of LDL-C every three minutes for 300 minutes. The lag time between Cu^{2+} exposure and oxidation of LDL-C was assessed and reported. Of the 6 cranberry phenolic fractions, only fractions containing proanthocyanidins were noted to have significantly different lag times of LDL-C oxidation compared to control. The other

fractions which included flavonols, anthocyanidins, or cinnamic acids showed no significant increase lag time indicating that only proanthocyanidins in isolated form were capable of protecting LDL-C from oxidation. This study demonstrates that isolated cranberry flavonols were not effective modifiers of LDL-C oxidation. A similar study investigating the effects of phenolic compounds on oxidation of LDL found that quercetin, a major polyphenol present in cranberries, had no effect on rates of LDL oxidation (50). This study similarly isolated LDL-C from 65 healthy volunteers and oxidized the LDL-C following treatment with 5 different concentrations of various isolated phenolic compounds including quercetin, catechin, hesperidin and ferulic acid. The LDL-C was then measured for markers of oxidative stress as quantified by TBARS. The data indicated no significant reduction in the oxidation of LDL-C following treatment with quercetin. In fact, the researchers actually observed a prooxidant effect of quercetin at all concentration levels. The researchers proposed that the chemical structure of quercetin was correlated to its apparent prooxidant activities at various concentrations particularly in the presence of Cu^{+} and not hydrogen peroxide. The data from this study support the theory that individual phytochemical supplementation may not be as effective as whole foods or extracts. Since epidemiological data support the role of fruits and vegetables in the prevention of CVD, perhaps it is a synergistic effect of the various nutrients, fiber and phytochemicals in whole fruits that contributes to this prevention.

Animal Studies

Animal studies have shown interesting findings in regard to cranberry supplementation and CVD risk factors including its effects on vasoconstriction, hypertension,

hyperglycemia and dyslipidemia. These studies provide additional insight into the potential effects of cranberry flavonoids on features of CVD beyond that of cell models as systemic effects can be observed. Juzwiak and colleagues conducted a randomized controlled supplementation study in rabbits to examine the effects of quercetin supplementation on hyperlipidemia and development of atherosclerosis (51). Following 2 weeks of acclimation, 30 male rabbits were assigned to one of 3 experimental groups: standard diet, high fat diet or high fat diet + 0.05 mg/kg/d quercetin supplementation. After 12 weeks, animals were fasted for 18 hours then sacrificed and TC, HDL-C, LDL-C, VLDL-C, TG, Cyt P450 and MDA were analyzed. Additionally, the aortic intima was dissected and evaluated for atherosclerotic plaques. After evaluation, the data revealed that the high fat diet group had significant increases in TC, HDL-C, LDL-C, VLDL-C and TG compared to the control group. However, the high fat diet + quercetin supplementation group had significantly reduced levels of TC, LDL-C, VLDL-C and TG compared to the high fat diet group. Also, Cytochrome P450, a liver enzyme involved in cholesterol metabolism, was significantly reduced in the high fat diet group compared to controls; however, no significant changes were observed in quercetin supplemented rabbits. Quercetin supplemented rabbits fed a high fat diet were noted to have lower MDA; however, no significant differences were observed between groups. Additionally, atherosclerotic plaques were significantly developed in the high fat diet rabbits with 24.6 +/-33.1% of the aorta covered. However, quercetin supplemented rabbits had significantly less area of the intima covered by atherosclerotic plaques (0.7 +/- 1.28%) compared to rabbits fed solely the high fat diet. This study indicates the hypolipidemic and antiatherosclerotic potential of quercetin and proposes a possible mechanism of the

lipid lowering effect. The researchers hypothesized that quercetin in the high fat diet possibly activated cytochrome P450 as evidenced by the difference in values between high fat diet groups. Cytochrome P450-dependent 7α-hydroxylase is involved in cholesterol metabolism and quercetin supplementation may stimulate the conversion of cholesterol to bile acids thus partially explaining its hypolipidemic effect.

Another study sought to examine the effects of cranberry juice supplementation on lipid profiles and antioxidant status in orchidectomized rats (52). Orchidectomized rats were investigated because research has identified low male sex hormones as an independent risk factor for hyperlipidemia and hypercholesterolemia in men thus directly contributing to the risk of CVD. This randomized control study divided 32 male rats into experimental groups: sham surgery + water, orchidectomy (ORX) + water, ORX + 27% cranberry juice or ORX + 45% cranberry juice and were sacrificed after four months. Antioxidant capacity, MDA, plasma and hepatic cholesterol and TG and liver SOD were assessed. Data showed that compared to sham group the ORX + water group had significantly lowered antioxidant capacity and liver SOD and significantly higher MDA, cholesterol and TG. The antioxidant capacity of cranberry supplemented groups, however, was not significantly different than the sham group. Also, MDA was significantly reduced in a dose dependent manner with cranberry juice supplemented rats. While this study demonstrated the possible action of cranberry juice supplementation in reducing hepatic triglycerides, it was not associated with any improvements to plasma lipid profile. Aside from dyslipidemia, hypertension is a major risk factor for CVD and thus cranberries effects on vasodilation were investigated.

In an ex vivo study by Maher et al, rat aortae were removed from animals and subjected to phenylephrine (PE) contraction and acetylcholine induced relaxation and tension was measured (10). Diluted cranberry juice was added before the next contraction and relaxation. The in vivo aspect of the study involved supplementing anesthetized rats with diluted cranberry juice to observe change in blood pressure and heart rate. The results showed significant vasodilation after cranberry juice exposure. The anesthetized rats showed a reduction in mean arterial blood pressure by 16% suggesting significant anti-hypertensive and vasodilatory effects of cranberries and their phytochemicals. A different study reported by Kim et al examined the effects of cranberry powder supplementation on biomarkers of oxidative stress (53). In this study, rats were divided into 4 groups: normal diet, atherogenic diet, atherogenic diet + 2% cranberry and atherogenic diet + 5% cranberry. The rats were fed ad libitum for 6 weeks and then sacrificed after a 12 hour fast. Blood was taken and analyzed for lipid profile, TBARs, SOD, protein carbonyl and total soluble phenolic compounds. The data revealed that HDL had been significantly increased in the 5% cranberry group and markers of protein and lipid oxidation had been significantly reduced in groups supplemented with cranberry powder. Although there were no significant changes in the LDL, triglycerides, total cholesterol, FRAP or SOD activity, this study did show a significant potential for cranberry to protect other biomolecules from oxidative damage. A different study evaluated the effect of quercetin on glucose uptake into pig brush border membranes (54). The results of this study indicated a significant decrease of glucose uptake into the brushborder in the presence of quercetin and suggested competitive inhibition with SGLT1 as a mechanism. This study illustrates a novel concept of the cranberry flavonoid

which may help control glucose absorption and transport as mediated through the glucose transporters (SGLTs).

The aforementioned studies illustrate the growing body of evidence supporting the role of cranberries in wellness and health promotion. Specifically, research has shown modest effects of cranberries and cranberry phenolics at improving lipid levels and markers of oxidative stress. Mechanistic studies at the cellular level have not shown significant benefit of isolated cranberry phenolic compounds. In fact, prooxidant effects were reported with isolated quercetin supplementation. Animal studies, however, have shown significant positive modifications to lipid levels with quercetin supplementation and modest results on antioxidant status when supplementing cranberry juice. Although results have been mixed, human studies have indicated a preventative role of cranberries as indicated by observed reductions in markers of oxidative stress as well as lipid levels. Studies have shown significant improvements in blood lipids in both healthy and diabetic subjects. While the results of the studies offer conflicting evidence as to the efficacy of cranberries and their phenolics, much of the research is preliminary. Because few studies have focused solely on berry flavonoids and even fewer have concentrated on cranberry phenolics, more studies are needed to ascertain the efficacy of whole cranberry products, cranberry extracts and capsules. Because cranberry products, particularly juice, are commonly available in the United States, the potential of cranberry juice to favorably modify risk factors associated with the development cardiovascular disease should be examined.

CHAPTER III

METHODS

Institutional Review Board Approval

The cranberry juice supplementation study, its protocol and all procedures were approved by the Oklahoma State University Institutional Review Board (IRB) and were conducted in accordance to the guidelines of the Declaration of Helsinki. All graduate research assistants (GRAs) and investigators were trained in human subject research practices and completed IRB training through Collaborative Institutional Training Initiative (CITI) prior to involvement in the study. Additionally, all GRAs received instruction on the process of consenting and explaining the study protocol to participants, safe food handling and preparation of cranberry juice or control, subject follow up and data collection. Prior to involvement or enrollment in the study, participants were instructed to read and sign an informed consent document.

Subjects

Subjects were recruited at Oklahoma State University using various media outlets. Advertisements were posted on the College of Human Environmental Sciences webpage as well as in the Oklahoma State University Headlines and flyers were posted in the Nutritional Sciences Department. Interested subjects were initially screened by the

principal investigator via telephone questionnaire and all potential participants were scheduled for a screening appointment to ensure all participants met inclusion criteria.

The initial screening consisted of blood pressure, weight, height and waist circumference measurements as well as blood draws. Participants were informed of the purpose of the study, the protocol and any potential risks or benefits associated with participating in the study prior to the screening blood draw. Additionally, participants were provided with an informed consent document which was read and signed before any other screening procedures were conducted. Participants unable to read the informed consent document were read the consent by a GRA or, if necessary, were given the option to have an interpreter explain the consent process as well as the purpose and protocol of the study. Inclusion and exclusion from the study was determined by the inclusion and exclusion criteria based on the results of the initial screen. Individuals were notified by phone of their participation status.

Adult participants meeting the following criteria were eligible for study inclusion: possession of any three of the five features of metabolic syndrome as outlined in the NCEP guidelines (waist circumference $\geq$35 inches in women, 40 in men, triglyceride level $\geq$ 150 mg/dL, HDL level $\leq$ 50 mg/dL in women, 40 mg/dL in men, blood pressure $\geq$ 130/85 mm Hg and fasting glucose $\geq$ 100 mg/dL), on stable medications and have normal white blood cells (WBC), platelet count, hemoglobin (Hb) as well as kidney, liver and thyroid function test. The exclusion criteria for our study included any form of pre-existing disease including diabetes, cardiovascular disease, cancer, liver or kidney diseases or anemia. Furthermore, subjects who were pregnant, nursing or taking any glucose or lipid lowering medication or

mega doses of fish oil or antioxidant supplements were excluded from the study. Tobacco usage and alcohol consumption were also criteria for exclusion.

Study Design

This study was a blind, cross-over control intervention. Ten subjects meeting the inclusion criteria were recruited and randomly assigned to either a treatment group or control group. Participants consumed two cups daily of placebo juice or cranberry juice for four weeks. Both the commercially processed and pasteurized cranberry and control juices were provided by Ocean Spray Cranberries Inc. (MA, USA). The placebo juice contained no cranberry juice and matched the cranberry juices in terms of macronutrient content. After four weeks, participants entered a two week washout period in which no treatment or placebo was administered. Following the washout period, participants were switched groups and followed the same procedure. Participants visited the research site three times weekly to pick up their supply of juice and confirm adherence.

Anthropometrics (height, weight and waist circumference), blood pressure and blood draws were obtained at screen and at the end of each intervention period. All blood draws were performed by a certified phlebotomist and participants were compensated $30 per blood draw excluding screening visit for study participation. Participants were asked to maintain their usual lifestyle, diet and physical activity during the study. Participants were asked to complete a three day food record during screen and each intervention period. Each weekly food record was to consist of one weekend day and two weekdays to provide an estimate of usual dietary intake. Subjects were instructed on how to accurately report type and amount of food as well as classification of type of meal (snack, lunch, supper, etc.) and location.

Materials

Anthropometrics: Participant body weight was determined using the Health-o-Meter tracking scale and the Gulick II tape measure was employed to determined waist circumference in inches at the super iliac crest. Participant height was measured in centimeters using a standiometer.

Blood Pressure: Using a portable blood pressure device, Spot Vital Signs Device, (Welch Allyn, Skaneateles Falls, NY) systolic and diastolic blood pressure measurements were recorded three separate times after a period of sitting. The average of the three measurements was recorded.

Blood Draws: Following an overnight fasting period, blood was collected from participants using anticoagulant EDTA and serum separator tubes. Serum samples were analyzed for glucose, insulin, lipid level, $HbA_{1c,}$ platelets, hemoglobin and hematocrit at Stillwater Medical Center (Stillwater, OK). Serum and plasma samples were separated by centrifugation, flushed with nitrogen and stored at -80^{o}C until analyses of oxidized LDL and myeloperoxidase (MPO).

Dietary Nutrient Intakes and Cranberry Beverage Polyphenol Content: Polyphenol content including polyphenols, anthocyanidins and proanthocyanidins as well as macronutrient profiles were provided by the manufacturers of the cranberry juice and placebo drinks (Ocean Spray). Food records completed by participants were analyzed to evaluate any dietary changes that might alter results of the trial. Following food record submission, dietary analysis was completed for each participant at week one and final week of study participation. Dietary analysis was completed using ESHA Food Processor 9.1.0 (ESHA

Research Inc., Salem, OR). Food records were analyzed for calories, total fat, protein, carbohydrates, fiber, vitamins A, D, E and K as well as iron and zinc.

Biomarkers of Oxidative Stress

Biomarkers of oxidative stress including oxLDL (oxLDL) and myeloperoxidase (MPO) were measured from serum samples obtained from participants at screen, end of placebo and end of juice intervention. Serum oxLDL was measured in duplicate using competitive ELISA (Mercodia, Uppsala, Sweden). In this assay, oxLDL from the participant's sample competed with a fixed amount of oxLDL in the well for binding of the biotin-labeled specific antibodies. The biotin-labeled antibody bound to the well was then detected by strepdavadin following a washing step that removed components of the sample that were unreactive. The wells were incubated and washed a second time then treated with 3, 3', 5, 5'-tetramethylbenzidine (TMB) to detect bound conjugate. Following the addition of stop solution to halt the reaction, the sample was read spectrophotometrically at 450 nm using the Synergy HT plate reader (BioTek Instruments, Inc., Winooski, VT).

25 µl of each sample diluted with 1,000 µl sample buffer

↓

50 µl of each sample dilution and calibrators placed in well plate in duplicate

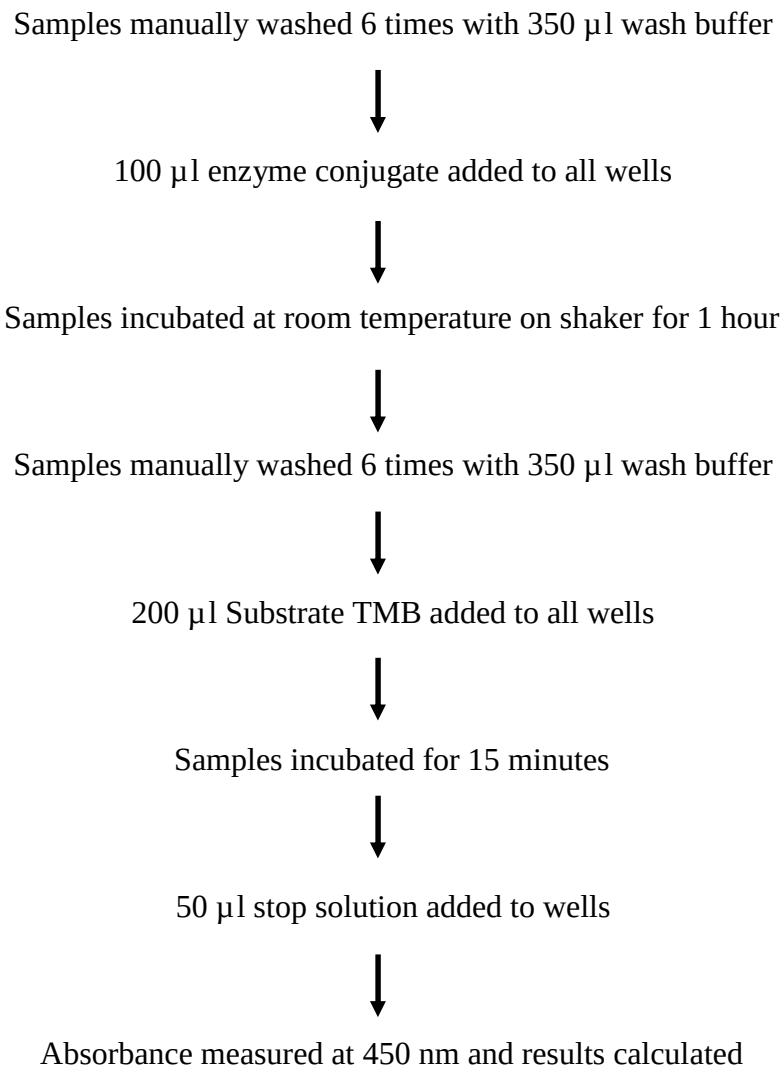
↓

50 µl antibody (mAb) added to all wells except the blank

↓

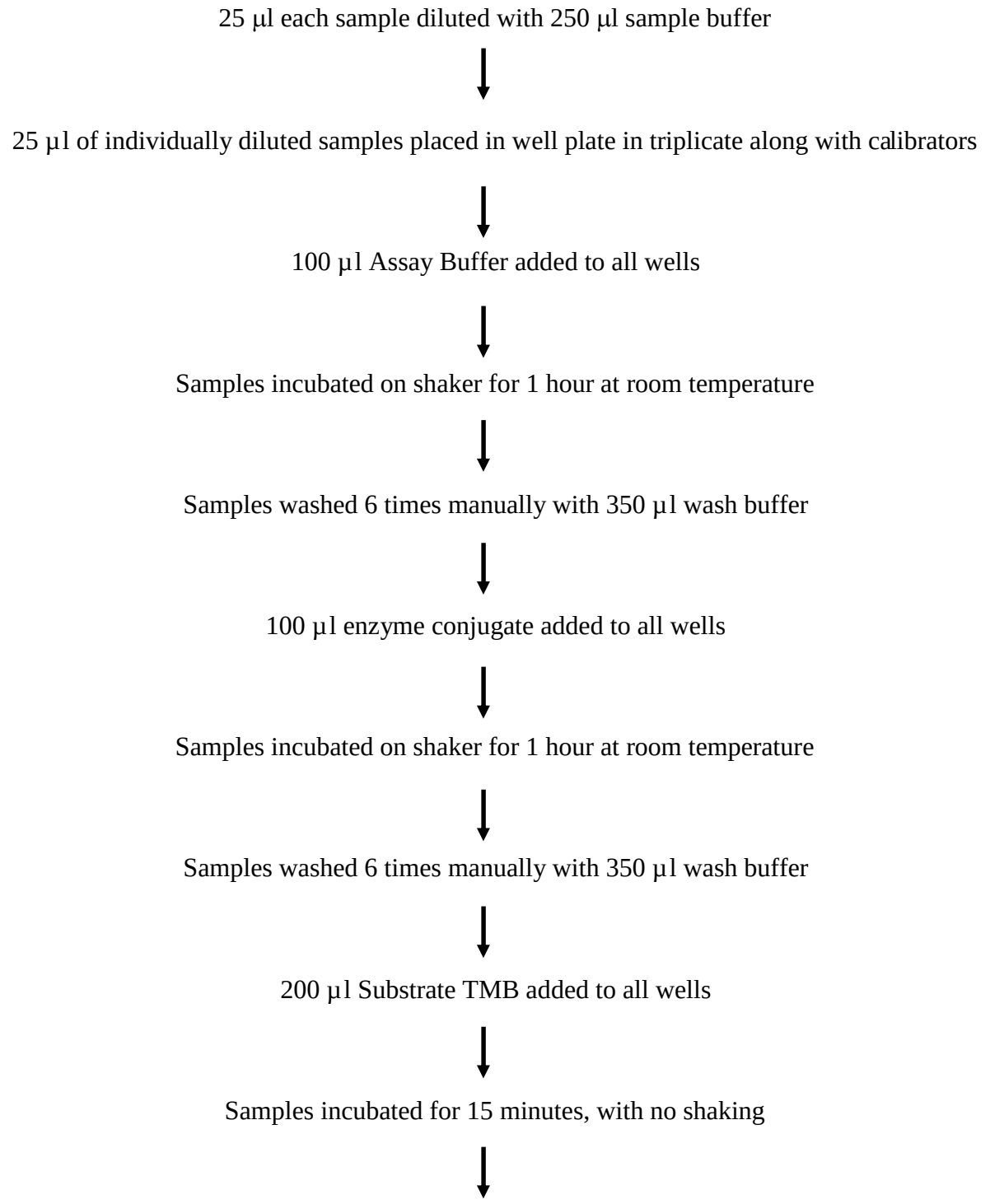
Samples incubated at room temperature on shaker for 2 hours

↓



Serum levels of MPO were quantified from participant samples using MPO solid phase ELISA (Mercodia, Uppsala, Sweden). This immunoassay directs two monoclonal antibodies against separate antigenic determinants on the MPO molecule. The MPO present in the sample reacts with anti-MPO antibodies bound to the wells as well as to the peroxidase-conjugated anti-MPO antibodies present in the solution during incubation. Following a washing step, peroxidase conjugated anti-MPO antibodies are added and after the second incubation and washing, the bound conjugate is detected by 3, 3', 5, 5'-

tetramethylbenzidine (TMB). Following the addition of stop solution to halt the reaction, the sample is read spectrophotometrically at 450 nm using the Synergy HT plate reader (BioTek Instruments, Inc., Winooski, VT).



50 µl stop solution added to all wells

Absorbance measured at 450 nm and results calculated

Statistical Analysis

Repeated measures ANOVA was performed on outcome measures to determine differences between screen, placebo and intervention anthropometrics, features of metabolic syndrome, lipid profiles and biomarkers of oxidative stress. Dietary records at screen and intervention were compared using paired t-tests. Statistical significance was set at $p < 0.05$ (two-sided test) and all data analyses were performed using SPSS 18.0 (SPSS Inc., Chicago, IL).

CHAPTER IV

FINDINGS

Composition of Cranberry Juice and Control Beverage

The composition of the cranberry and control juices was provided by Ocean Spray Cranberries Inc. (MA, USA) and outlined in **Table 3**. Both beverages were low in calories and contributed only an additional 40 calories to daily energy intake. While the analysis provided by the manufacturer did not specify micronutrient content, the cranberry juice beverage was reported to be high in total phenolics and anthocyanidins.

Baseline Characteristics

The baseline characteristics of the participants are shown in **Table 4** (mean ± SD). Ten participants were screened and enrolled in the study, all subjects were female with ages ranging from 40 to 64 years (50.5 ± 7.0). As classified by BMI, all subjects were obese or overweight with a mean BMI of 39.87±7.77 kg/m^2. At screen, three participants reported taking multivitamin or mineral supplements and three reported taking blood pressure lowering medication. For continuity, dietary supplement usage was continued throughout the trial.

Anthropometrics and Clinical Parameters As outlined in the inclusion criteria, all participants presented with a waist circumference of 35 inches or greater. Throughout the study, no significant changes were observed in waist

circumference, BMI or body weight. Clinical parameters are outlined in **Table 5**. Some features of metabolic syndrome were affected by cranberry juice supplementation including a significant reduction of HDL cholesterol ($p = 0.024$) and a significant increase in fasting glucose when compared to baseline ($p = 0.000$). Changes were observed in blood pressure following cranberry juice supplementation reducing systolic and diastolic blood pressure significantly when compared to baseline ($p = 0.036$, $p = 0.037$ respectively). At baseline, systolic blood pressure met criteria for diagnosis of metabolic syndrome; yet, after cranberry juice intervention, systolic blood pressure was below that required for metabolic syndrome. Other lipoprotein levels were affected by cranberry juice supplementation with non significant reductions to cholesterol and LDL-C by 2.6% and 4.4% respectively.

Biomarkers of Oxidative Stress

Two biomarkers of oxidative stress were evaluated to determine the efficacy of cranberry juice as an antioxidant in individuals with metabolic syndrome. There were no significant changes observed to oxidized LDL or MPO following cranberry juice intervention. Although oxidized LDL was reduced 21.3% following cranberry juice intervention compared to baseline, this reduction was not statistically significant ($p = 0.205$). Furthermore, nonsignificant increase in MPO of 22.16% was also observed from baseline ($p = 0.129$)

Dietary Analysis

From baseline to end of study, participant's average total caloric intake decreased 12.4% (**Table 6**). At baseline, macronutrient composition of average participant intake was 52.27% carbohydrate, 32.16% fat and 15.57% protein. Mean cholesterol intake did not change significantly over the intervention and was moderate with 276.75 ± 233.20 mg/day and 246.17 ± 170.26 mg/day respectively at baseline and end of intervention. Changes in micronutrients from baseline to week 10 include observed decreases in total carotenoids and vitamin C by 85.5% ($p = 0.271$) and 29.1% ($p = 0.199$) respectively. Copper intake was increased 16.05% ($p = 0.413$) from baseline. Intake of other micronutrients was not significantly different from baseline. In general, fiber intake was low throughout the intervention with mean intake meeting roughly 50% of the recommended intake for women.

Table 3: Composition of Cranberry Juice and Placebo Beverage

	Placebo (per 240 ml serving)	Juice (per 240 ml serving)
Brix	4.1 ^{o}B	4.2 ^{o}B
Calories	40	40
Sugars: fructose	5.3 g	4.8 g
Sugars: glucose	1.9 g	1.8 g
Sugars: sucrose	0.1 g	0.1 g
Sweetener: Sucralose	Proprietary	Proprietary
Sweetener Acesulfame	Proprietary	Proprietary
pH	2.9 g	2.9 g
Titratable Acidity	1.44 g	1.56 g
Acids: Quinic	0.65 g	0.72 g
Acids: Citric	0.79 g	0.82 g
Acids: Malic	0.55 g	0.48 g
Ascorbic Acid (Vitamin C)	60 mg	60 mg
Total Phenolics (by HPLC)	0 mg	229 mg
Total Anthocyanins (by HPLC)	0 mg	12.4 mg
Cy-3-Galactoside	0 mg	3.1 mg
Cy-3-Glucoside	0 mg	0.2 mg
Cy-3-Arabinoside	0 mg	3.0 mg
Pn-3-Galactoside	0 mg	3.8 mg
Pn-3-Glucoside	0 mg	0.2 mg
Pn-3-Arabinoside	0 mg	2.1 mg
Proanthocyanidins (by DMAC)	0 mg	119 mg
Haze	5.1 NTU	27.2 NTU
Color: Hunter L*	23	24
Color: Hunter a*	49	53
Color: Hunter b*	39	40

Source: Ocean Spray Cranberries Inc. (MA, USA)

Table 4: Baseline Characteristics of Participants

Characteristics	Mean	SD
Age	50.5	7.024
BMI (kg/m^2)	39.871	7.766
ALT (U/l)	24.2	6.512
AST(U/l)	27.8	14.505
BUN	13.7	3.268
Cr	0.73	0.095
M/F (n/n)	0/10	
Dietary supplement use (n/n)	3/10	
Medication (n/n)	3/10	
Aspirin use (n/n)	0/10	

N= 10

Table 5: Effects of Cranberry Juice Supplementation on Clinical Features of Metabolic Syndrome, Glucose, Insulin and Lipids

Clinical Features	Screen	Placebo	Intervention
Glucose (mg/dL)	94.8 ± 9.85	96.9 ± 9.20	102.2 ± 12.01*†
Insulin (μU/ml)	17.81 ± 9.69	16.39 ± 6.46	19.61 ± 8.46
HOMA-IR	4.302 ± 2.65	3.986 ± 1.80	5.041 ± 2.50
Cholesterol (mg/dL)	201.7 ± 34.98	203 ± 36.73	196.4 ± 30.36
Triglycerides (mg/dL)	139.6 ± 71.05	146.1 ± 52.19	155.5 ± 69.99
LDL-cholesterol (mg/dL)	121.7 ± 28.17	124 ± 29.73	116.3 ± 23.03
HDL- cholesterol (mg/dL)	52.1 ± 6.19	49.3 ± 5.93	48.9 ± 8.24*
VLDL (mg/dL)	27.8 ± 14.27	29.2 ± 10.47	31.2 ± 14.13
Systolic blood pressure (mm Hg)	131.4 ± 19.67	127 ± 15.17	126.7 ± 19.16*
Diastolic blood pressure (mm Hg)	82.4 ± 10.04	82.9 ± 9.45	79.2 ± 8.74*
Waist circumference (cm)	43.3 ± 3.47	43 ± 3.68	43.1 ± 3.57

Data Expressed as Mean ± standard deviation
* Significantly different compared to baseline ($p \leq 0.05$)
†Significantly different compared to placebo ($p \leq 0.05$)

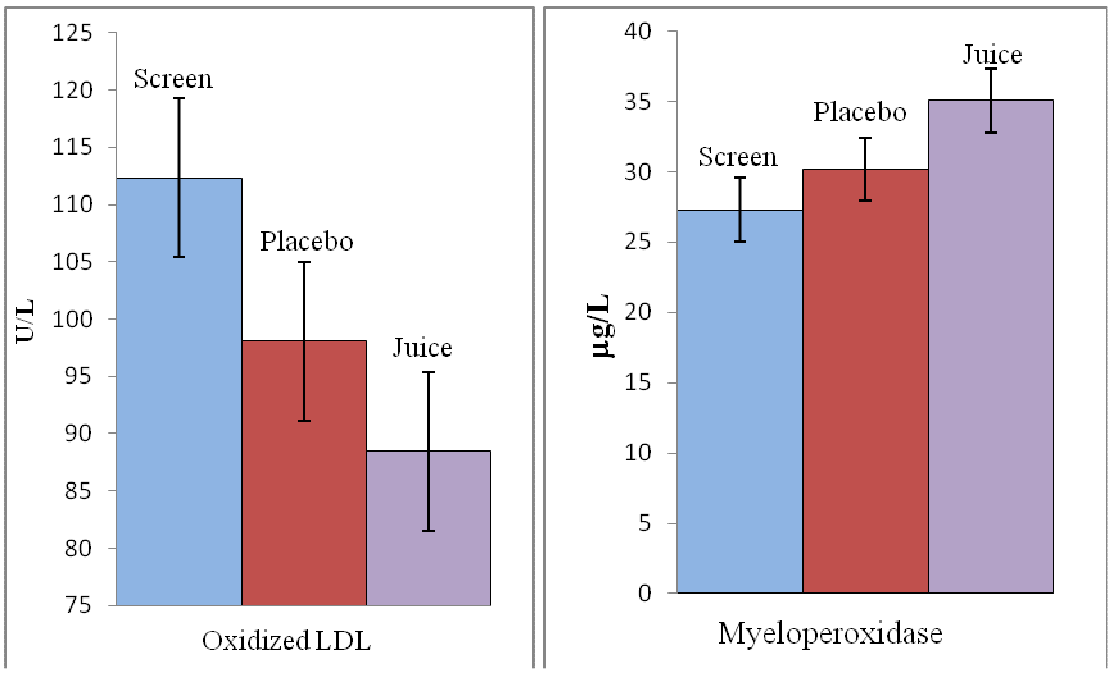
Table 6: Dietary Analysis of Participants' Intake from Screen and Week 10

Nutrient	Beginning of Study	End of Study
Energy (kcal)	2173.26 ± 1043.66	1904.49 ± 405.95
Protein (g)	85.47 ± 32.15	68.95 ± 17.86
Carbohydrate (g)	287.03 ± 134.48	259.47 ± 59.76
Fiber (g)	13.67 ± 8.50	13.57 ± 9.28
Total Fat (g)	78.50 ± 58.63	71.06 ± 28.49
Saturated fat (g)	28.97 ± 14.96	25.07 ± 9.78
Monounsaturated Fat (g)	11.62 ± 9.03	15.36 ± 18.07
Polyunsaturated fat (g)	6.34 ± 5.05	7.81 ± 5.65
Cholesterol (mg)	276.75 ± 233.20	246.17 ± 170.26
Carotenoids (RE)	519.05 ± 511.54	75.25 ± 45.17
Vitamin C (mg)	97.09 ± 75.95	68.84 ± 56.82
Vitamin E (mg)	6.85 ± 6.12	6.68 ± 4.57
Iron (mg)	12.99 ± 4.93	12.21 ± 6.57
Copper (mg)	0.68 ± 0.39	0.81 ± 0.39
Zinc (mg)	6.95 ± 8.39	6.58 ± 5.10

Data expressed as mean ± standard deviation
N=4

Figure 1. Effects of low calorie cranberry juice supplementation on biomarkers of oxidative stress

A **B**



(A)Oxidized LDL and (B) Myeloperoxidase
Data presented as mean ± SD
N=8

CHAPTER V

CONCLUSION

The present study illustrates the effects of cranberry juice supplementation on clinical features of metabolic syndrome and biomarkers of oxidative stress in participants. Many in vitro and in vivo studies have shown the antioxidant, anti-hypertensive and anti-hyperlipidemic properties of cranberries. To our knowledge, our study is the first study to examine and report the anti-hypertensive and antioxidant impact of cranberry juice supplementation in individuals with metabolic syndrome. Metabolic syndrome is significantly associated with increased risk of CVD morbidity most likely due to the increased oxidative stress and hyperlipidemia typically seen in individuals with metabolic syndrome (55). Therefore, modulation of clinical parameters associated with metabolic syndrome by incorporating functional foods like antioxidant rich fruits and vegetables may reduce the risk of CVD development.

One objective of our intervention was to examine the effect of low calorie cranberry juice supplementation on the clinical features of metabolic syndrome including: glucose, triglycerides, HDL, blood pressure and waist circumference. Our study found no significant changes to TG or cholesterol. These findings are consistent with a previous

study in which no changes were observed in lipid profile including TG and cholesterol in healthy volunteers supplemented with 750 ml of cranberry juice daily for two weeks (46). However, it is important to note that, at baseline, participants had normal lipid profiles and were supplemented for only two weeks which may not be sufficient to see significant changes, particularly in individuals who are normolipidemic.

Our study did observed a significant decrease in HDL following cranberry intervention compared to baseline ($p = 0.024$). In fact, mean HDL at screen was moderate at 52.1 mg/dL; however, following cranberry supplementation, mean HDL was 48.9 mg/dL. The American Heart Association interprets HDL levels <50 mg/dL as low for women (www.heart.org). Low HDL levels are associated with heart disease risk as these particles remove cholesterol from the arteries and deliver it to liver for excretion or reutilization. In contrast to our findings, a cranberry juice intervention by Ruel et al found significant increases to HDL as well as a non-significant reduction in triglycerides in sedentary male participants (44). The variability in findings may be attributed to differences in patient population, sample size or duration of study. Ruel et al supplemented 31 men with increasing doses of cranberry juice over a period of 12 weeks while our study supplemented 10 women for 4 weeks. It is possible that the study duration was not sufficient to observe significant modifications to clinical parameters or that other dietary or lifestyle factors may have inhibited a change from being observed.

Data from our intervention did indicate a significant reduction to systolic ($p = 0.036$) and diastolic ($p = 0.037$) blood pressure compared to baseline. Similar antihypertensive effects of berry flavonoids, including cranberry juice, have been well documented in animal models and a human intervention completed by Erlund et al observed significant

decreases in systolic and diastolic blood pressure following consumption of various berries and berry products for 8 weeks (10, 56, 57).

Insulin resistance and related hyperglycemia have been suggested as the primary abnormal characteristic of metabolic syndrome (22). It is proposed that the observed T2D causes metabolic alterations that result in dyslipidemia and hypertension thus increasing the risk of CVD. Given this framework, glycemic control is of utmost importance for individuals with metabolic syndrome. The results from our study indicated that blood glucose was significantly higher following cranberry intervention compared to both baseline ($p = 0.00$) and placebo ($p = 0.003$). Because the cranberry juice and placebo beverage were nearly identical in terms of macronutrient content, these results were unanticipated. Participants were not instructed to compensate for the additional carbohydrates the juice provided and thus may not have adjusted carbohydrate intake accordingly. Furthermore, our finding differs from a study completed in type 2 diabetic patients in which various cranberry juices and placebos were administered while blood glucose was monitored. Following consumption of juices, blood glucose of participants who consumed the low calorie cranberry juice were not statistically different from baseline while those who consumed the regular juice had significantly higher glucose levels compared to baseline. The low calorie juice administered in the previous study was a 27% low calorie juice matching the juice utilized in our study. It seems unlikely that the cranberry juice was the sole cause of the observed increase in blood glucose levels in our study; however, future studies should instruct participants to compensate appropriately for additional carbohydrate consumption.

Oxidative stress plays a key role in the development of CVD by inducing lipid peroxidation. Oxidation of LDL-C and its subsequent uptake by macrophages in the intima is a critical early step in the development and progression of atherosclerosis (58). Therefore, presence of elevated oxLDL in the serum is an indicator of lipid peroxidation and, consequently, a good marker of oxidative stress associated with atherogenesis. Although OxLDL was reduced in the serum by 25% following cranberry juice intervention as compared to baseline, this change was not signifcant ($p = 0.205$). However, the potential of cranberry juice to inhibit LDL-C oxidation is well documented in cell culture studies (47,49). However, only one human study has reported a significant reduction in plasma oxLDL following cranberry juice intervention (37). Again, this study was a 12 week intervention with a larger cohort of 31 men.

Myeloperoxidase (MPO) is a monocyte secreted enzyme associated with both inflammation and oxidative stress. Epidemiological evidence supports MPO as a risk factor for CVD as elevated plasma MPO is associated with as much as a 20 fold increase in risk of CAD (59). MPO has the ability to oxidatively modify lipids such as LDL and increase production of free radicals. Our study found no significant changes in MPO following cranberry juice intervention ($p = 0.129$). These findings are consistent with another study which supplemented freeze dried blueberry beverage to participants with metabolic syndrome (60). This study found no significant changes in MPO following 8 weeks of supplementation.

Participants were asked to maintain three day food records for each week for the duration of the study. Only four participants submitted complete food records to be analyzed for differences between screen and end of study. Overall, mean caloric intake did not

significantly differ from screen to week ten. The macronutrient composition was within the recommended range advised by the Institute of Medicine (IOM) which suggests that 45-65% of total calories should come from carbohydrate sources, 20-35% from fat, and 10-35% from protein (61). Regarding micronutrient intake, average vitamin E, copper and zinc intakes were below that recommended by the IOM (62). Analysis of other micronutrient intake found that although total carotenoid and vitamin C intake decreased 85.5% and 29.1% from baseline, intake was not significantly different from baseline due to the small percentage of completed records. However, from those records, vitamin C intake at screen was higher than the RDA but was below the RDA at end of study. Both vitamin C and carotenoids are principal antioxidant micronutrients which have the ability to reduce oxidative stress and prevent lipid peroxidation. Decreases in intake observed from screen to week ten, although not significant, may have interfered with the cranberry juice supplementation and its biological effects. It is possible that the lack of stable carotenoid antioxidant intake throughout the study may have blunted the effects of the cranberry juice thereby contributing to the observed non-significant data.

Our study was a four week crossover-control study in which participants with metabolic syndrome were supplemented with either cranberry juice or cranberry control beverage. This intervention has a variety of public health implications. For example, both cranberry drinks used in the study were low calorie and did not significantly contribute to the daily caloric intake of participants. Although blood glucose was raised significantly following cranberry supplementation, 27% low calorie cranberry juice provides less carbohydrates than regular cranberry cocktail juices and are more palatable than 100% unsweetened juices. Furthermore, the provided polyphenol content supplied by Ocean

Spray Cranberries Inc. (MA, USA) listed the total phenolics of the low calorie juice to be 229 mg/240 ml. This is a considerable amount of polyphenols in view of the minimal caloric contribution of the juice. Finally, the cranberry juice is affordable and widely available commercially. Many Americans do not consume the recommended servings of fruits and vegetables each day and due to the commercial availability, considerable polyphenol content and minimal caloric contributions of the cranberry juice, it may be advisable for individuals to incorporate cranberry products, such as low calorie cranberry juice, into a healthy diet.

The primary objective of our study was to examine the effects of low calorie cranberry juice supplementation on clinical features of metabolic syndrome and biomarkers of oxidative stress in obese adults.

Our null hypotheses were:

1. Low calorie cranberry juice supplementation will have no effects on features of metabolic syndrome (waist circumference, BP, HDL, blood glucose or TG)
2. Low calorie cranberry juice supplementation will have no effects on biomarkers of oxidative stress associated with metabolic syndrome.

Based on the results, we reject the null hypothesis that low calorie cranberry juice supplementation will not affect features of metabolic syndrome because HDL, systolic and diastolic blood pressures were significantly reduced and blood glucose levels were significantly increased following cranberry intervention compared to baseline. We fail to reject the null hypothesis that low calorie cranberry juice supplementation will have no

effect on biomarkers of oxidative stress associated with metabolic syndrome. Despite the downward trend noted in the OxLDL values, there were no statistically significant changes to OxLDL or MPO following cranberry intervention.

Limitations of our study that must be noted include the relative small sample size, duration, sampling method and nature of the study. Due to the fact that only ten participants were enrolled at the campus at Oklahoma State University, the results of our study may not be widely generalized. Supplementation of a longer duration in a larger cohort may be recommended to reveal additional beneficial effects and increase validity of our findings. Furthermore, dietary intake and compliance was self-reported. Future studies may monitor subjects more closely to ensure adherence to intervention regimen and accurate completion of food records.

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APPPENDICES

Oklahoma State University Institutional Review Board

Date:	Thursday, August 27, 2009	**Protocol Expires:**	**8/26/2010**
IRB Application No:	HE0856		
Proposal Title:	Cranberry Flavonoid Consumption and Biomarkers of Lipid Peroxidation and Inflammation in Subjects with Metabolic Syndrome (MeS)		
Reviewed and Processed as:	**Modification/Continuation**		

Status Recommended by Reviewer(s) **Approved**

Principal Investigator(s) :

Arpita Basu
301 HES
Stillwater, OK 74078

Approvals are valid for one calendar year, after which time a request for continuation must be submitted. Any modifications to the research project approved by the IRB must be submitted for approval with the advisor's signature. The IRB office MUST be notified in writing when a project is complete. Approved projects are subject to monitoring by the IRB.

☒ The final versions of any printed recruitment, consent and assent documents bearing the IRB approval stamp are attached to this letter. These are the versions that must be used during the study.

Signature :

Sheila Kennison, Chair, Institutional Review Board

Thursday, August 27, 2009
Date

INFORMED CONSENT DOCUMENT

Project Title: Cranberry flavonoid consumption and biomarkers of lipid peroxidation and inflammation in subjects with Metabolic Syndrome (MeS).

Investigators:

Arpita Basu, PhD, RD
Karah Sanchez, MS, RD
Marci Wilkinson, BS

Purpose: This is a research study to find out the health effects of cranberries. You are being asked to participate because you may run the risk of developing diabetes and heart disease in future, because of being overweight, or having high blood pressure, blood glucose, or blood lipid levels. Cranberries have been shown in other studies to protect the cells against damage that occurs as a result of being overweight, or having high blood glucose or lipids. In this research study we will find out whether cranberry juice will reduce those damage or risks.

Procedures: You may qualify for the study if you have 3 of the following 5 features-

- Your waist measures greater than 35 inches (if you are a female) or 40 inches (if you are a male)
- You have high blood pressure
- You have high blood glucose
- You have high blood lipids
- You have low levels of good lipids

- In order to qualify you should not have any other serious health problems or take medicines to lower your lipids or glucose levels. If you qualify, you will be taking 2 cups of cranberry juice or control juice every day, each juice for a period of 4 weeks. The control juice will have filtered water, natural flavors and colors, and splenda. You will be drinking cranberry juice for 4 weeks, take a break for 1 week, and then drink control juice without cranberries for the next 4 weeks. You will be asked to maintain your usual diet and lifestyle throughout the 9 weeks. The order of the juices may vary and you may drink either of two drinks in the first 4 weeks and then come back for the second drink in the next 4 weeks. You will be making 3 days/week visits to the Department of Nutritional Sciences at Oklahoma State University for the supply of juices. You will be asked to drink one cup in the morning and then another cup in the evening. We will provide you the drink in containers. You will also keep a diary of everything you eat for 3 days of the week, every week, during the 9-week study period.

Visits: You will be making the following visits for blood draws and 3-day food records

Screening- You will be asked to sign the consent form, and we will measure your height, weight, blood pressure, and waist. You will be asked to fast the previous night and about 3-4 tablespoons of blood will be taken for measuring your blood sugar, lipids, blood cell counts, and do some tests to find out how well your liver and kidney are working. If you qualify, we will let you know over the telephone and ask you to come back for the 9-week study. You will also be asked to record your food intake before you start the study and will also be told how to do it. You will be notified

within 3 days of the screening visit whether or not you qualify for the study.

- 1st week- start the first drink (cranberry juice or control juice), turn in 3-day food record at baseline
- 2nd week- draw about 2 tablespoons of fasting blood to do some safety tests which will indicate any health risks of the juices and will include liver, kidney, and thyroid functions tests
- 4th week- turn in 3-day food records, draw about 3-4 tablespoons of fasting blood for measuring your blood sugar, lipids, and do some tests to find out how well the cells in your body are working. We will also measure your body weight, blood pressure, and the amount of fat in your body, and do some safety tests.
- 5th week- break after the first drink for 4 weeks
- 6th week- start the second drink, turn in 3-day food records
- 7th week- draw about 2 tablespoons of fasting blood to do some safety tests which will indicate any health risks of the juices and will include liver, kidney, and thyroid functions tests
- 9th week- This will be your final visit; turn in 3-day food records, draw about 3-4 tablespoons of fasting blood for measuring your blood sugar, lipids, and do some tests to find out how well the cells in your body are working. We will also measure your body weight, blood pressure, and the amount of fat in your body, and do some safety tests which will indicate any health risks of the juices and will include liver, kidney, and thyroid functions tests

If you do not qualify for the study after the screening visit, you will be informed over the telephone and you will also receive a copy of your blood reports. However, if you do not qualify, all other information we have collected will be immediately destroyed.

Risks of Participation: You may experience slight pain during the blood draw. But, a trained phlebotomist will be there for any help. Also, you will not be allowed to participate in the study if you have any blood-related health problems. You may experience slight stomach problems like flatulence if you are not used to taking 2 cups of cranberry juice per day. But, these symptoms may soon go away.

Benefits: You may benefit from the cranberry juice, which may reduce some cell damage in your body. Since, this is a research study, we cannot guarantee benefit before we get study results. But, the results of this study will greatly benefit research by providing information on the health benefits of cranberries.

Confidentiality: The records of this study will be kept private. You will be assigned a research code so that your personal data is not identifiable with your name and other personal information. Your name will not appear on any reports that contain your data and will be referred to using the code. Any written results will discuss group findings and will not include information that will identify you. Research records will be stored securely and only researchers and individuals responsible for research oversight will have access to the records. It is possible that the consent process and data collection will be observed by research oversight staff responsible for

safeguarding the rights and wellbeing of people who participate in research.

Compensation: You will be compensated for $30 at two, four, seven, & nine weeks of the study, a total of $ 120. This payment will be made to you in cash and you will need to sign a form as a receipt. No funds have been set aside by Oklahoma State University to compensate you in the event of illness or injury resulting from this study.

Contacts: If you have any questions about the research and the subject's rights, please contact Arpita Basu, PhD, at 405-744-4437 (9AM-5PM, Monday –Friday) or at 916-607-4143 (any time).If you have questions about your rights as a research volunteer, you may contact Dr. Shelia Kennison, IRB Chair, 219 Cordell North, Stillwater, OK 74078, 405-744-1676 or irb@okstate.edu.

Participant Rights: Your participation in this research is voluntary and you may discontinue the research activity at any time without reprisal or penalty. No risks will be involved due to your withdrawal from the study. Your participation may be terminated if you develop any allergy towards the cranberry or control juice or if you fail to make the visits as per schedule.

Signatures:

I have read and fully understand the consent form. I sign it freely and voluntarily. A copy of this form has been given to me.

_____ _____

Signature of Participant Date

I certify that I have personally explained this document before requesting that the participant sign it.

_____ _____

Signature of Researcher Date

Okla. State Univ.
IRB
Approved 8/27/09
Expires 8/26/10
IRB # HE-09-86

Are you overweight? Do you have high blood pressure or blood sugar?

in this study toda

* Find out if you qualify for this 9 week study which will test the health effects of cranberry juice.

You may qualify for this study if you are a male or female with at least three of the following:

- A waist circumference of greater than **40 in.** in men or **35 in.** in women
- HDL less than **40 mg/dL** in men or **50 mg/dL** in women
- Blood pressure ≥ **130/85** mmHg or are on blood pressure medications
- Triglycerides ≥ **150** mg/dL
- Fasting blood glucose ≥ **100** mg/dL but ≤ **126** mg/dL

CONTACT DR. ARPITA BASU

405-744-4437

All assessments will be free of charge. Study participants will receive a compensation per visit.

Day/ Date of Appointment: _____ *Time:* _____

SCREENING QUESTIONNAIRE FOR CRANBERRY STUDY

NAME: _____

ADDRESS: _____

_____

PHONE (WORK): _____

PHONE (HOME): _____

AGE: _____ DATE OF BIRTH: _____ GENDER: _____

SCREENING QUESTIONS:

Do you currently take any cholesterol/triglyceride lowering medications? YES NO

Are you pregnant or lactating? YES NO N/A

Do you smoke? YES NO

Do you currently take vitamins or nutritional supplements? YES NO
What are they? _____
_____

Have you taken antioxidant **supplements** regularly in the past 3-6 months? YES NO

Do you take more than 1 g/day of fish oil capsules? YES NO

Do you exercise ≥ 60 min/day? YES NO

Do you drink more than 1 oz of alcohol/day? YES NO
(1 oz alcohol = 2 beers or 10 oz of wine or 2 ½ oz liquor)

Do you have diabetes? YES NO UNSURE
We will confirm with fasting blood glucose

Do you have hypo/hyperthyroidism? YES NO UNSURE
We will check TSH

Day/ Date of Appointment: _____ *Time:* _____

Do you have any gastrointestinal problems? YES NO

Do you have anemia? YES NO

Are you suffering from any other disorder or illness? YES NO
(Cardiovascular disease, rheumatoid arthritis, etc.)

Do you have high blood pressure? YES NO
If controlled, what medications does the patient take?
_____

Are you taking any other medications on a regular basis? YES NO

If you are taking medications, what are they? And, how long have you been taking them?
_____
_____

Do you take aspirin? How often? Dose? _____
_____

Do you take estrogen or oral contraceptives? YES NO N/A

Is the subject <u>ELIGIBLE</u> based on the questionnaire? YES NO

<u>ELIGIBILITY REQUIRES 3 OF THE 5 FEATURES:</u>

FEATURES OF METABOLIC SYNDROME (Check all that apply):

1. _____ **Waist circumference** **(Male ≥ 40 inches)** (Value:_____)
(Female ≥ 35 inches)

2. _____ **Hypertension controlled by anti-hypertensive medication**
_____ **Systolic Blood Pressure** **(≥ 130 mmHg)** (Value:_____)
_____ **Diastolic Blood Pressure** **(≥ 85 mmHg)** (Value:_____)

3. _____ **HDL Cholesterol** **(Male ≤ 40 mg/dL)** (Value:_____)
(Female ≤ 50 mg/dL)

4. _____ **Triglycerides** **(≥ 150 mg/dL)** (Value:_____)

5. _____ **Fasting Blood Glucose (≥ 100 mg/dL and < 126 mg/dL)** (Value:_____)

Oklahoma State University
Nutritional Sciences
Cranberry Study

As a part of this study, you will be asked to keep a Diary of *everything* you eat and drink for **3 days**. These 3 days should include 2 weekdays and 1 weekend day, *example*: Thursday, Friday and Saturday.
Begin with the first food or beverage in the morning and write down what you eat as you go through the day.
The Nutritionist will review your completed Food dairy.

When you come back, please bring in any bottles/packages of dietary or nutritional supplements you have taken within the past week.
This would include any pills, powders, capsules, oils, tablets, or liquid vitamin/mineral supplements, herbal supplements, herbal teas or tinctures or any other type of dietary supplement you have taken.

GENERAL INSTRUCTIONS FOR RECORDING FOOD INTAKE

1. Please record on the Food Diary Form the place (home, home of a friend, restaurant) of each meal and snack.

2. Record one food item per line on the Food Diary Form. Space is provided on both sides of the form. Be sure to include gum, candy and beverages.

3. Record the amount and food item on the Food Diary Form using common household measurements, for example: Tablespoons, cups, package size etc.

4. Remember to record everything you possibly can about a food. The more detail you include the better.

5. When you record an item, please note if it was baked, boiled, broiled, fried, or roasted. This is extremely important, especially for meats.

6. Record any additions to a food item. This **would** include sugar, relish, margarine, butter, catsup, pickles, mayonnaise, mustard, gravies, cream, etc., which were served with the food.

7. When eating out, record the menu item and amount eaten. Refer to Hints for Eating Out.

8. List the method of mixing a package mix if it is different form the directions given on the package. You may record this on a Recipe Form.

9. Use the Recipe Form to record any homemade items you have prepared. Measure each ingredient and record the method of preparation on the bottom of that form.

10. If you have any questions, please call Arpita Basu at 405-744-4437.

HINTS FOR EATING OUT

1. Record the name of the restaurant.

2. Quiz the wait staff regarding portion sizes.

3. Record amounts in standard household measurements, ie: teaspoons (ts), tablespoons (Tb), ounces, cups, etc.

4. For items such as bacon, rolls, and cucumbers, record the number of each item eaten.
   For example: 3 small white rolls
   4 cucumber slices
   2 medium bacon slices

5. For meats, record the dimensions of the cooked meat. Do not include the bone.
   For example: 2 slices of roast beef 4" x 3" x ¼". State the weight of the meat if it is mentioned on the menu.

6. Refer to the Food Description Flow Charts to describe your food.

7. For national fast food restaurants, (i.e. McDonald's, Arby's, Burger King), record the name of the sandwich/item you ate (i.e. Big Mac, Whopper).

Oklahoma State University
Nutritional Sciences
Cranberry Study
Food Diary

Name: _____ ID#_____ Protocol No:_____

Date of Record:_____ Day of Week:_____

Please record everything you eat today. Please include descriptions, brand names, and weighed and measured amounts (Please save labels). In the first column under meal and place, please put what meal you ate and where you ate it. You may use the codes at the bottom of the page for convenience. Thank you.

Meal* Place*	Amount	Food & Beverage Description	Office Use Only

*Meal Codes:
Breakfast - BR
Morning Snack – MS
Lunch – LU
Afternoon Snack –AS
Supper – SU
Evening Snack – ES

*Place Codes:
Home – HO
Restaurant – RE (Please Specify name of Restaurant)
Friends –FR
Work- W

VITA

Jennifer Paige Ortiz

Candidate for the Degree of

Master of Science

Thesis: CRANBERRY FLAVONOID SUPPLEMENTATION AND BIOMARKERS OF OXIDATIVE STRESS IN SUBJECTS WITH METABOLIC SYNDROME

Major Field: Nutritional Sciences

Biographical:

Personal: Born in Midwest City, Oklahoma on July 22, 1987, the daughter of William and Melodie Fulmer

Education: Graduated from Santa Fe High School, Edmond, Oklahoma in 2005; received Bachelor of Science in Dietetics from Oklahoma State University, Stillwater, Oklahoma in May of 2009. Completed the requirements for the Master of Science in Nutritional Sciences at Oklahoma State University, Stillwater, Oklahoma in December, 2010.

Experience: Dietetic Intern with Oklahoma State University, 2009 to present. Employed by Oklahoma State University, College of Human Environmental Sciences Center for Student Success as a graduate teaching assistant, 2009 to present. Employed by Oklahoma State University, Department of Nutritional Sciences as a graduate research assistant, 2010.

Professional Memberships: American Dietetic Association, Oklahoma Dietetic Association

Name: Jennifer Paige Ortiz Date of Degree: December, 2010

Institution: Oklahoma State University Location: OKC or Stillwater, Oklahoma

Title of Study: CRANBERRY FLAVONOID SUPPLEMENTATION AND BIOMARKERS OF OXIDATIVE STRESS IN SUBJECTS WITH METABOLIC SYNDROME

Pages in Study: 60 Candidate for the Degree of Master of Science

Major Field: Nutritional Sciences

Scope and Method of Study:

Metabolic syndrome (MeS), a collection of risk factors including dyslipidemia, hypertension, visceral adiposity, and hyperglycemia, is also a condition associated with increased oxidative stress and CVD risk. Cranberry flavonoids, such as those present in cranberry juice, are potent antioxidants. The objective of this randomized crossover controlled trial was to analyze any difference in features of MeS and biomarkers of oxidative stress including OxLDL and MPO, in subjects following a 4-week intervention of low calorie cranberry juice in comparison to controls. 10 subjects with MeS were recruited and randomized to either cranberry group (2 cups of cranberry juice/day) or control group (2 cups of flavored juice/day) in a crossover design with a two week washout period. Anthropometrics, blood pressure and blood draws were obtained at screen as well as at the end of each intervention period. From blood draws, lipid profiles, oxidized LDL and myeloperoxidase (MPO) were determined. Subjects were asked maintain 3-day food records. Repeated measures ANOVA were performed to detect differences between groups at each intervention period with statistical significance set and 0.05.

Findings and Conclusions:

Significant reductions were observed in HDL as well as systolic and diastolic blood pressures following low calorie cranberry juice intervention compared to baseline. Additionally, a significant increase in blood glucose was observed following low calorie cranberry juice intervention compared to baseline. No significant differences were observed in biomarkers of oxidative stress including OxLDL and MPO. The results of the present study do not suggest that short term low calorie cranberry juice supplementation positively effects oxidative stress in subjects with metabolic syndrome. However, the present study was a pilot study and further research should be conducted to examine the efficacy of low calorie cranberry juice at positively modifying CVD risk factors including blood pressure and lipid profiles.

ADVISER'S APPROVAL: Dr. Arpita Basu