METABOLIC RISK FACTORS FOR CARDIOVASCULAR DISEASE IN OKLAHOMA ADULTS WITH ABDOMINAL ADIPOSITY AND DYSLIPIDEMIA: A CROSS-SECTIONAL STUDY.

By

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Bachelor of Science in Nutritional Sciences

Oklahoma State University

Stillwater, OK

2010

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
December, 2013

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ACKNOWLEDGEMENTS

First, I would like to thank Dr. Arpita Basu for developing the concept of this study as well as for providing me the opportunity to be involved in the research and analysis of this study which has enabled me to complete my Master's Thesis. I am especially grateful for all the support, encouragement, and guidance I have received from Dr. Basu. Next, I would like to thank Dr. Nancy Betts and Dr. Deana Hildebrand for serving on my thesis committee and supporting me as I pursued my Master's Degree. Additionally, a special thanks goes to Dr. Betts for offering her time and skills to perform the statistical analysis for this study. I also appreciate each of the study participants because without them and their willingness to participant, this study would not have been possible. I would like to thank the faculty and staff of the Oklahoma State University Nutritional Sciences Department for the encouragement and guidance they have offered me throughout my time at Oklahoma State University. Finally, I am thankful to my family and friends who have always had confidence in my ability to succeed and have supported me in all my professional and academic endeavors.

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Date of Degree: DECEMBER, 2013

Title of Study: METABOLIC RISK FACTORS FOR CARDIOVASCULAR DISEASE IN OKLAHOMA ADULTS WITH ABDOMINAL ADIPOSITY AND DYSLIPIDEMIA: A CROSS-SECTIONAL STUDY.

Major Field: NUTRITIONAL SCIENCES

Scope and Method of Study: Dietary intakes of fruits and vegetables have been shown to lower risks of cardiovascular complications in both epidemiological and clinical studies. In particular, the polyphenol subclass of flavonoids has been found to exert anti-atherosclerotic, anti-hypertensive, and anti-oxidative properties. Our study aims to further investigate the relationships among cardiovascular disease (CVD) risk factors, total servings of fruits and vegetables, as well as flavonoid intakes in Oklahoma adults with abdominal adiposity and dyslipidemia. Thirty participants (5 males, 25 females) were recruited at the General Clinical Reserch Center at the Oklahoma University Health Science Center and Department of Nutritional Sciences at the Oklahoma State University. Blood draws and anthropometrics were performed and participants completed a 3 day food records for dietary analysis.

Findings and Conclusions: Serum total cholesterol, LDL-cholesterol and glucose levels were significantly different across tertiles of ox-LDL ($p < 0.05$); total- and LDL-cholesterol were significantly higher in the highest vs. lowest tertiles. Waist circumference, serum glucose, HbA1c, quercetin intake, and total servings of fruits and vegetables were significantly different across tertiles of CRP ($p < 0.05$); elevated levels in highest vs. lower tertiles. This cross-sectional study shows significant differences among measures of lipid oxidation, CRP, and flavonoids (kaempferol, myricetin, quercetin) with measures of lipids and glycemic control in Oklahoma adults with abdominal adiposity and dyslipidemia. Further investigation with a large population should be conducted to confirm these findings.

TABLE OF CONTENTS

Chapter Page

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Cardiovascular Disease (CVD) refers to multiple conditions of heart and vascular complications. The term is often used for damage caused to either the heart or blood vessels by atherosclerosis. Emerging research shows that obesity, hypertension, diabetes mellitus, dyslipidemia, smoking, aging, diets rich in saturated fats and reduced physical activity are the established risk factors for atherosclerosis and cardiovascular disease (1). All these metabolic and degenerative disorders are also characterized by inflammation and oxidant burden. Oxidative stress and inflammation play a pivotal role at all stages of atherosclerosis and the subsequent development of CVD (2).

Research has shown that patients with elevated basal levels of C-reactive protein (CRP) are at an increased risk of diabetes, hypertension, and cardiovascular disease (3,4). CRP expression occurs during the acute phase response to tissue injury or inflammation in the hepatic cells of mammals. High sensitivity-CRP (hs-CRP) assay, detects low concentrations of the protein, and is considered useful in determining the potential risk level for CVD. However, it is not known whether hs-CRP is merely an indicator of CVD or if it actually plays a role in causing cardiovascular diseases.

Experts recommend the combination of hs-CRP tests and the lipid profile as a reliable approach to predict CVD risk. Diet and lifestyle factors continue to be the cornerstone in health and prevention of chronic diseases. Consumption of fruits and vegetables is associated with the primary prevention of CVD as well as improved management of diabetes (5). Many of the health benefits associated with diets high in fruits and vegetables are attributed to their phytochemical content, particularly flavonoids (6). Dietary bioactive compounds, especially phytochemicals, have been shown to play a crucial role in attenuating biomarkers of oxidative stress and inflammation.

According to the National Health and Nutrition Examination Survey (NHANES) 1999-2002 data on 24 hour dietary recalls, the mean flavonoid intake among U.S. adults is 1897 mg/day (7). This phytochemical sub-class exhibits protective effects against metabolic syndrome, a precursor for CVD and diabetes mellitus (DM). However, most epidemiologic studies investigating flavonoids and CVD risk have examined only one or two sub-classes of flavonoids. Also, epidemiologic investigation of flavonoid intakes in relation to CVD risk in Oklahoma adult population is limited (8). Thus, there exists a need to investigate the relation between total fruits and vegetables consumption, total flavonoid intake, dyslipidemia, and CRP in adults with abdominal adiposity.

Purpose:

The purpose of this study was to investigate the associations among hs-CRP, lipid oxidation [oxidized low-density lipoprotein (oxLDL), malondialdehyde (MDA)], and flavonoids [Flavonols: kaempferol (KAE), myricetin (MYR), quercetin (QUER), with measures of lipids [total cholesterol, triglycerides (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), very low-density lipoprotein (VLDL)] and glycemic

control [glucose, hemoglobin A1c (HbA1c)]. We also aimed to examine for differences among waist circumference, measures of lipids (total cholesterol, TG, LDL, HDL) and glycemic control (glucose, HbA1c), QUER intake, and servings of fruits and vegetables across tertiles of CRP and ox-LDL in our study participants with abdominal adiposity and dyslipidemia.

Hypotheses:

The following hypotheses are being examined in our study.

1. Measures of lipid oxidation (ox-LDL, MDA), CRP, and flavonoids (KAE, MYR, QUER) will have significant correlations with measures of lipids (total cholesterol, TG, LDL, HDL, VLDL) and glycemic control (glucose, HbA1c).

2. Participants with higher mean ox-LDL will have higher waist circumference, TG, LDL, fasting serum glucose, and HbA1c and lower HDL, QUER intakes, and consumption of fruits and vegetables.

3. Participants with higher mean CRP will have higher waist circumference, TG, LDL, fasting serum glucose, and HbA1c and lower HDL, QUER intakes, and consumption of fruits and vegetables.

CHAPTER II

REVIEW OF LITERATURE

High dietary intakes of fruits and vegetables, containing a variety of polyphenolic phytochemicals, have been shown to lower the risk of cardiovascular complications in both epidemiological and clinical studies. In particular, the polyphenol sub-class of flavonoids has been found to exert anti-atherosclerotic, anti-hypertensive, and anti-oxidative properties (8). However, most research in flavonoid consumption focuses on the associations and the effects of only one or two sub-classes, and limited research has been conducted in human subjects with abdominal adiposity and dyslipidemia. Thus, our study aims to further investigate the differences between surrogate risk factors of CVD and total intake of fruits and vegetables, as well as flavonoid intake in subjects with abdominal adiposity and dyslipidemia.

Metabolic syndrome and cardiovascular disease

Metabolic syndrome (MetS) is defined as a cluster of at least three conditions including the following: increased blood pressure, elevated fasting glucose levels, excessive body fat around the waist, or dyslipidemia (high triglycerides, and low high-density lipoprotein), (9). Risk factors characterized to increase the chance of metabolic syndrome may be summarized as follows: age (advancing age), race (Hispanic and Asian decent), obesity (abdominal obesity and a Body Mass Index ≥30), history of diabetes

(individual or family history of type 2 diabetes mellitus and gestational diabetes), and a diagnosis of high blood pressure and cardiovascular disease (9, 10). MetS affects one in five adults and its prevalence increases with age. The estimated prevalence of MetS in U.S. adults (20 years and older) is 76 million (11).

Cardiovascular disease (CVD) refers to multiple conditions of heart and blood vessel disorders. However, the term is often used to describe damage caused to either the heart and/or blood vessels by atherosclerosis (1). Factors that increase risk of CVD include obesity, hypertension, high serum cholesterol levels, tobacco use, insulin insensitivity, and physical inactivity. Globally, CVD, especially atherosclerosis, is the most critical health threat, contributing to more than one-third of global morbidity. According to the American Heart Association, one in three American adults have one or more type(s) of CVD. Less than half of the population with recorded CVD is 60 years or over in age (12). In 2009, direct and indirect costs for CVD were estimated around 475.3 billion dollars (13). Once thought as an elderly disease, increasing interest for prevention and intervention has grown in the last 10 years because of a high prevalence of cardiovascular complications in young and middle aged adults.

CVD has become an escalating health problem among Oklahomans. In 2009, the age-adjusted prevalence of CVD among Oklahomans was 7.2 percent of the state's adult population. Compared to national CVD related death statistics, Oklahoma ranks the third highest state in CVD related death (14). In addition, CVD related disease is the primary diagnosis resulting in over 49,000 hospitalizations, totaling over $2 billion in hospital charges in Oklahoma during 2008. This information is not unexpected when Oklahomans, in general, rank high in modifiable risk factors for the development of

cardiovascular related diseases. A recorded 25 percent of the Oklahoma adult population is current smokers and studies have shown that smokers are two to four times more likely to develop coronary heart disease (14). The prevalence of type 2 diabetes mellitus (T2DM) in adults in Oklahoma is 11 percent, and heart disease death rates increase two to four times in adults with diabetes when compared to non-diabetic adults. Furthermore, 40.4% of Oklahoma adults were observed to have high serum cholesterol levels which has shown to be directly associated with the development of CVD (15).

Abdominal adiposity

Abdominal adiposity, i.e. central obesity, is the accumulation of abdominal fat resulting in an increase in waist size. Increasing abdominal obesity tends to have an association with increasing Body Mass Index (BMI) (16, 17). BMI is calculated by dividing total body weight in kilograms by the total height in meters squared. Obesity is defined as having a BMI score of greater than 30. As a chronic condition, the development and incidence of obesity can be affected by an interaction of demographical, behavioral, physiological, socioeconomic, and genetic factors (18).

There is a strong correlation between central obesity and CVD. The excessive accumulation of adipose tissue greatly increases the risks of a number of metabolic disorders including but not limited to the following: dyslipidemia, insulin resistance, chronic inflammation, endothelial dysfunction, and hypertension (19). These negative health effects have been associated with waist circumference greater than 40 inches in men and greater than 35 inches in women (20).

Researchers have observed an increase in prevalence of abdominal adiposity in U.S. adults (9). A gradient of increasing waist circumference was found in the first National Health Examination Survey (NHES I), the third National Health and Nutrition Examination Survey (NHANES III), and 1999-2000 National Health and Nutrition Examination Survey (NHANES 1999-2000) in U.S. men and women. After age-adjustment, the overall prevalence of abdominal fat in men was found to have increased by 25.6 percent and in women by 40.5 percent between the years 1960 to 2000 (21). Body-fat distribution has been identified as an independent predictor of health risk. Individuals with excess abdominal fat are at an increased risk of negative health consequences due to obesity (17, 22). Data, from the Centers for Disease Control and Prevention, indicate a 34 percent incidence of obesity among the U.S. adult population (more than double the percent of incidence 30 years ago) (22).

Dyslipidemia

Dyslipidemia is a disorder in the normal levels of lipids in the blood and distinguished by a triad of lipid level abnormalities including elevated triglycerides and low density lipoprotein cholesterol and decreased high density lipoprotein cholesterol (23). In Western societies, most dyslipidemias are hyperlipidemias (elevation of lipids in the blood) often due to individual diet and lifestyle choices. However, genetic predispositions for high serum cholesterol levels have been recorded in some families. Prevalence of dyslipidemia tends to increase with high BMI (24).

Undeniable evidence exists that the lipid levels that make up the abnormal triad are independently associated with increased CVD risks. Dyslipidemia adversely affects endothelium function (9). Alterations in endothelial function contribute to pathogenesis

and clinical manifestations of CVD (1) which will be discussed further in the next section.

Oxidative stress and inflammation

Increased oxidative stress and inflammation contribute to the development of atherosclerotic cardiovascular diseases. Oxidative stress is caused by an imbalance of free radicals in the living system (25). Free radicals are molecules or molecular fragments that contain at least one unpaired electron in their molecular orbitals and can be divided into two categories as follows: reactive oxygen species (ROS) and reactive nitrogen species (RNS).

Formed during biochemical processes, ROS and RNS can play beneficial and harmful roles in the human body. The beneficial roles include defending against infectious agents and cell signaling responses. Free radicals also stimulate protein synthesis. Conversely, free radicals in excess can cause damage to major proteins, enzymes and other compounds (26). Production of free radicals promotes further generation of these molecules. In other words, a balance of free radicals in the system is essential for a homeostatic state in living systems.

Adequately functioning antioxidant systems control the accumulating production of free radicals (27). Endogenous and dietary antioxidant compounds scavenge free radicals to be disposed through excretion systems. These antioxidant defenses function as either enzymatic or non-enzymatic mechanisms in the body. Enzymatic compounds (glutathione peroxidase, superoxide dismutase) and non-enzymatic compounds (glutathione, α-tocopherol, ascorbic acid, carotenoids, flavonoids) work together to

generate antioxidant systems. However when excessive amounts of free radicals are developed beyond the capability of the antioxidants, inflammation can occur and chronic oxidative stress can cause damage to vascular tissues that processes to atherosclerotic diseases (1).

Inflammation is the natural acute immune system response to any stimulus such as trauma, stress, immune challenge, bacterial, viral, or fungal toxin, resulting in massive outpouring of primary inflammatory cytokines from monocytes (28). Monocytes are immune cells that travel through the blood stream to identify injured tissues. Once the injured tissue has been located pro-inflammatory cytokines are released into the system to up-regulate inflammation to attract more monocytes to the stressed area. Monocytes transform into macrophages that engulf the harmful agent (29).

However, if inflammation advances to a chronic state, damage to tissues can occur. In this circumstance, the endothelium becomes compromised from the continuous inflammation and migration of adhesion molecules, on the endothelial level, that attach to the monocytes (26, 29). The inflamed monocytes roll over the endothelium until an opening into the intima is located, where they migrate into the cell to become macrophages. The macrophages (with no foreign substance to engulf) will start engulfing oxidized low-density lipoproteins (ox-LDL) to form foam cells (26, 27, 28). This foam cell accumulation converts to atherosclerotic plaque which continues the progression by the release of compounds that stimulate further generation of adhesion molecules and large amounts of ox-LDL. Smooth muscle tissue migrates to form a fibrous cap to protect the vein against the atherosclerotic plaque. However continuous

inflammation can weaken the fibrous cap wall, possibly resulting in a thrombus clot. If the thrombus breaks free, risks of CVD increase (29).

C-reactive protein

C-reactive protein (CRP) expression occurs during an acute phase response to tissue injury or inflammation in the living system (3, 7). The liver synthesizes CRP in response to factors released by adipocytes. The primary factor responsible for rapid elevations in the serum concentration of CRP is a rise in plasma concentration of interleukin- 6 (IL-6) (3). The IL-6 cytokine acts as both a pro-inflammatory and an anti-inflammatory molecule in which macrophages and adipocytes are the predominate producers in inflammation. The production of this inflammatory molecule from adipocytes has been suggested as a reason why obese individuals have higher endogenous levels of CRP (1).

The physiological role of CRP resembles that of an antibody by attaching to phosphocholine expressed on the surface of dying cells in order to activate the complement system via the C1Q complex (7). Correspondingly, the protein enhances phagocytosis (engulfing mechanism) of macrophages which expresses a receptor for CRP complexes (3).

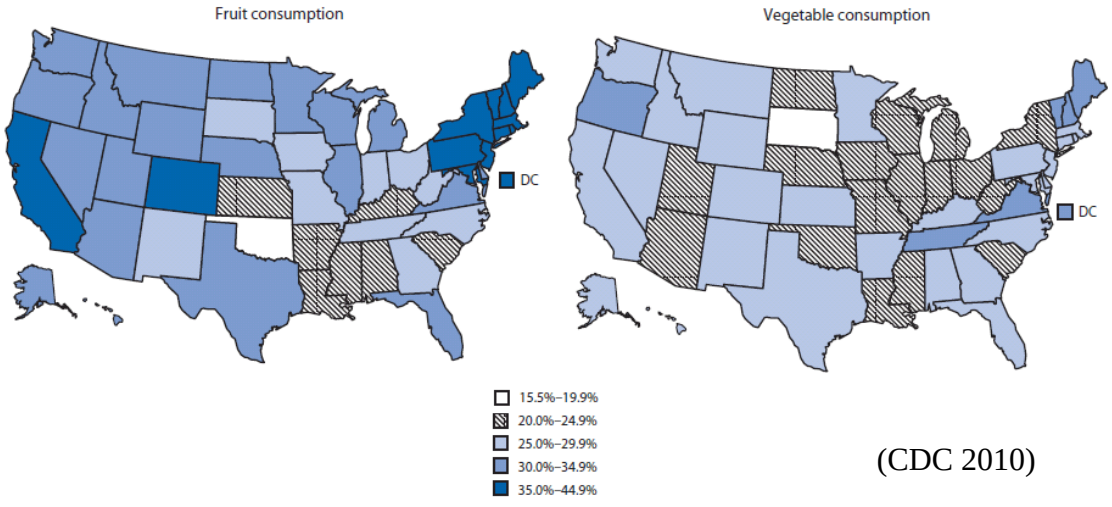
Recent research suggests that patients with elevated basal levels of CRP are at an increased risk of diabetes, hypertension, and CVD (3, 7). Two different tests are used to measure the level of serum CRP; the standard test and the high sensitivity-C reactive protein (hs-CRP) test. The standard test measures a wide range of CRP but is less sensitive in the lower ranges, while the hs-CRP test can accurately detect low concentrations of the protein. The latter is considered the most useful in determining the

potential risk level for CVD (7). Experts encourage the combination of hs-CRP tests along with a subject's lipid profile as a reliable approach to predict risk. However, a rise in levels can be caused by a number of other underlying factors. It is important for researchers to use a combination of examination techniques to determine an individual's risks for CVD (1).

Dietary intakes of fruits and vegetables

According to the *Healthy People 2010* objectives for consumption of fruits and vegetables includes targets of increasing the proportion of U.S. individuals who consume at least two servings of fruits daily by 75 percent and increasing the proportion of U.S. individuals who consume at least three servings of vegetables daily by 50 percent (30). According to a CDC report, the average American falls short of these consumption objectives (14). In 2009, an estimated 32.5 percent of U.S. adults consumed fruits at least two times per day and only 26.3 percent of adults consumed vegetables at least three times per day.

Figure 1. Consumption of fruits and vegetables among US adults

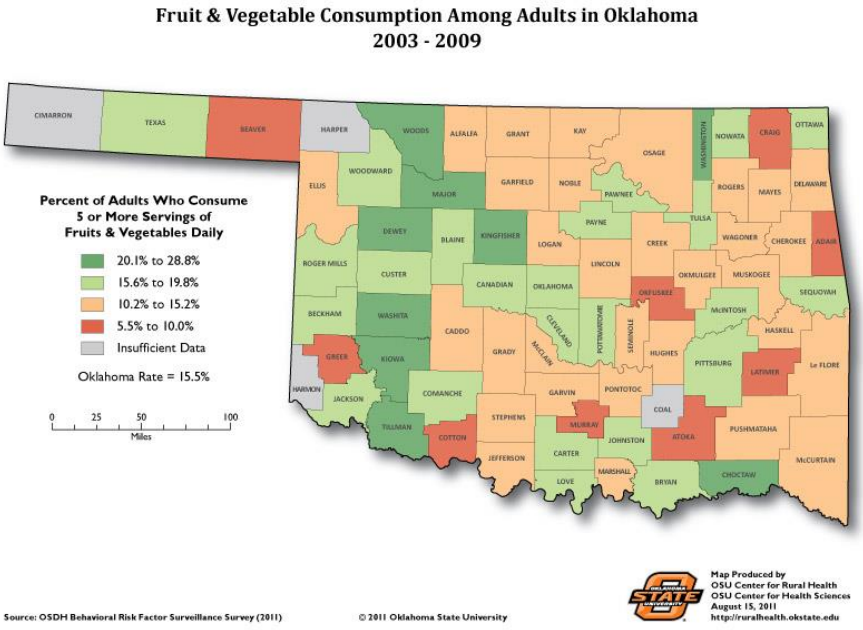


(CDC 2010)

Based on the CDC's findings, no state met *Healthy People 2010* objectives for the consumption of fruits or vegetables. Oklahoma ranks 51st for the consumption of fruits and 47th for the consumption of vegetables, compared to all 50 states and the District of Columbia (14).

According to the *2011 Oklahoma State of the State Health Report*, only 14.6 percent of Oklahoma adults consumed fruits and/or vegetables greater than five times per day. Also 50 of the 77 counties in Oklahoma received a failing grade for their average consumption of fruits and vegetables. No significant differences were determined in consumption among ethnic, economic, or age groups. Higher education status only slightly raised the intake percent from a grade of failing "F" to above average "C" when analyzed at the college graduate level (31).

Figure 2. Consumption of fruits and vegetables among adults in Oklahoma



(OSDH 2011)

Furthermore, according to the CDC *State Indicator Report on Fruits and Vegetables*, the percentage of Oklahomans who report consuming greater than one serving per day of fruits and vegetables was 50.2 percent (fruits) and 26.8 percent (vegetables) (32).

There are many obstacles influencing the consumption of fruits and vegetables in Oklahoma. Access continues to be a significant obstacle in the quest to increase total consumption of fruits and vegetables in this population. According to CDC statistics, only 51 percent of Oklahoma census territories have food retailers that stock such items within a half mile boundary of both rural and urban settings. This situation could be related to only 0.3 percent of total cropland acreage in Oklahoma is used for the harvest of fruits and vegetables (32). Consumers must rely on out-of-state producers which can limit selection and availability depending on season, environmental conditions, cost, and related factors. Furthermore the increasing cost of food items may compel many Oklahomans to choose less nutrient-dense, high-calorie foods in order to feed their families in lieu of purchasing produce (33). According to the USDA *Consumer Price Index (CPI) for Food 2013*, the average cost per portion of fruits and vegetables ($0.46) is double the amount of the average cost per portion of high-density snack foods ($0.23) (33).

Flavonoids

Flavonoids are one of the largest phytochemical sub-classes and can be categorized into several varieties, as listed in Table 1. These compounds are water-soluble polyphenolic molecules containing 15 carbon atoms arranged in two benzene rings which are joined together with a short three carbon chain. Over 4,000 flavonoids have been identified, many of which occur in fruits and vegetables. Flavonoids provide much of the flavor and

color of the edible plants and the foods and beverages that are derived from them. Like other phytochemicals, they are not considered essential nutrients (i.e., proteins, fats, carbohydrates, vitamins, minerals, and water) and for this reason are often referred to as "nonnutritive" compounds (34).

These plant compounds have aroused considerable interest recently because of their potential beneficial effects on human health, particularly obesity and metabolic syndrome. They have been reported to have antiviral, anti-allergy, antiplatelet, anti-inflammatory, anti-tumor and antioxidant activities, which spark researchers to investigate the potential of flavonoid-rich diets and individual flavonoids (4).

Table 1: Flavonoid Subclasses and Common Dietary Sources

Class	Flavonoid	Common Sources
Flavonol	Kaempferol	Apples, Onions
	Myricetin	Lettuce, Tea
	Quercetin	Tomatoes
Flavone	Apigenin	Apples, Celery
	Luteolin	Lettuce, Oregano
Flavanone	Hesperetin	Citrus Fruits: Oranges
	Naringenin	Grapefruits, Lemons
Flavan-3-ol	Catechin	Apples, Cranberries
	Epigallocatechin-3-gallate (EGCG)	Chocolate, Tea
Anthocyanidins	Cyanidin	Blueberries, Cranberries
	Malvidin	Raspberries, Strawberries
	Peonidin	
	Petunidin	

Adapted from the USDA Flavonoid Database (67)

According to the Department of Health and Human Services, diet plays a role in 10 of the leading causes of death, including DM2, coronary heart disease, stroke, certain types of cancer, and atherosclerosis (35). Epidemiologic studies have suggested beneficial effects of flavonoids in diets high in fruits and vegetables by decreasing the risk of chronic diseases.

Observational studies on the dietary intakes of fruits and vegetables

Fruits and vegetables contain a wide range of potentially cardioprotective components including vitamins, fiber, other antioxidants, and non-nutrient phytochemicals. The World Health Organization noted that inadequate intake of fruits and vegetables is one of the leading causes of chronic disease and overall death and mortality worldwide (20). On the whole, observational and case-control studies focusing on consumption of fruits and vegetables in relation to CVD risk have consistently shown inverse associations.

Intake of fruits and vegetables and cardiovascular disease risk

Higher consumption of fruits and vegetables has consistently shown inverse associations with CVD risk when compared to lower intakes. A cohort study by Hung et al. (36) investigated the correlation between consumption of fruits and vegetables and risk of chronic disease in participants from the Nurses' Health Study and Health Professional Follow-Up Study comprising of 121,700 women (mean: 42.5 y/o) and 51,529 men (57.5 y/o) respectively . CVD incidence was significantly lower at the highest quartile intake of fruits and vegetables (≥ 8 serving per day), when compared to the lowest quartile intake (<1.5 serving per day). Relative risk of CVD was 0.88 for an increment of five servings per day of total intake of fruits and vegetables, significantly. (36).

Similar trends were found by Bazzano et al. (37) when examining the association between frequency of the intake of fruits and vegetables and CVD risk in 9,608 participants (<60 y/o: 6312 and >60 y/o: 2844) in the National Health and Nutrition Examination Survey Epidemiologic Follow-Up Study (NHANES I). Intake of fruits and vegetables greater than three times per day compared to less than once per day was associated with a 27 percent lower risk of stroke, a 42 percent lower stroke mortality, a

24 percent lower ischemic heart disease mortality, a 27 percent lower CVD mortality, and a 15 percent lower all-cause mortality after adjustment for established CVD risk factors (37).

Intake of fruits and vegetables and coronary heart disease

The relationship between risk of Coronary Heart Disease (CHD) and the consumption of fruits and vegetables was investigated, by Joshipura et al. (38), in 84,231 middle aged women from the Nurse's Health Study and 42,148 middle aged men from the Health Professional's Follow-Up Study. A trend of 20 percent lower incidence of disease was witnessed in participants that consumed greater than four servings of fruits and vegetables per day. Consumption of fruits and vegetables appeared to have a significant protective effect against CHD. In addition, a significant inverse relationship was found for general heart disease (38).

In agreement with the previous study, Nikolić et al. (39) studied the relationship between dietary intake of fruits and vegetables and the risk of CHD in a case-control study including 290 cases (194 men, 96 women; mean age 59.98 y/o) of first event acute coronary syndrome and 290 paired controls. The controls were matched by sex, age, and admitted to the same regional hospitals as cases without diagnosis of coronary heart disease. The benefits from consumption of fruits and vegetables increased significantly by the number of servings consumed. A trend of 60 percent lower risk of CHD was found in those in the upper tertile of consumption of fruits (> 5 items per day) when compared to the lowest tertile (< 1 item per day). A 70 percent lower risk of CHD was associated with consumption of vegetables of at least three times per day when compared to subjects who did not consume any vegetables (39).

To further investigate this inverse association, Oude Griep et al. (40) examined the manner in which fruits and vegetables were consumed (raw and processed) in relation to CHD incidence. The cohort study observed 8,988 men (mean 42.6 y/o) and 11,081 women (mean 49 y/o) participants' habitual food consumption through a semi-quantitative food frequency questionnaire (FFQ). The average daily intake of fruits and vegetables in the total study population was 378 grams per day (g/d), of which 188 g/d was consumed as raw and 190 g/d processed (including cooked fruits and vegetables). An inverse association for CHD was present for both the intake of raw and processed fruits and vegetables. The highest total fruits and vegetables consumption (> 475 g/d) was inversely associated with CHD incidence compared to participants with the lowest consumption (< 241 g/d). Compared to participants with the lowest intake, an inverse association was observed for a high intake of raw fruits and vegetables (<192 g/d vs. >262 g/d, respectively) with CHD as well as a high intake of processed fruits and vegetables (< 113 g/d vs. >233 g/d). An inverse trend was observed between CHD incidence and intake of fruits greater than 328 g/d and intake of vegetables greater than 162 g/d; however this was not statistically significant (40).

Intake of fruits and vegetables and C-reactive protein

C-reactive protein (CRP) levels have been shown to be affected by consumption of fruits and vegetables. A study by Esmaillzadeh et al. (41) observed an inverse association of both intake of fruits and intake of vegetables with plasma CRP concentrations in postmenopausal women (n=486 [mean age 50 y/o]). The higher intakes of both fruits and vegetables consumed (> 5 servings/d) showed a lower incidence of obesity along with lower plasma CRP concentrations; thus supporting the anti-inflammatory effects of

consuming a diet rich in fruits and vegetables. The association became more evident when combining the data for total dietary fruits and vegetables consumption. The study observed a 39 percent lower concentration in CRP levels in the highest intake of fruits and vegetables (> 5 servings/d) compared to the lowest intake of less than one serving per day (41).

This anti-inflammatory effect due to fruits and vegetables was also observed in another epidemiologic study by Gao et al. (42) examining the relationship between intakes of fruits and vegetables with plasma CRP concentrations in 445 Hispanic elders (mean 69.5 y/o) and 154 neighborhood-based non-Hispanic white elders (mean 68.8 y/o). The Massachusetts Hispanic Elders Study (MAHES) cross-sectional study assessed dietary habits with a FFQ designed for the specific study population. A significant inverse dose-response association between the intake of fruits and vegetables and plasma CRP was observed. The prevalence of high plasma CRP (> 10 mg/L) was significantly greater among subjects in the lowest quartile of consumption of fruits and vegetables [1.4 (0.2-2.2) servings/d], compared to the highest quartile [5.5 (4.4-14.8) servings/d] (42).

Furthermore, a cross-sectional study investigating the correlation among intake of fruits and vegetables with inflammatory and oxidant status found similar results. This study by Root et al. (43) examined 1000 adult subjects [394 male (mean age 45 y/o), 606 female (mean age 47 y/o)]. The participants self-reported the frequency of intakes of fruits and vegetables and multiple markers of inflammatory and oxidant status. Higher intakes of combined fruits (> 2 servings/d) and vegetables (> 3 servings/d) were associated with lower concentrations of CRP and other established inflammatory markers when compared to the lowest intakes (< 2 servings/d and <3 servings/d) (43).

Observational studies on the dietary intakes of flavonoids

Epidemiological evidence suggests that flavonoids may explain the cardio-protective benefits of increasing consumption of fruits and vegetables. Several prospective studies have reported statistically significant inverse associations between total flavonoid intake and the intake of specific sub-classes of flavonoids and CVD incidence, mortality, or risk factors. Thus the following evidence suggests that phytochemicals may play a crucial role in reducing CVD risks.

Flavonoid intake and cardiovascular disease risk

Research provides conflicting evidence on correlations between flavonoid intake and overall cardiovascular disease risk. A study by Mink et al. (6) observed that higher flavonoid intake to be inversely associated with CVD mortality in postmenopausal women. Women (n= 34,489; mean age 62 y/o) from the Iowa Women's Health Study 1986 (IWHS) were selected for the purpose of evaluating the relationship between individual high flavonoid content foods and specific mortality endpoints. A follow up questionnaire was distributed after 16 years to collect information from the participants or their relatives regarding cause and rate of death. Of these participants, there were 7,091 total deaths, with 2,316 CVD related deaths, 1,329 CHD related deaths, and 469 stroke related deaths. In the analysis of total flavonoid and flavonoid sub-class intakes, dietary flavanones and anthocyanidins were significantly associated with a reduced risk of death due to CVD and CHD. However there was no association between total flavonoid intake or any of the sub-classes and stroke mortality. Further investigation found a similar association in the analysis of CHD mortality and foods rich in flavonoids. The age- and energy-adjusted relative risks were significantly reduced in participants reporting

consumption of apples, pears, oranges, grapefruit, blueberries, red wine, celery, strawberries, chocolate, bran, and other fruit juices. Additionally, apples, pears, grapefruit, and red wine remained significantly inversely associated with CHD mortality in the multivariate-adjusted model (6).

A large prospective cohort study of U.S. adults by McCullough et al. (44) further observed that high intake of total flavonoids, and majority of flavonoid sub-classes, were associated with a lower risk of fatal CVD in both men and women after adjusting for several confounding factors. A total of 98,469 participants (38,180 men [mean age 69.9 y/o] and 60, 289 women [mean age 68.5 y/o]) free of chronic disease were chosen from the Cancer and Prevention Study II Nutrition Cohort study. The energy-adjusted mean total flavonoid intake for both men and women was 268 mg/d, corresponding to the 10^{th}-90^{th} percentile distributions. The participants with total flavonoid intakes in the highest quintile (≥359.7 mg/d) had an 18 percent lower risk of fatal CVD when compared with the lowest quintile(<121/5 mg/d). Significant inverse associations were observed for anthocyanidins (median: 22.2 mg/d), flavan-3-ols (median: 63.7 mg/d), flavones (median: 3.0 mg/d), flavonols (median: 27.5 mg/d), and proanthocyanidins (median: 379.4 mg/d). In examining men and women separately, strong inverse associations were seen in both gender groups. The strongest inverse association in women was observed with flavones for fatal CHD. In men, total flavonoid intake was associated with lower risk of fatal stroke. Interestingly, comparing total flavonoids and proanthocyanidin intake in the third tertile (median intakes 201.9; 132.0 mg/d respectfully) compared with the bottom tertile (median intakes 94.5; 53.1 mg/d respectfully), men of 70 years or older in age had a lower risk of fatal CVD compared with men younger than 70 years of age. Many of the

associations were nonlinear, with low risks seen at even modest intake, suggesting that consumption of even small amounts of flavonoid-rich foods may be beneficial for reducing risk of fatal CVD (44).

In contrast, Mursu et al. (45) examined the relationship between flavonoid intake and the CVD mortality along with the risk of ischemic stroke. Finnish men (n= 1950, mean age 52.4 y/o) from the Kuopio Ischemic Heart Disease Risk Factor Study (KIHD) were used to examine the relationship. During the average follow-up time of 15.2 years, men with no previous CHD or stroke experienced 102 ischemic strokes and 153 CVD related deaths. When comparing the most commonly consumed flavonoids in quartiles, neither total flavonoid nor flavonoid subclasses were associated significantly with CVD mortality (45).

Similarly, Sesso et al. (46) evaluated the intakes of total flavonoids, flavonols and flavones among 38,445 women (mean age: 53.9 y/o) free of CVD (at baseline) from the Women's Health Study. A follow-up questionnaire was used to collect information on CVD risk and incidence. After adjustment for dietary and lifestyle factors, the results indicated that a higher flavonoid intake (median intake: 47.44 mg/d) was not associated with a reduced risk of CVD compared to lowest flavonoid intake (median intake: 8.88 mg/d). No single sub-class of commonly consumed flavonols or flavones showed a clear inverse association with the risk of either CVD or important vascular events (46).

Flavonoid intake and hypertension

High flavonoid intakes have been shown to have inverse correlations with blood pressure. In examining the habitual intake of flavonoid sub-classes, Cassidy et al. (47) found that

specific classes of flavonoids, specifically anthocyanins, were associated with a reduction in hypertension risk. Three prospective cohort studies using similar questionnaires were chosen to explore the association between flavonoids sub-classes and risk of incident hypertension as follows: 1976 Nurses' Health Study (NHS I), 1986 Health Professionals Follow-Up Study (HPFS), and 1991 Nurse's Health Study (NHS II). The participants (46,672 women mean age of 55y [NHS I], 87,242 women mean age 36y [NHS II], and 23,043 men mean age of 56y [HFPS]) were included in the analysis based on a set of inclusion criteria. In a combined cohort analysis, an 8 percent reduction risk of hypertension was associated with a high intake of anthocyanins when comparing the highest intake (Quintile 5) with the lowest intake (Quintile 1). Interestingly, comparing the same quintiles, the magnitude of the association was greater in participants under 60 years of age. The inverse association was observed in the NHS I and NHS II cohorts; however, no association was found in HPFS. Also, only in NHS II (younger adult women) was a linear association observed. Additionally, no significant evidence was shown in the other major flavonoid sub-classes in association with reduction in incident of hypertension. However, some individual compounds within the major sub-classes were found to be associated with lower rates of hypertension. For instance, high apigenin (flavones subclass) intake was found to have a 5 percent reduction in rates of hypertension in individuals in the 5^{th} quintile compared to 1^{st} quintile. Catechin and epicatechin (flavan-3-ols) intakes were associated with lower rates in participants under 60 years of age. These data reinforce the importance of dietary intervention strategies for reduction of surrogate risk factors for cardiovascular complications before middle age (47).

Flavonoid intake and C-reactive protein

High intakes of total flavonoid and individual sub-classes have been associated with lower serum C-reactive protein (CRP). A study by Chun et al. (7) examined the associations between dietary flavonoid intake and serum CRP concentrations after adjusting for known factors that affect inflammation levels in humans. The analysis consisted of a 24 hour dietary flavonoid estimated intake and serum CRP concentrations from a total of 8335 adult individuals (mean age: 44.5 y/o) from the NHANES 1999-2002. A significant inverse correlation among serum CRP concentration was seen with total flavonoid intake and individual flavonol, anthocyanidins, and isoflavone sub-classes after adjusting for confounding factors (7).

Clinical studies of dietary flavonoids

The majority of studies of dietary intervention of flavonoids focus on the following specific risk factors of CVD: hypertension, lipid metabolism, glucose metabolism, and inflammation. These cardio-protective effects are specifically shown in flavonoid rich dietary intakes of cocoa, green tea (EGCG), berries (anthocyanidins), and soy (isoflavones).

Effects of flavonoids on hypertension and vascular function

Cocoa and chocolate

Chocolate products have been shown to have beneficial effects on the vascular system and accounts for the majority of total flavonoid intake in Western countries. A randomized, controlled, single-blinded, parallel-group trial conducted by Taubert et al. (48) demonstrated the effect of low dose habitual cocoa intake on blood pressure in 44 pre-hypertensive adults (20 men, 24 women). The participants (aged 53 to 73 years)

were randomly assigned to consume either a dark chocolate bar containing 30 mg of polyphenols (22 participants) or a matching polyphenol-free white chocolate bar (22 participants). The participants were counseled to maintain their usual dietary and physical activities, and instructed to abstain from consuming other cocoa products during the course of the 18 week study. Participants completed a 7-day cocoa-free run-in period and an overnight (12 hour) fasting period before starting the intervention. The chocolate products were consumed two hours after the evening meal. Blood pressure (BP) was recorded between 8 and 10 am to examine the effects of the intervention (baseline, 6, 12, and 18 weeks). BP was taken in intervals (0, 60, 120, 240, 360, and 480 minutes) after first dose to assess acute effects from the chocolate treatments. The results showed that consuming dark chocolate progressively lowered systolic (SBP) and diastolic (DBP) blood pressure significantly compared to the baseline. No significant changes were seen at six weeks. However, after 12 weeks of dark chocolate intake, both SBP (mean of -2.4 mm Hg) and DBP (mean of -1.3 mm Hg) declined compared to the baseline. Further decline of SBP (mean of -2.9 mm Hg) and DBP (mean -1.9 mm Hg) was observed after consumption of the dark chocolate for 18 weeks. SBP and DBP remained unchanged throughout the duration of the study for the participants consuming the white chocolate. This study showed the positive effects on blood pressure from consuming flavon-3-ol rich cocoa (48).

A randomized, single-blind, cross-over study was conducted by Grassi et al. (49) to examine the vascular-protective effects of dark chocolate in 20 never-treated hypertension stage 1 adult patients (10 men, 10 women [mean age 43.65 y]) and 15 healthy control adult subjects (7 men, 8 women [mean age 33.9 y]). Participants were

instructed to maintain their usual diet; however, they were requested to abstain from consuming flavonoid-rich foods and beverages during the 30 week (Phase 1: 15 weeks; Phase 2: 15 weeks) duration of the study. All participants entered a 7-day-cocoa-free-run-in period before starting the first phase. The groups were randomly assigned to consume either a dark chocolate (DC) 100 gram bar (21.91 mg catechin, 65.97 mg epicatechin, 0.59 mg QUER, 0.03 mg KAE) or a 90 gram flavonol-free white chocolate (WC) bar daily. At the end of Phase 1, all participants completed another 7-day-cocoa-free period. During Phase 2, participants were crossed over to the corresponding treatment after completion of the second cocoa-free period. Before and after each study phase, BP was recorded by 24-hour ambulatory blood pressure monitoring (ABPM) at 15 min intervals during the daytime (6 am to 10 pm) or 20 min intervals during the nighttime (10 pm to 6 am). BP was similar at baseline for all hypertensive patients. The results showed that the consumption of DC significantly decreased SBP (-11.0 mm Hg) and DBP (-6.4 mm Hg) in the hypertensive patients compared to the consumption of WC. Flow-mediated dilation (FMD) of the brachial artery was also improved after the treatment of DC. In the hypertensive patients, FMD increased to almost normal values (8.9 percent) compared to the WC treatment. Even the healthy controls showed improved FMD after DC intake compared to WC intake. Significant improvements in glucose and insulin responses were observed in both groups after the DC treatment compared to the WC treatment (49). The above findings support a potentially beneficial action of cocoa flavonols on BP in hypertensive and healthy adults.

Tea-derived epigallocatechin-3-gallate (EGCG)

Epigallocatechin-3-gallate (EGCG) from green tea has shown significant inverse effects on endothelial dysfunction. The endothelium plays a central role in the regulation of vascular homeostasis, and maintenance of the normal vasodilator properties of the endothelium may reduce cardiovascular risk. A double-blind, placebo-controlled, crossover, five week study by Widlansky et al. (50) examined the effects of EGCG on vascular function in 42 adult subjects (29 men, 13 women) with Coronary Artery Disease. Subjects were randomly assigned to receive either 150 mg EGCG capsules (TEAVIGO) or placebo gelatin capsules. Both the EGCG-first group and the placebo-first group were instructed to take a capsule twice a day for two weeks, followed by a one-week washout period. Finally, a cross-over of the treatments occurred for an additional two weeks of study after the washout period. The participants' vascular functions were tested at baseline, two hours after initial treatment, and after two weeks of treatment. FMD of both groups increased significantly after treatment of EGCG compared to baseline. The placebo treatment had no effect on FMD. Additionally, EGCG (300 mg/d) improved brachial artery flow-mediated dilation in patients with coronary artery disease (50).

Further significant beneficial effects on endothelial function were shown after green tea consumption in a study by Alexopoulos et al. (51). The randomized, single-blind, sham procedure-controlled, crossover design study investigated the effects of tea consumption in 14 healthy individuals. Participants consumed either 450 ml of boiled water, green tea in 450 ml of boiled water or 125mg of caffeine (the amount contained in 6 g of green tea) in 450 ml of boiled water. A baseline FMD of the brachial artery was measured before

each intervention and at intervals of 30 minutes, 90 minutes, and 120 minutes after intervention. CRP, IL-6 and total plasma oxidative status were measured at baseline and at 120 minutes. There was a significant increase in FMD with tea consumption (by 3.69 percent, peak at 30 min), and no significant change was observed with caffeine consumption (increase by 1.72 percent peak at 30 min). No effect was observed on CRP concentration, IL-6 concentration, or total plasma oxidative status (51).

Soy isoflavones

Soy (isoflavones) consumption has been found to have cardio-protective benefits. A randomized cross-over clinical trial was conducted by Azadbakht et al. (52) to determine the effects of soy consumption on markers of endothelial function and inflammation in postmenopausal women with metabolic syndrome. All 42 women participants had visceral adiposity, dyslipidemia, hypertension, insulin resistance and elevated blood levels of inflammatory markers including CRP, interleukin (IL) and tumor necrosis factor (TNF-α). The participants were randomly assigned to consume one of three diets for eight week intervals. The diets were defined as follows: the control diet (Dietary Approaches to Stop Hypertension [DASH]), with 55 percent carbohydrates, 17 percent protein, and 28 percent total fat and 1 serving of red meat per day), the soy nut diet (DASH diet replacing the red meat serving with roasted soy nut), and the soy protein diet (DASH diet replacing the red meat serving with soy protein powder or tofu). Anthropometric measures, fasting blood samples and 3-day food records were recorded at baseline, 4, 8, 12, 16, 20, 24, 28, 32,and 36 weeks. Each participant followed the three diets and had two washout periods (four weeks each washout) before starting a corresponding diet. The results revealed a significant difference in CRP levels when

participants consumed the soy nut diet (-8.9 percent) and the soy protein diet (-1.6 percent) compared to the control group. In addition, differences in nitric oxide levels were displayed due to consumption of the soy nut diet (9.8 percent) and the soy protein diet (1.7 percent) compared to the control diet. Neither soy protein nor soy nut consumption changed weight or waist circumference significantly compared with the control diet. Thus, the results indicate that replacement of red meat in the diet by soy nut or soy protein improves some markers of endothelial function and inflammation in postmenopausal women with metabolic syndrome (52).

While studying the effects of dietary soy/isoflavones on blood pressure profiles, Teede et al. (53) determined that processed soy protein dietary supplementation had no effect on arterial function and blood pressure parameters in hypertensive subjects. A double-blind, placebo controlled, cross-over trial was conducted in 41 hypertensive adult participants (26 men, 15 women). Subjects received a soy cereal comprised of 23.0 percent Sorghum (cereal grain), 4.4 percent Nutragen (soy isoflavone), 59.6 percent soy concentrate, 11.9 percent sugar and 0.18 percent salt or the placebo equivalent (41.2 percent Sorghum, 46.2 percent gluten, 12.4 percent sugar and 0.18 percent salt). The cereal was consumed for breakfast during three months and the participants were then crossed over to the corresponding cereal for the final three month duration of the study. Cardiovascular risk assessments and biomedical assessments were conducted at three months and six months. No effect was noted due to consumption of test cereal on arterial stiffness, arterial compliance, 24 hour ambulatory blood pressure parameters, or endothelial function (53).

Effects of flavonoids on lipid metabolism

Cocoa and chocolate

Cocoa has been shown to exert beneficial effects on blood lipids in humans. A randomized, cross-over study in 23 healthy adults (10 men, 13 women), conducted by Wan et al. (54), investigated the effects of cocoa powder and dark chocolate on blood lipids. Subjects were fed one of two controlled experimental diets defined as follows: 1) average American diet (AAD) and 2) cocoa powder (22 g) and dark chocolate diet (CP-DC) for four weeks followed by a two week wash-out period before the cross-over to the corresponding diet for four weeks. Subjects continued their regular diet during the two week wash-out period. The experimental diets were similar in macronutrient content and differed only in contents of flavonoids, and polyphenols due to the cocoa powder and dark chocolate bar. Fasting blood samples were collected at baseline, and before and after each experimental diet period to examine effects on serum total cholesterol, HDL, LDL, VLDL, and triglyceride (TG) concentrations. No significant difference was seen in total cholesterol, LDL, VLDL, or TG concentrations after intake of CP-DC diet. However after consumption of CP-DC diet, subjects had a significant increase in HDL concentrations (0.05 mmol/L) compared with the AAD diet (54).

Baba et al. (55) further conducted a single-blind, controlled study on the effects of cocoa powder on serum lipid concentrations in mildly hypercholesterolemic adult males. The subjects (n= 25) were divided into two groups according to BMI and serum total, LDL, and HDL concentrations. The subjects were further divided in to subgroups randomly and assigned to consume either a cocoa containing drink (26 g cocoa powder and 12 g sugar/d) or a control drink (12 g sugar/d) twice a day. Fasting serum VLDL, LDL, HDL

concentrations were taken at baseline and 12 weeks. Home deliveries of food were made to ensure that the same foods were consumed in the three days before collection of blood samples. The HDL concentration increased significantly by 23.4 percent after consumption of the cocoa drink compared with baseline concentrations and in the subjects that consumed the control drink. Also LDL concentration decreased significantly by 12.6 percent in the cocoa group compared to control group. No significant differences in resulting effects were found between subjects in the higher BMI group compared to lower BMI group (55).

Cocoa powder and dark chocolate consumption have also been shown to exert favorable effects on lipid oxidation susceptibility. A study reported by Mathur et al. (56) investigated the effects of cocoa rich supplementation on lipid oxidation susceptibility in healthy adults. Subjects (13 men, 12 women) were instructed to consume both a dark chocolate bar (36.9 g) and a cocoa powder drink (30.95g [651 mg procyanidins/d]) daily for six weeks. The intervention was followed by a 6-week-cocoa-free wash-out period to accesses the impact on lipid oxidation after treatment. Subjects were advised to consume a low flavonoid diet and abstain from consuming other cocoa containing products throughout the entire 12 week study period. Fasting blood samples were obtained at the baseline, after the intervention period, and after the wash-out period. The results showed that the cocoa product supplementation significantly decreased oxidization of low-density lipoprotein (LDL) with a 9.8 percent longer lag time of conjugated double bond diene formation compared with the baseline. However, serum total antioxidant capacity did not have a correlation with LDL oxidation lag time, rate of LDL oxidation, or conjugated

diene formation. Thus, the results show that the cocoa product supplementation can decrease susceptibility to lipid oxidation (56).

Tea-derived epigallocatechin-3-gallate (EGCG)

Green tea consumption has been shown to have beneficial effects on serum lipid levels. A study by Coimbra et al. (57) found significant improvement in the lipid profiles of 29 subjects (7 men and 22 women) after participating in a study to evaluate the effect of green tea on the lipid profile. The biochemical evaluations included cholesterol, TG, HDL-C, LDL-C, Apo A-I, ApoB, and LP(a). These measurements were performed at the beginning of the study, after three weeks of drinking one liter of water daily, and after consuming one liter of green tea (1.75 g of tea leaves per 200mL of water) daily for four weeks. Fasting venous blood samples revealed no significant reduction in lipid profiles after the water phase. The results showed a significant reduction in total cholesterol (-2.8 percent), LDL (-8.9 percent), Apo B (-3.6 percent) and a significant increase in HDL (4.0 percent) and Apo A-I (5.1 percent) after green tea consumption compared to the water phase. The data suggests green tea intake protects against CVD by improving blood lipid profiles (57).

Berry Anthocyanidins

Berries have been shown to modulate lipid levels in several clinical studies using freeze-dried forms of the berry intervention. A single-center, intervention study by Basu et al. (58) examined the effects of consuming freeze-dried strawberry powder (FSP) daily on serum concentrations of lipids, biomarkers of oxidative stress, and inflammation in 16 women with metabolic syndrome. The shakes contained 25g per cup FSP mixed in eight ounces of water and were consumed at least six hours apart for a total of four weeks.

Fasting blood samples were collected at the baseline, and four weeks. A significant reduction in serum total cholesterol and LDL levels was observed after the intervention period compared to baseline. However, no significant differences were found in fasting glucose, triglycerides, HDL- and VLDL-cholesterol levels (58).

Similar effects of strawberries were observed in a randomized, single-blind, placebo-controlled, 12 week crossover intervention trial conducted by Burton-Freeman et al. (59) to examine the effects of freeze-dried strawberry powder with a challenge of high fat diet (HFD) on blood lipid concentrations in hyperlipidemic adults. Following a 7-day-berry-free run-in period, participants (10 men, 14 women) were randomly assigned to one of the two trial arms as follows: daily consumption of active freeze-dried strawberry beverage (Str) or placebo strawberry-flavored beverage (Pbo). All subjects consumed the HFD consisting of breakfast items that reflected typical American dietary patterns for energy and macronutrient intakes during the entire duration of study. Subjects consumed their assigned beverages for six weeks, followed by a cross-over period of the alternate beverage for an additional six weeks. No wash-out period occurred between the six week intervention intervals. Blood samples were obtained after the initial run-in period and after each six week intervention interval to analyze the fasting and postprandial total cholesterol, LDL, HDL, TG, and ox-LDL concentrations. Under both experimental conditions, significant increases in concentrations of TG, HDL, and oxLDL were observed in the postprandial state. However, significantly lower concentrations of TG, HDL, and ox-LDL were seen when participants consumed the Str beverage along with the HFD diet compared to the Pbo. The LDL concentrations were only affected significantly by the Str in the beverage postprandial phase in men compared to the Pbo

(120.1 mg/dL vs. 122.8 mg/dL). No significant effects on total cholesterol were observed in relation to the Str beverage. Furthermore, only in men did the postprandial ox-LDL concentrations lower significantly at all points (180, 240, 360 minutes) after consumption of the Str beverage compared to the Pbo. No significance in ox-LDL concentrations were found among women (59). Thus, Str beverages have a beneficial effect on postprandial blood lipid concentrations in hyperlipidemic men.

Soy isoflavones

Significant lipid lowering effects have been observed in studies examining the health effects of soy isoflavones. A randomized, cross-over study by Jenkins et al. (60) investigated the effects of soy foods containing different concentrations of isoflavones on blood lipid levels in 41 hyperlipidemic adults. The subjects were recruited to participate in three different diet phases: the control phase (dairy-/egg protein), the soy-protein high isoflavones phase (73 mg/d), and the soy-protein low isoflavones phase (10 mg/d). During all three phases subjects followed a self-selected National Cholesterol Education Program Step II diet (< 7 percent energy from saturated fat and < 200 mg dietary cholesterol/d) for base meals. The subjects were randomized as follows: the control phase (low-fat dairy products and egg substitute), and the soy phases (low-fat soymilk, and soy hot dogs, burgers, old cuts, and tofu burgers). The soy phases were controlled for isoflavones concentrations by the amounts allowed to be consumed during each phase. Each of the phases lasted for a length of four weeks, and then was followed by a two week wash-out period before the cross-over period to an alternate diet. Fasting blood samples were obtained at the start and the end of each four week diet phase to analyze the effects of diet on total cholesterol, triacylglycerol, and HDL cholesterol concentrations.

The results showed an equal decrease in blood lipid concentrations during both soy phases. This decrease was significantly greater than the control phase compared to all the baseline blood lipid concentrations. Both low- and high-isoflavones soy phases had significantly lower values of total cholesterol, total:HDL ratio, LDL:HDL ratio than the control compared to all baselines. In addition, LDL concentrations significantly decreased during the high-isoflavone phase compared to the control phase. The Low-isoflavone phase showed a lower LDL concentration when compared to the control phase; however, it was not statistically significant (60). These outcomes show that intake of isoflavones can have a beneficial effect on blood lipid concentrations.

Effects of flavonoids on glucose metabolism

Cocoa and chocolate

Dietary cocoa flavonoids have been shown to have blood glucose lowering effects and in improving insulin sensitivity. A randomized, single-blind, cross-over two phase study by Grassi et al. (61) was conducted in 15 healthy adults (7 men, 8 women [mean age 33.9 years]). The participants completed a 7-day-cocoa-free run-in period before being randomly assigned to consume either 100 g dark chocolate bars (~500 mg polyphenols) or 90 g white chocolate bars during Phase 1 (15 days). Phase 2 (15 days) began after a second cocoa-free-wash-out period in which participants consumed the corresponding treatment. An oral-glucose-tolerance test (OGTT) was performed after the cocoa-free-run-in period and after each phase (participants fasted overnight and ingestion of last chocolate was at least ≥12 hours). The assessments of blood glucose and insulin were performed in 30 minute intervals from baseline to 180 minutes after the glucose load. The results showed that ingestion of the dark chocolate bar significantly lowered the

homeostasis model assessment of insulin resistance (HOMA-IR) values compared to the ingestion of the white chocolate bar. The quantitative insulin sensitivity check index (QICKI) values increased after the ingestion of the dark chocolate bar compared to the ingestion of white chocolate bar. Also, the insulin sensitivity index (ISI) values increased significantly with ingestion of dark chocolate compared to white chocolate. These findings indicate that ingestion of dark chocolate could improve insulin sensitivity in healthy adults (61).

Similar results were observed in hypertensive patients in another randomized, single-blind, cross-over two phase study by Grassi et al. (62). This study aimed to examine the effects of flavonoid-rich dark chocolate (FRDC) and flavonol-free white chocolate (FFWC) on glucose tolerance and insulin sensitivity in 19 hypertensive adult subjects (11 men, 8 women [mean age 44.8 y/o])) with impaired glucose tolerance (IGT). After starting with a 7-day-cocoa-free-run-in period, the study randomly assigned subjects in Phase 1 (15 days) to consume each day in two half-bar dose at breakfast and lunch either a FRDC bar (100g) or a FFWC bar (100g). Following a second 7-day-cocoa-free period, Phase 2 was started in which subjects consumed the corresponding treatment. A glucose tolerance test (OGTT) was performed following a 10 to 14 hour overnight fast at the end of the first run-in period and after both intervention phases. The plasma glucose and insulin assessments were performed in 30 min intervals from the baseline to 180 min after the glucose load. The results indicated that after 15 days of consuming the FRDC bar, the HOMA-IR values decreased and the QUICKI values increased significantly compared to the baseline and ingestion of the FFWC bar. No changes were shown in either the HOMA-IR values or the QUICKI values after ingestion of the FFWC bar. The

ISI values increased compared with the baseline (2.03) and ingestion of the FFWC bar (1.99). Thus, the results concluded that the ingestion of dark chocolate has the ability to enhance insulin sensitivity in hypertensive patients with IGT (62).

Tea-derived epigallocatechin-3-gallate (EGCG)

Flavonoids from green tea (EGCG) have shown blood glucose lowering effects and in improving insulin sensitivity. A randomized, control, cross-over study by Fukino et al. (63) was performed in 60 healthy adults (49 men, 11 women) to investigate the effects of green tea-extract powder on glucose abnormalities. Subjects were randomly divided into the Early intervention group or the Later intervention group. The Early group consumed an one packet daily supplement of a mixture of green tea-extract and green tea powder (ratio 9:1) for the first two months of the study's duration. The Later group consumed the one packet daily supplement in months three and four after the first phase. The supplement mixture (1/3 to 1/4 of packet used at a time [~456 mg catechins]) was dissolved in eight ounces of hot water and consumed at the end of every meal. Biochemical measures (fasting blood glucose, insulin, and HbA1c) were obtain at the baseline, two, and four months. A significant reduction was observed in HbA1c level associated with the intervention in a time-dependent manner in both the Early and Later intervention groups. However, no significant changes were found in blood glucose or insulin levels associated with green tea intervention (63).

Effects of flavonoids on inflammation

Cocoa and chocolate

Cocoa has been demonstrated to have beneficial effects on lowering biomarkers of inflammation. A single center controlled trial by Monagas et al. (64) investigated the

effects of 100 g of dark chocolate (DC) consumed daily for one week on CRP levels in 28 healthy adults (9 men, 19 women). Each dose of DC contained 70 percent cocoa and provided 700 mg of flavonoids daily. The subjects were asked to abstain from consuming any outside sources of dark chocolate or other flavonoid-rich products for two weeks prior to the intervention and during the duration of the study (7 days). Fasting blood samples were obtained and analyzed pre- and post-intervention to examine hs-CRP levels. The seven day consumption of the DC significantly decreased hs-CRP levels by 23 percent in women. However, there was no significant association with DC treatment in men or total men and women combined. This study demonstrated that short-term dark chocolate intake can significantly lower biomarkers of inflammation in healthy adult women (64).

Berry anthocyanidins

Among the berry fruits, strawberries are a rich source of anthocyanins which have been shown to possess anti-inflammatory effects in humans. A single-center, randomized single-blind, placebo-controlled, cross-over trial by Edirisinghe et al. (65) was conducted in 26 healthy adults (10 men, 14 women). The subjects completed a 7-day-berry-free-run-in period before starting the study. The subjects consumed a controlled meal accompanied by either an active strawberry beverage or a strawberry-flavored placebo beverage, randomly. At the next controlled meal time, subjects consumed the corresponding beverage which allowed subjects to serve as their own control. Both of the beverages contained Strawberry Nesquik powder, skim milk, and were matching in total energy. However, only the active strawberry beverage contained 10 g of freeze-dried strawberry powder (~81.6mg/10g of anthocyanin) from California, USA. Under

supervision, the meals and the beverages were consumed within 20 minutes. Blood samples were obtained for analysis of hs-CRP levels before the meal and after the meal at multiple point intervals (30 minute to 120 min and then hourly thereafter for a total of 6 hours). The results indicated that postprandial hs-CRP significantly decreased due to the active strawberry beverage when compared to the placebo. Thus, this study showed that the intake of an anthocyanin rich strawberry beverage can have beneficial effects on CRP levels in both adult men and women (65).

Conclusions

Epidemiologic and clinical evidence suggests that protection against cardiovascular disease risk factors might be the result of the interaction of several dietary antioxidant phytochemicals including flavonoids. The mechanisms by which they may exert these functions could be summarized as follows: lipid lowering, antioxidant and anti-inflammatory effects. Importantly, a review of the effect of phytochemicals on cardiovascular disease risk factors suggests that the favorable effects may be related to whole foods and beverages containing these flavonoids versus isolated doses of flavonoids, thus suggesting a synergistic action among different flavonoids and nutrients in foods and beverages. However, most epidemiologic studies on flavonoids and CVD risk have examined only one or two classes of flavonoid sub-classes. In addition, epidemiologic investigation of flavonoids in relation to CVD risks in Oklahoma adult populations is limited. Thus, there exists a need to investigate the relationship between total intake of fruits and vegetables, total flavonoid intakes, dyslipidemia, and CRP, in adults with abdominal adiposity and this leads to the hypothesis of our current study.

CHAPTER III

METHODOLOGY

Institutional review board approval

This study was conducted according to the guidelines of the Declaration of Helsinki, and approval was obtained from the Oklahoma State University Institutional Review Board (IRB) for all procedures and the corresponding human ethics committee at the University of Oklahoma Health Sciences Center. Prior to involvement in the study, all investigators and graduate research assistants (GRA) completed the IRB training for human subjects research practices through the Collaborative Institutional Training Initiative (CITI) and also received training on the process of consenting and data collection. All participants provided signed informed consent before their enrollment in the study.

Participants

Thirty adult participants with abdominal adiposity and dyslipidemia were recruited at both the General Clinical Research Center (GCRC) at Oklahoma University Health Sciences Center Oklahoma City, and the Oklahoma State University Campus, Stillwater. Interested participants were screened to examine if they fit the criteria of the study. A telephone questionnaire was used for initial screening, followed by an onsite interview to ensure qualification and compliance. The qualification was confirmed based on specific

measurements such as waist circumference and a fasting lipid profile. Upon qualification, participants were enrolled into the study. This study required participation of adults (>21 years) with abdominal adiposity as well as dyslipidemia.

Inclusion criteria: Participants with enlarged waist circumference indicative of abdominal adiposity (men >40 inches, women >35 inches) and dyslipidemia (2 of 4 criteria: fasting total cholesterol >200mg/dL, triglycerides >150mg/dL, LDL-cholesterol >100mg/dL, or HDL-cholesterol (men <40mg/dL, women <50mg/dL) were included in the study. Participants on stable multivitamin/mineral supplements or prescription medications (except hypolipidemic, hypoglycemic, and steroid agents) were also included. In addition, males and females, as well as individuals from any ethnic group, who qualified, were included in the study.

Exclusion criteria: Individuals were excluded if they had any form of pre-existing disease including cancer, heart disease, diabetes (fasting glucose ≥126mg/dL, liver, or renal disorders, or anemia. Subjects were also excluded if they were pregnant, nursing, taking mega doses of antioxidants/fish oil supplements (> 1g/day), taking hypolipidemic, hypoglycemic, and steroid medications. Moreover, individuals with abnormal metabolic levels of hemoglobin (normal range: 12.0-18.0g/dL), white blood cells (normal range: 4.0-11.0 K/mm^3), or platelets (140-440 K/mm^3), hypo/hyperthyroidism (normal range for thyroid stimulation hormone: 0.35-4.940 uiu/mL), abnormal liver enzymes (normal range for aspartate amiontransferase: 7-40 units/L; alanine aminotransferase: 10-45 units/L), and abnormal kidney function (normal creatinine: females- 0.7-1.2mg/dL; males- 0.8-1.2mg/dL; normal BUN: 1-59 years- 7-18mg/dL; >59 years- 8-21mg/dL) were excluded

from this study. Individuals who smoked or used any other form of tobacco were excluded as well as those who consumed greater than one serving of alcohol per day.

Cross-sectional study design

Participants were asked to maintain their typical diet, physical activity, and lifestyle during the study. Registered Dietitians (at both sites) instructed the participants on correct completion of the detailed 3-day food records, including one weekend day to reflect average normal dietary intake. The participants were instructed on how to use the food records and how to accurately record any food portions consumed. The participants recorded/described the type of food and amount of food consumed, as well as location (home, restaurant, with friends, or at work) and type of meal or snack during which the food was eaten (breakfast, morning snack, lunch, afternoon snack, dinner, or evening snack). The participants were also instructed to record the names of specific restaurants where the food was consumed and the names of the menu items eaten. The submission of recipes and nutrition labels for foods prepared at the participant's home were requested in order to ensure accurate dietary analysis.

Dietary analyses

A 3-day average for each participant's intake of fruits and vegetables and average total flavonoid intake were analyzed using the U.S. Department of Agriculture's Food and Nutrient Database (66) and U.S. Department of Agricultures' Flavonoid Databases (67), respectively. The following flavonoid sub-classes were included in the analysis: anthocyanides, flavones, flavan-3-ol, and flavonol (kaempferol, myricetin, and quercetin). To obtain the 3-day estimated flavonoid intake values, the reported frequency

of standard portion sizes were multiplied by the flavonoid compound content of each food (per 100 grams) and flavonoid intakes across total dietary food and beverage items. Gram amounts of each food item were weighted out on the Ozeri ZK011 Precision Pro Scale (San Diego, California). If a participant consumed a food item not included in the database, the item was omitted and recorded as missing data to be explained in the discussion session.

Anthropometrics and blood pressure

Height, weight, blood pressure, and waist circumference were measured by trained personnel at GCRC and at NSCI. Weight was measured with the SECA 644 Multifunctional Hand Rail Scale (SECA) and recorded to the nearest 0.1 kg. Height was measured using the Accustat Genentech Stadiometer and recorded to the 0.1 cm. Systolic and diastolic blood pressures were measured in millimeters Hg using Spot Vital Signs Device. Participants were asked to lay down and relax for 8-10 min, followed by three blood pressure measurements recorded in five minute intervals. Waist circumference was measured at the superior iliac crest using the Gulick II Tape Measure. All measurements were conducted in a fasting state.

Clinical analysis

Blood draws were performed by trained nurses at GCRC and by trained phlebotomists at Stillwater Medical Center at OSU. Serum separator tubes (SST) and tubes containing the anticoagulant EDTA were used in collecting 45-60 mL of blood from the participants after a period of fasting. Serum and plasma samples were stored at -80 °C until analysis. In order to separate plasma and serum, centrifugation at 1464 g was performed for 10

minutes at 4° C using the Centrifuge 5810 R (Eppendorf, Hamburg, Germany) in Human Nutrition Laboratory at Oklahoma State University Nutrition Science Department or the University of Oklahoma Medical Center laboratory. Analysis of each participant's serum glucose, lipids, hemoglobin and hematocrit, platelets, and liver, renal, and thyroid function tests were performed at Stillwater Medical Center (Stillwater, OK) and the University of Oklahoma Medical Center laboratory (Oklahoma City, OK).

Lipid oxidation analysis

Biomarkers of lipid oxidation in blood samples that were measured for this study included oxidized LDL (ox-LDL), and malondialdehyde (MDA). The serum ox-LDL was measured in triplicate using an ox-LDL competitive ELISA (Mercodia, Uppsala, Sweden) which is based on the monoclonal antibody 4E6 (mAb-4E6). In this procedure, the ox-LDL in the participant's blood sample competes with a pre-determined amount of ox-LDL in the microtiter well for binding with biotin-labeled specific antibodies. Following a washing to remove un-reactive sample components, the biotin labeled antibody was identified with streptavidin. In the final steps, the bound conjugate was detected through its reaction with 3,3,5,5- tetramethylbenzidine (TMB), stop solution was added, and the sample was read spectrophotometrically using the Synergy HT Plate reader (BioTek Instruments, Inc., Winooski, VT) at 450 nm.

The serum levels of MDA were determined with the Bioxytech® LPO-586™ assay (OxisResearch™ Inc., Foster City, CA). This assay detects lipid peroxidation based on the reaction of MDA with the chromogenic reagent, N-methyl-2-phenylindole at 45°C. Methanesulfonic. The sample was read spectrophotometrically using the Synergy HT plate reader (BioTek Instruments, Inc., Winooski, VT) at 586 nm.

C-reactive protein analysis

The concentrations of human C-reactive protein (CRP) were measured as a biomarker of inflammation. The procedure for the determination of serum levels of CRP utilizes the quantitative sandwich enzyme immunoassay technique with a monoclonal antibody that is specific for CRP. The standards and samples are added to the wells and the CRP present in the plasma becomes bound by the immobilized antibody. Subsequent washing removes any unbound substances and an enzyme-linked monoclonal antibody specific for CRP is then added to the wells. After a second wash, addition of a substrate solution causes color to develop in proportion to the amount of CRP that was bound in the first step of the procedure. A stop solution stops the color development and the intensity of the color is measured spectrophotometrically with Synergy HT plate reader at 450 nm.

Statistical analysis

Pearson Correlation analyses were used to examine the correlations among measures of lipid oxidation (ox-LDL, MDA), CRP, flavonoids (KAE, MYR, QUER), fruits and vegetables intakes, and measures of lipids (total cholesterol, TG, LDL, HDL, VLDL) and glycemic control (glucose, insulin, HbA1c). To ascertain whether waist circumference, measures of lipids and glycemic control differed by CRP and ox-LDL levels, CRP concentrations and ox-LDL concentrations were defined by tertiles as follows: CRP [Tertile 1 (0.1 to 2.63 mg/L); Tertile 2 (2.631 to 7.3 mg/L); Tertile 3 (7.31 to 25.6 mg/L)]; oxLDL [Tertile 1 (87 to 125.33 U/L); Tertile 2 (125.34 to 140 U/L); Tertile 3 (140.1 to 156 U/L)]. An ANOVA was used to determine if lipids (total cholesterol, TG, LDL, HDL, VLDL), glycemic control (glucose, insulin, HbA1c), waist circumference, and intakes of flavonoids, and fruits and vegetables differed by tertiles of CRP and ox-

LDL concentrations. Due to missing data among several flavonoid sub-classes, only quercetin was included in the final analysis examined by ox-LDL and CRP tertiles. Variables were compared using SPSS® 16.0 for Windows (SPSS Inc., Chicago, IL, USA). Alpha was set as 0.05 for statistical significance.

CHAPTER IV

RESULTS

Baseline characteristics of participants

A total of 30 adults with abdominal adiposity and dyslipidemia were included in the analysis. The baseline characteristics of the participants are illustrated in Table 2. The participants' age (mean±SD) was 48±9.6 years old and ranged from 27 to 72 years. The participants were predominantly female (n= 25) with few male participants (n= 5). Table 2 also displays the daily medication and supplement use by the participants.

All participants were obese as determined by their body mass index (BMI). The mean±SD for BMI was 37.2±6.3 kg/m² for all participants. Each male participant had a waist circumference greater than 40 inches and each female participant had a waist circumference greater than 35 inches. The mean±SD waist circumference of all participants was 42.7±4.5 inches (43.4±3.5 M, 42.5±4.7 F). All participants had dyslipidemia indicated by mean±SD serum values as follows: total cholesterol 213±37 mg/dL (normal: <200mg/dL), TG 173±74 mg/dL (normal: 40 to 160 mg/dL M; 35 to 135 mg/dL F), LDL 185±294 mg/dL (normal: 60 to 180 mg/dL), HDL 47±16 m/dL (normal: >45 mg/dL M; >55 mg/dL F), VLDL 34±15 mg/dL (normal: 7 to 32 mg/dL), and LDL/HDL ratio 3±1 (normal: <3.6 M, <3.2 F).

The mean±SD serum glucose level (94±15 mg/dL) was in the normal range (< 100 mg/dL); whereas the mean±SD HbA1c level (5.9±0.6%) was in the pre-diabetes range (5.7-6.4%). The participants were found to be pre-hypertensive with measurements of systolic and diastolic blood pressures above 120/80 mm Hg. The mean±SD systolic and diastolic blood pressure values were 130±13/84±8 mm Hg.

The average reported servings of fruits and vegetables, derived from 3-day food records, for each participant were calculated using the USDA Food and Nutrient Database. The average reported serving of fruits was 0.66 cups, average reported serving of vegetable 1.20 cups, and total reported servings of fruits and vegetables was 1.86 cups which is lower than the desirable daily intake of greater than five servings (about 5 cups) of fruits and vegetables per day. The average reported flavonoid intakes of the participants, derived from 3-day food records, were analyzed using the USDA Flavonoid Database. The mean±SD reported intakes of the flavonoid sub-classes were as follows: kaempferol 928±7 mg/100g, myricetin 567±7 mg/100g, and quercetin 5980±238 mg/100g. The reported intakes of the other flavonoid subclasses had too many missing values to be included in the analysis.

The dietary flavonoid containing foods most commonly reported as consumed by the participants are shown in Supplemental Figure 1.

Correlations among measures of lipid oxidation, CRP, and flavonoids with measures of lipids and glycemic control

The correlations among measures of lipid oxidation (ox-LDL, MDA), CRP, and flavonoids (KAE, MYR, QUER) with measures of lipids (total cholesterol, TG, LDL, HDL, VLDL) and glycemic control (glucose, HbA1c) are illustrated in Table 3. The

Pearson Correlation statistic was used to examine these correlations. The results showed that ox-LDL had significant positive correlations with total cholesterol (0.406; $p < 0.05$) and HDL-cholesterol (0.362; $p < 0.05$). However, no significant correlations of ox-LDL were observed with TG, LDL, VLDL, glucose, HbA1c, reported total servings of fruits and vegetables, servings of fruits, and servings of vegetables. Likewise, MDA had significant positive correlations with total cholesterol (0.436; $p < 0.05$) and HDL-cholesterol (0.432; $p < 0.05$). However, no significant correlations were observed with TG, LDL, VLDL, glucose, HbA1c levels. No correlations of MDA were observed with reported total servings of fruits and vegetables, serving of fruits, and servings of vegetables. The results showed that the CRP levels were not correlated with any of the variables. However, all three flavonols (KAE, MYR, QUER) showed positive correlations with HbA1c levels (0.572, 0.572, 0.474, respectively; $p < 0.05$).

Differences in metabolic parameters and intakes of fruits and vegetables among increasing tertiles of ox-LDL and CRP.

The comparisons among waist circumference, measures of lipids (total cholesterol, TG, LDL, HDL), glycemic control (glucose, insulin, HbA1c), CRP, QUER, and reported servings of fruits and vegetables across tertiles of ox-LDL are illustrated in Table 4. Serum ox-LDL levels were defined as follows: Tertile 1: (lowest [87 – 125.33 U/L]); Tertile 2 (125.34 – 140 U/L), and Tertile 3: (highest [140.1 – 156 U/L]). The results showed that the participants in the highest tertile of ox-LDL had significantly higher total cholesterol, and LDL cholesterol when compared to the lowest tertiles ($p < 0.05$). The participants in the middle tertile (Tertile 2) had significantly higher glucose than the highest tertile ($p < 0.05$). No significant differences were observed in waist

circumference, HbA1c, TG, HDL, and intakes of QUER, fruits and vegetables among tertiles of ox-LDL (Table 4).

The comparisons among waist circumference, measures of lipids (total cholesterol, TG, LDL, HDL), glycemic control (glucose, insulin, HbA1c), QUER, and reported servings of fruits and vegetables across increasing tertiles of CRP are illustrated in Table 5. The tertiles were defined as follows: Tertile 1 (lowest [0.1 – 2.63 mg/L]), Tertile 2 (2.631 – 7.3), and Tertile 3 (highest [7.31 – 25.6 mg/L]). The results showed that the participants in the highest tertile of CRP had higher waist circumference than the middle tertile (Tertile 2). Again, those with the highest CRP levels had significantly higher glucose and HbA1c when compared to the lower tertile ($p < 0.05$). Finally, the highest tertile of CRP was observed to have higher QUER and reported servings of fruits and vegetables vs. lower tertiles ($p < 0.05$). However, no significant differences were observed in measures of lipids (total cholesterol, TG, LDL, and HDL) among tertiles of CRP (Table 5).

Table 2. Baseline characteristics of participants **(n=30)**	
Gender	
Male (n)	5
Female (n)	25
Age (years)	48.0±9.6
Anthropometrics	
Weight (kg)	101.7±21.5
Height (cm)	166.4±7.0
BMI (kg/m²)	37.2±6.3
Waist Circumference (inch)	42.7±4.5
Males	43.4±3.5
Females	42.5±4.7
Blood Pressure	
Systolic blood pressure (mm Hg)	130.0±13.0
Diastolic blood pressure (mm Hg)	84.0±8.0
Glycemic Control	
Glucose (mg/dL)	94.0±15.0
Insulin (mg/dL)	21.0±17.0
HbA1c (%)	5.9±0.6
Lipid Panel	
Total Cholesterol (mg/dL)	213.0±37.0
Triglyceride (mg/dL)	173.0±74.0
LDL-cholesterol (mg/dL)	185.0±294.0
HDL-cholesterol (mg/dL)	47.0±16.0
VLDL-cholesterol (mg/dL)	34.0±15.0
LDL/HDL ratio	3.0±1.0
Inflammation and Oxidation Markers	
High sensitivity CRP (mg/dL)	7.0±6.0
Oxidized LDL (U/L)	130.0±16.0
Reported Medication and Supplement Use (n)	
Medication	
Hypertension	6
Anti-depressant	4
Pain Medication	4
NSAID	4
Supplement	
Calcium	3
Multivitamin/mineral	3
Herb/other	4
Reported Servings of Fruits & Vegetables	
Fruits (cup)	0.7
Vegetables (cup)	1.2
Total Fruits & Vegetables (cup)	1.9
Reported Flavonoid Intake	
Kaempferol (mg/100g)	928±7.0
Myricetin (mg/100g)	567±7.0
Quercetin (mg/100g)	5980±238

Data presented as mean±SD. Reported intakes of fruits and vegetables (3 day averages) were quantified based on the USDA Food and Nutrient Database. Reported intakes of flavonoids (3 day averages) were quantified based on the USDA Flavonoid Database.

Table 3. Correlations among measures of lipid oxidation (ox-LDL, MDA), CRP, and flavonoids (KAE, MYR, QUER) with measures of lipids (total cholesterol, triglycerides, LDL, HDL, VLDL), and glycemic control (glucose, HbA1c).

	ox-LDL (n=30)	MDA (n=30)	CRP (n=30)	KAE (n=26)	MYR (n=26)	QUER (n=30)
Total Cholesterol (mg/dL)	0.406*	0.436*	-0.077	-0.108	-0.143	-0.080
Triglyceride (mg/dL)	0.348	0.270	-0.158	0.139	0.232	0.347
HDL-cholesterol (mg/dL)	0.362*	0.432*	-0.141	-0.144	-0.138	-0.151
LDL-cholesterol (mg/dL)	-0.012	0.010	0.268	-0.221	-0.337	-0.339
VLDL-cholesterol (mg/dL)	0.345	0.269	-0.159	0.139	0.231	0.346
Serum Glucose (mg/dL)	-0.272	0.133	0.266	0.304	0.418*	0.395*
HbA1c (%)	0.055	0.119	0.176	0.572**	0.572**	0.474**
Fruit & Vegetables (cup)	-0.205	-0.075	0.271	0.058	0.226	0.316
Fruits (cup)	-0.306	-0.047	0.272	0.083	0.173	0.261
Vegetables (cup)	-0.032	-0.072	0.041	0.010	0.167	0.245

Reported intakes of fruits and vegetables (3 day average) were quantified based on the USDA Food and Nutrient Database. Reported intakes of flavonoids (3 day average) were quantified based on the USDA Flavonoid Database. *Pearson Correlation is significant at the 0.05 level (2-tailed), **Pearson Correlation is significant at the 0.01 level (2-tailed). ox-LDL (oxidized low-density lipoprotein), MDA (malondialdehyde), CRP (C reactive protein), KAE (kaempferol), MYR (myricetin), and QUER (quercetin).

Table 4. Differences in waist circumference, glycemic control, lipids, quercetin, and fruits and vegetables among tertiles of ox- LDL.

	Tertile 1 (87-125.33 U/L)	Tertile 2 (125.34-140 U/L)	Tertile 3 (140.1-156 U/L)	Overall	p[1]
n=	10	10	10	30	
Waist Circumference (inch)	44.56±4.3	42.3±4.8	41.19±3.9	42.68±4.5	NS[2]
Serum Glucose (mg/dL)	92.8±14.3[a,b]	102.7±8.3[a]	86.1±15.7[b]	93.87±14.5	0.009
HbA1c (%)	5.89±0.51	5.89±0.6	6.0±0.62	5.93±0.56	NS[2]
Total Cholesterol (mg/dL)	192.7±21.6[a]	218.2±44.3[a,b]	227.0±34.1[b]	212.63±36.5	0.035
Triglyceride (mg/dL)	155.7±71.3	170.4±50.4	191.3±96.3	172.47±73.9	NS[2]
LDL-cholesterol (mg/dL)	117.9±15.4[a]	133±43.7[a,b]	148.5±29.2[b]	133.13±33.1	0.040
HDL-cholesterol (mg/dL)	43.8±12.8	50.9±19.0	46.9±17.2	47.2±16.2	NS[2]
Quercetin (mg/100g)	196.67±130.5	243.74±194.5	157.53±173.4	199.32±166.2	NS[2]
Fruits & Vegetables (cups)	2.18±1.8	1.86±0.7	1.53±1.1	1.86±1.3	NS[2]

Data presented as mean±SD. Reported intakes of fruits and vegetables (3 day averages) were quantified based on the USDA Food and Nutrient Database. Reported intakes of flavonoids (3 day averages) were quantified based on the USDA Flavonoid Database. [1]ANOVA for categorical variables, and [2]NS= No Significance. [a,b]For each variable, values with different superscripts letters are significantly different across tertiles of ox-LDL (oxidized low-density lipoprotein) ($p < 0.05$).

Table 5. Differences in waist circumference, glycemic control, lipids, quercetin, and fruits and vegetables among tertiles of CRP.					
	Tertile 1 (0.1-2.63 mg/L)	Tertile 2 (2.631-7.3 mg/L)	Tertile 3 (7.31-25.6 mg/L)	Overall	p[1]
n=	10	11	9	30	
Waist Circumference (inch)	42.0±4.4[a,b]	41.03±3.8[a]	45.46±4.4[b]	42.68±4.5	0.025
Serum Glucose (mg/dL)	86.8±15.7[a]	94.0±11.4[a,b]	101.56±13.9[b]	93.87±14.5	0.027
HbA1c (%)	5.69±0.3[a]	5.81±0.4[a]	6.34±0.7[b]	5.93±0.6	0.01
Total Cholesterol (mg/dL)	218.8±44.3	212.73±39.6	205.67±23.8	212.63±36.5	NS[2]
Triglyceride (mg/dL)	163.2±80.6	194.91±78.4	155.33±60.7	172.47±73.9	NS[2]
LDL-cholesterol (mg/dL)	136.8±38.7	136.36±37.5	125.11±20.4	133.13±33.1	NS[2]
HDL-cholesterol (mg/dL)	49.6±22.2	43.18±5.4	49.44±18.1	47.2±16.2	NS[2]
Quercetin (mg/100g)	153.7±140.8[a]	144.15±138.9[a]	317.43±176.9[b]	199.32±166.2	0.026
Fruits & Vegetables (cups)	1.51±0.9[a]	1.39±1.0[a]	2.81±1.5[b]	1.86±1.3	0.021

Data presented as mean±SD. Reported intakes of fruits and vegetables (3 day averages) were quantified based on the USDA Food and Nutrient Database. Reported intakes of flavonoids (3 day averages) were quantified based on the USDA Flavonoid Database. [1]ANOVA for categorical variables, and [2]NS= No Significance. [a,b]For each variable, values with different superscripts letters are significantly different across tertiles of CRP (C-reactive protein) ($p < 0.05$).

CHAPTER V

CONCLUSION

Our cross-sectional study shows significant associations among measures of lipid oxidation, and flavonols (KAE, MYR, QUER), with measures of lipids, and glycemic control in Oklahoma (OK) adults with abdominal adiposity and dyslipidemia. Also, we found significant differences in these CVD risk factors across tertiles of ox-LDL and CRP. In general, participants with higher ox-LDL and/or CRP were shown to have higher levels of serum cholesterol, glucose, and HbA1c. To our knowledge no previous study has specifically examined the differences among biochemical variables of CVD risks in this sub-group of adults in OK.

Although OK is an agricultural state, very little of the total cropland acreage is used to harvest fruits and vegetables (32). Consumers thus rely on out-of-state producers which can limit selection and availability depending on season, environmental conditions, cost, and related factors. Epidemiologic investigation of flavonoids in relation of CVD risks in OK adults populations is limited. Thus, there exists a need to investigate the relationship among total servings of fruits and vegetables, total flavonoid intakes, dyslipidemia, and CRP levels in OK adults with general obesity and abdominal adiposity. This forms the scope of our study.

A total of 30 adults with abdominal adiposity and dyslipidemia were included in our analysis. All participants were obese as determined by their BMI and had dyslipidemia as evidenced by their serum lipid levels. Although serum glucose levels were found to be within the normal range, the mean HbA1c was in the pre-diabetes range. Also, the participants were found to be pre-hypertensive; but free of any other disease conditions. The reported servings of fruits and vegetables by the participants were found to be lower than the desirable daily intake of greater than five servings of fruits and vegetables per day, which is not surprising as the consumption of fruits and vegetables in OK adults has been documented to be inadequate (14).

Oxidized LDL (ox-LDL) has been shown to be a surrogate risk factor for CVD (28). The oxidation of LDL occurs when LDL cholesterol particles react with free radicals. The ox-LDL becomes reactive with the surrounding tissues, which can produce tissue damage. These ox-LDL particles enter directly into the endothelium of arterial tissue and promote atherosclerosis by inducing accumulation macrophages and adhesion molecules to the damaged area. This process causes inflammation at the site of the damaged artery. The accumulation leads to formation of plaque within the arterial wall. If uncontrolled, the plaque buildup causes thickening of arterial wall and restricts blood flow throughout the damage area (68).

We noted that participants in the highest tertile of ox-LDL also had the higher total cholesterol and LDL-cholesterol concentrations when compared to lowest tertile which are in accordance with previously reported studies (69, 70, 71). Higher concentrations of LDL cholesterol have been shown to be associated with higher ox-LDL serum levels. A study by Wu et al. (69) in adults with dyslipidemia observed significant increases in lipid

oxidation in subjects with higher total cholesterol and LDL levels compared to healthy adults (69). Another study in adults with dyslipidemia, reported by Oqunro et al. (70), also observed significant increases in susceptibility of lipid oxidation directly related to high total and LDL-cholesterol levels (70). Furthermore a study by Lankin et al. (71) showed a similar correlation among levels of ox-LDL and LDL-cholesterol. Concentrations of ox-LDL, in subjects with CVD, were significantly higher in the subjects with highest LDL levels compared to subjects with lower LDL levels (71). These observations support our study findings.

Interestingly, we noted that participants in the middle tertile of ox-LDL had significantly higher serum glucose levels than the highest tertile which is not entirely in accordance with previously reported studies (72, 73). However, this may be attributed to the small sample size within each tertiles of our analyses. Studies have shown higher susceptibility of lipid oxidation in relation to high serum glucose levels. A study by Likidlilid et al. (72) investigated oxidative stress in type 2 diabetic subjects. The subjects (19 uncontrolled T2DM subjects, 26 controlled T2DM subjects, 20 subjects with T2DM and CVD complications, and 20 healthy controls) with higher serum glucose levels also had significantly higher ox-LDL levels (72). Another study by Nivedita et al. (73) investigating the association between hyperglycemia and lipid oxidation levels observed similar results. DM adult subjects (n= 100; 50 uncontrolled DM, 50 controlled DM) were compared to 50 healthy adults and the susceptibility of LDL oxidation was found to be significantly higher in both diabetic groups compared to the healthy control group (73).

No significant differences were observed among intakes of fruits and vegetables and quercetin across increasing tertiles of ox-LDL. These outcomes are contradictory to a similar study by McCullough et al. (44), who observed a significant inverse association between flavonol intake (median 27.5 mg/d) and measures of oxidative stress in both adult males and females (44). This could be explained by our small sample size of a population that is already known for their low intake of fruits and vegetables (14). Also, these findings could possibly be affected by missing data.

Several studies have documented inflammation as a central contributor to the progression of cardiovascular complications (3, 25). The predictive value of serum CRP as a risk factor for cardiovascular events allows researchers to use it as a main CVD risk assessment tool. We found that waist circumference was significantly higher in the highest tertile of CRP vs. the lower middle tertile. Again, those in the highest tertile of CRP had significantly higher serum glucose and HbA1c when compared to the lower tertiles. Multiple studies have shown inflammation can be affected by visceral adiposity (74, 75, 76). Tsuriya et al. (74) observed significant increases in serum CRP levels with increased waist circumference in adult men and women (74). Another study by Verrijken et al. (75) examined inflammation in relation to elevated visceral adipose tissue and observed that CRP levels were significantly higher in participants defined as obese compared to participants with normal waist circumference (75). A study investigating the relationship between glycemic control and inflammation by Bahceci et al. (76) also observed elevated serum CRP concentrations in T2DM males with high levels of serum glucose and HbA1c concentrations compared to healthy males (76).

Interestingly, we observed higher quercetin and reported total servings of fruits and vegetables among participants in the highest tertile of CRP. This is surprising because flavonoids have been shown to decrease inflammation according to multiple studies (7, 77, 78). Our findings were also contradictory to studies that provide evidence that high intakes of fruits and vegetables reduce plasma CRP concentrations (25, 41, 43). Again, small sample size, methodological errors in flavonoid estimations, particular dietary habits, and missing data may explain these differences among our study and previously reported findings.

Based on our results we partially support the hypothesis that measures of lipid oxidation, CRP, and flavonoids (KAE, MYR, QUER) will have significant correlations with measures of lipids and glycemic control. The measures of lipid oxidation (i.e ox-LDL and MDA) were shown to be significantly correlated with total cholesterol levels and HDL-cholesterol levels; however no other correlations were observed with ox-LDL and MDA. Also, no correlations were observed among either of the measures of lipids and glycemic control with CRP levels. Finally, significant correlations were observed in HbA1c levels with all three flavonoids; and among serum glucose concentrations with MYR and QUER.

On the other hand, our data largely supports the hypotheses that participants with higher ox-LDL and CRP levels will have significantly different waist circumference, measures of lipids, and glycemic control, when compared to lower levels. The highest tertile of ox-LDL was observed to have significantly higher levels of total cholesterol and LDL-cholesterol. Conversely, the middle ox-LDL tertile had significantly higher serum glucose than the highest tertile in our study. In regards to CRP, participants with the

higher concentrations were shown to have significantly a larger waist circumference, and higher measures of glycemic control (serum glucose, HbA1c), QUER, and reported servings of fruits and vegetables when compared to the lower tertiles.

Our study has certain limitations that must be taken into consideration in the interpretation of our findings. First, this investigation was conducted in a small sample of participants. Second, the biological measurements that were obtained in this cross-sectional study were collected at only one time point and may not be reflective of overall inflammatory status or oxidative stress in participants with abdominal adiposity and dyslipidemia. Third, the USDA food composition databases, in particularly the Flavonoid Database, are lacking in some dietary choices and preparation methods which could affect our dietary analyses. Any reported dietary choices that were not in accordance with database content or preparation methods had to be excluded from the final analysis and therefore may not be a true representation of the flavonoid intakes. Fourth, the reported dietary items the participants consumed were not prepared using similar methods and could affect the balance of anti- and pro-inflammatory biomarker factors expression in the participants. Fifth, this study focused on flavonoid intake, not bioavailability and metabolism in the human body or changes during processing and food preparation. Finally, we have not completely adjusted for other variables that could be confounding our results, such as total energy intake. Thus, our findings warrant further investigation in larger prospective studies.

Future studies should include a more comprehensive analysis of biomarkers of oxidative stress and inflammation, such as, 4-hydroxynonenal (HNE), advanced glycation end products (AGEs) or F_2-isoprostances, as these markers are also elevated in patients with

dyslipidemia (79). Additionally, it may be beneficial to include a more extensive food record, such as having participants complete a food frequency questionnaire (FFQ) that includes flavonoid containing food products. This would give a better representation of the amount and type of polyphenolic compounds typically consumed in the participants' overall dietary habits. Furthermore, oxidative stress and pro-inflammatory responses may be affected by a variety of stimuli including stress, increased activity, a high-fat diet, or bacterial infections (26). These need to be addressed in larger studies.

In conclusion, this cross-sectional study demonstrates differences in serum concentrations of total cholesterol, LDL-cholesterol, and serum glucose across tertiles of oxLDL. In addition, this study shows higher levels of waist circumference, measures of glycemic control, quercetin intake and servings of fruits and vegetables across tertiles of CRP in OK adults with abdominal adiposity and dyslipidemia. Thus, our small cross-sectional study provides evidence of elevated surrogate risk factors of CVD, especially elevated waist circumference, serum glucose and HbA1c, total and LDL-cholesterol in obese adults with higher ox-LDL and CRP levels. These findings remain significant when compared to lower levels of these two surrogate markers of CVD risks. Thus, the utility of serum ox-LDL and CRP in identifying individuals with specific CVD risks, including dietary habits need to be further investigated in larger prospective studies.

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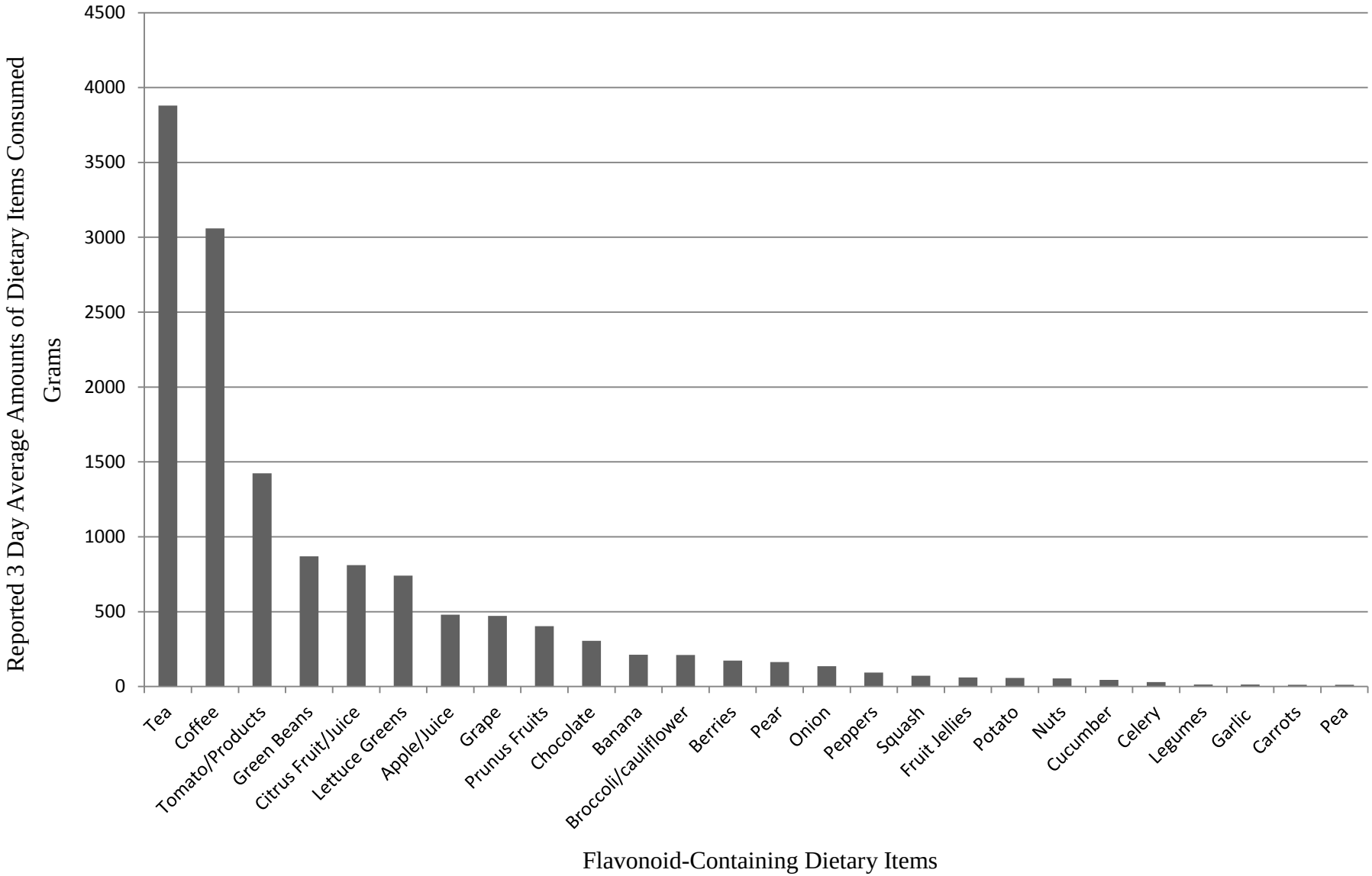
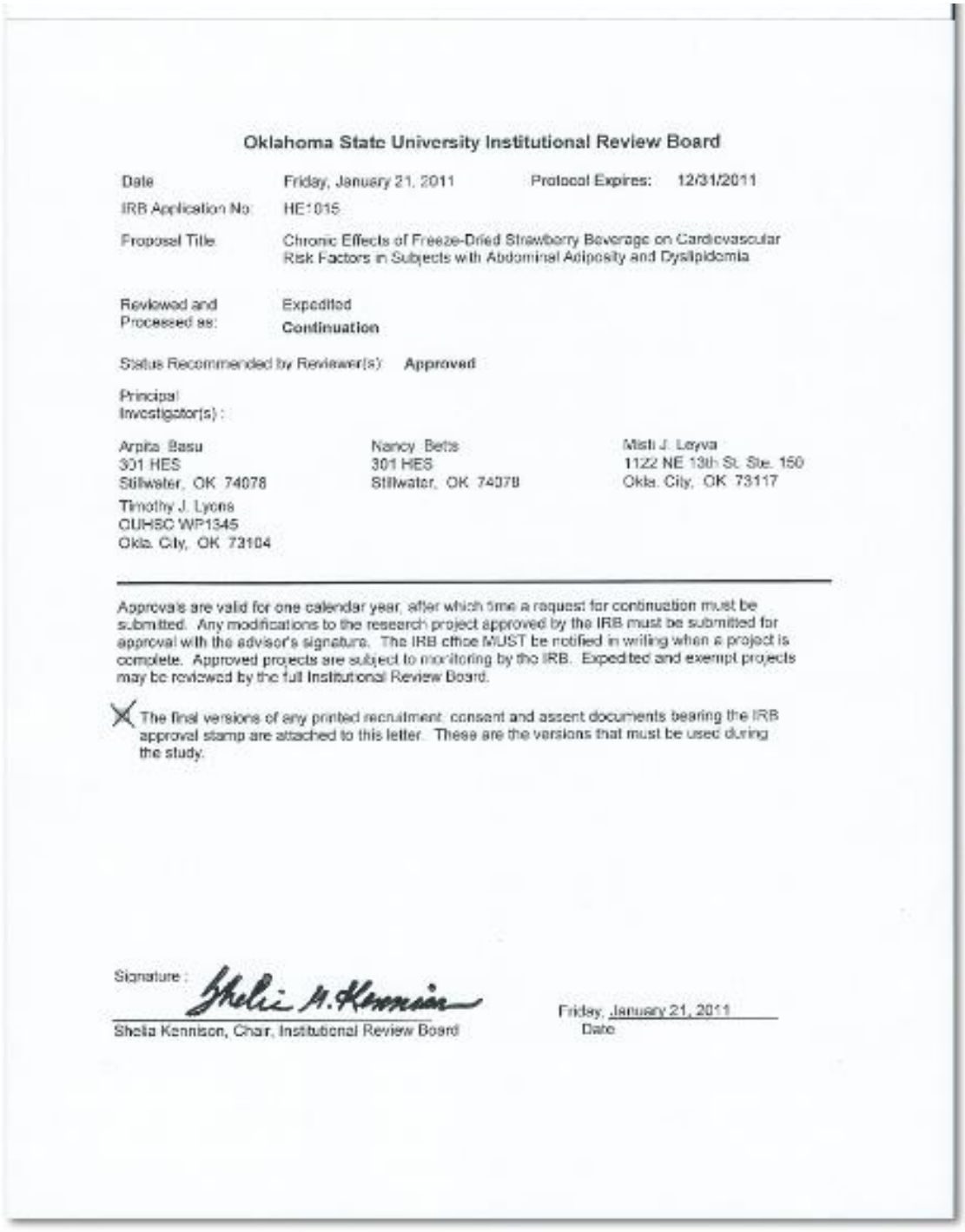
APPENDICES

Appendix A. Supplemental Figure 1. Frequently consumed flavonoid-containing dietary items



Appendix B. Institutional review board approval



Oklahoma State University Institutional Review Board

Date	Friday, January 21, 2011	Protocol Expires:	12/31/2011
IRB Application No	HE1015		
Proposal Title	Chronic Effects of Freeze-Dried Strawberry Beverage on Cardiovascular Risk Factors in Subjects with Abdominal Adiposity and Dyslipidemia		
Reviewed and Processed as:	Expedited **Continuation**		

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

Principal Investigator(s):

Arpita Basu
301 HES
Stillwater, OK 74078

Nancy Betts
301 HES
Stillwater, OK 74078

Misti J. Leyva
1122 NE 13th St. Ste. 150
Okla. City, OK 73117

Timothy J. Lyons
OUHSC WP1345
Okla. City, OK 73104

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. Expedited and exempt projects may be reviewed by the full Institutional Review Board.

X 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: Sheli H. Kennison

Sheila Kennison, Chair, Institutional Review Board

Friday, January 21, 2011
Date

Appendix C. Screening questionnaire

Day/ Date of Appointment _____ *Time:* _____

SCREENING QUESTIONNAIRE FOR STRAWBERRY 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

Have you ever been allergic to strawberries? YES NO

Do you take more than 1 g/day of fish oil capsules? 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? _____
_____

*Is the subject **ELIGIBLE** based on the questionnaire?* YES NO

ELIGIBILITY REQUIRES THE FOLLOWING FEATURES:

(Check all that apply):

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

And 2 of the following:

2. _____ Total cholesterol **(>200 mg/dL)** **(Value:_____)**

3. _____ LDL cholesterol **(>100 mg/dL)** **(Value:_____)**

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

5. _____ HDL-cholesterol **(Male ≤ 40 mg/dL)** **(Value:_____)**
(Female ≤ 50 mg/dL)

Appendix D. Consent form

701-A

Consent Version 03, 4/30/2010

OUHSC IRB No: 15109
OSU IRB No: HE-10-15

Consent Form
University of Oklahoma Health Sciences Center (OUHSC)
Oklahoma State University (OSU)

Title: Chronic effects of freeze-dried strawberry beverage on cardiovascular risk factors in subjects with abdominal adiposity and dyslipidemia.

Sponsor: California Strawberry Commission

Principal Investigator: Timothy J Lyons MD, FRCP (OUHSC)
Co-Principal investigator: Misti J Leyva MS, RD (OUHSC)
Sub-Investigator: Arpita Basu, PhD, RD (OSU)

This is a clinical trial (a type of research study). Clinical trials include only patients who choose to take part in them. Please take your time to make your decision. Discuss this with your family and friends.

Why Have I Been Asked To Participate In This Study?
You are being asked to take part in this trial/study because you have been diagnosed with dyslipidemia and being overweight. Dyslipidemia is a condition where you have increased levels of bad lipids and/or low levels of good lipids. This condition puts you at a high risk of developing heart disease.

Why Is This Study Being Done?
The purpose of this study is to find out about the effects of strawberry drink intake on blood lipid levels. In the study, certain markers in blood will be associated with cell damage due to dyslipidemia and increased body weight.

What is the Status of the Drugs (Devices or Procedures) involved in this study?
This study involves the use of strawberry powder and dietary fiber (cellulose and Metamucil) which will be made into a drink with ice, vanilla essence, Splenda and water. The strawberry powder is not approved by FDA as a drug.

How Many People Will Take Part In The Study?
About 60 people will take part in this study at both of the sites.

APPROVED
JAN 0 3 2011
OUHSC IRB

Okla. State Univ.
IRB
Approved 1/21/11
Expires 12/31/11
IRB # HE1015

APPROVAL EXPIRES
DEC 3 1 2011
OUHSC IRB

What Is Involved In The Study?

You will be randomized to receive either low or high dose strawberry high dose control drink (made of dietary fiber and sugar). Randomizatio are put in a group by chance, like a flip of a coin. A computer program at sponsor will make this random assignment. Neither you nor the investigator which group you will be in.

If you take part in this study, you will have the following tests and procedures:

This is a 12- week study that will be conducted at the General Clinical Research Center (GCRC) at Oklahoma City and at the Nutritional Sciences Clinical Assessment Unit at Oklahoma State University at Stillwater.

Screening visit:
During your first visit we will do some tests and measurements to determine if you qualify for the study. This will involve:

- reading and signing the consent form;
- measuring your height, weight, blood pressure, and waist size
- drawing about 3-4 tablespoons of fasting blood for measuring your blood sugar, lipids, blood cell counts to do some tests to find out how well your cells, liver, kidney, and thyroid are working; and
- provide you with guidelines and forms for a 3-day food record.

If you qualify, we will let you know over the telephone and ask you to come back for the strawberry or control drink and 3 visits/week and follow-up visits for 12 weeks.

You will be taking 2 cups of strawberry or control drink everyday for 12 weeks. Each cup of the strawberry drink will be made of 12.5g strawberry powder in low dose group, or 25g strawberry powder in high dose group, in addition to a quarter cup of ice, vanilla essence, Splenda and three-fourth cup of water. Each cup of control drink will be made of 1/3 teaspoon fiber (dietary cellulose + Metamucil) and 2 teaspoons sugar in low dose group, or 3/4 teaspoon fiber (dietary cellulose + Metamucil) and 4 teaspoons sugar in high dose group, in addition to a quarter cup of ice, vanilla essence, Splenda, and three-fourth cup of water.

You will be making 3 visits/week to the study site for the strawberry or control drink. 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, during the first, 6th, and final week, throughout the 12-week study period.

Visits:
Though you will be coming to the clinic 3 days/week for the drink, the study also includes the following visits:

- 1st week- turn in 3-day food records, short talk on how you are doing on this study.
- 6th week- turn in 3-day food records, draw about 3-4 tablespoons of fasting blood for measuring your blood sugar, lipids, and find out how well the cells in your body are working. We will also measure your body weight, blood pressure, and waist size.
- 12th 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 find out how well the cells in your body are working. We will also measure your body weight, blood pressure, and waist size.

How Long Will I Be In The Study?

You will be in the study for 12 weeks. The duration of the screening and follow-up visits will be between 1/2-1 hour. The duration of the 3 days/week visits to the clinic will be about 10-15 minutes each.
There may be anticipated circumstances under which your participation may be terminated by the investigator without regard to your consent. This may occur if you fail to follow the study requirements, such as, making 3 days/week visits to the study site. You can stop participating in this study at any time. However, if you decide to stop participating in the study, we encourage you to talk to the researcher and your regular doctor first.

What Are The Risks of The Study?

While on the study, you are at risk for these side effects. You should discuss these with the researcher and/or your regular doctor. There also may be other side effects that we cannot predict.

Likely: the risks involved with drinking 2 cups of strawberry or fiber drink per day may include stomach aches, gas, or headaches which may happen daily or less if you are not used to taking strawberries or fiber.

Less likely: you may develop some allergies, the daily strawberry supplement may change the color of stools.

There is also the risk involved with pain, bruising and infection during blood draw.

Are There Benefits to Taking Part in The Study?

If you agree to take part in this study, there may or may not be direct medical benefit to you. We hope that the information learned from this study will benefit other overweight patients with dyslipidemia in the future.

What Other Options Are There?

You may choose not to participate in the study. You may also obtain strawberries outside of the study if you choose not to participate.

What About Confidentiality?

Efforts will be made to keep your personal information confidential. You will not be identifiable by name or description in any reports or publications about this study. All personal information will be coded using numbers in the order people are enrolled in the study and all files will be kept in locked cabinets in the offices of the study researchers at OUHSC and OSU. Stored data in the computer will be protected by passwords known only to the study researchers who will also have access to these data and files. All information linked to specific names will be coded and names will be deleted after data collection is complete. After that, only numerical codes will be used to identify subjects. We cannot guarantee absolute confidentiality. Your personal information may be disclosed if required by law. You will be asked to sign a separate authorization form for use or sharing of your protected health information.

There are organizations that may inspect and/or copy your research records for quality assurance and data analysis. These organizations include the US Food & Drug Administration, the California Strawberry Commission, and the OUHSC & OSU Institutional Review Board. However, all data will be coded and no personally identifiable information will be shared with the California Strawberry Commission or the FDA.

What Are the Costs?

The study sponsor will pay for all costs related to your participation in this study.

Will I Be Paid For Participating in This Study?

You will not be paid for participating in this study but you will be reimbursed $30 every week for twelve weeks of the study to cover travel (3 days/week visits) and expenses; a total of $ 360. No additional payment will be made for blood draws at screen, 6 and 12 weeks of the study.

What if I am Injured or Become Ill While Participating in this Study?

In the case of injury or illness resulting from this study, emergency medical treatment is available. No funds have been set aside by Oklahoma State University or The University of Oklahoma Health Sciences Center (General Clinical Research Center), or the California Strawberry Council to compensate you in the event of injury.

What Are My Rights As a Participant?

Taking part in this study is voluntary. You may choose not to participate. Refusal to participate will involve no penalty or loss of benefits to which you are otherwise entitled. If you agree to participate and then decide against it, you can withdraw for any reason and leave the study at any time. You may discontinue your participation at any time without penalty or loss of benefits, to which you are otherwise entitled.

We will provide you with any significant new findings developed during the course of the research that may affect your health, welfare or willingness to continue your participation in this study.
You have the right to access the medical information that has been collected about you as a part of this research study. However, you may not have access to this medical information until the entire research study has completely finished and you consent to this temporary restriction.

Whom Do I Call If I have Questions or Problems?

If you have questions about the study or have a research-related injury, contact Dr. Arpita Basu, PhD at 405-744-4437 (9AM-5PM, Monday-Friday) or at 405-612-2414 (anytime) or Timothy Lyons, MD at 405-271-5896 (8AM-5PM), or 405-255-3340 (anytime), or the General Clinical Research Center (GCRC) at 405-271-4272 (8:00AM-5:00PM).

If you cannot reach the Investigator or wish to speak to someone other than the investigator, contact Dr. Shelia Kennison, IRB Chair, 219 Cordell North, Stillwater, OK 74078, 405-744-3377 or irb@okstate.edu, or the OUHSC Director, Office of Human Research Participant Protection at 405-271-2045.

Signature:

By signing this form, you are agreeing to participate in this research study under the conditions described. You have not given up any of your legal rights or released any individual or entity from liability for negligence. You have been given an opportunity to ask questions. You will be given a copy of this consent document.

I agree to participate in this study:

PARTICIPANT SIGNATURE (age ≥18) *(Or Legally Authorized Representative)*	Printed Name	Date
SIGNATURE OF PERSON OBTAINING CONSENT	Printed Name	Date

IRB Office Version Date: 07/07/2009

AUTHORIZATION TO USE or DISCLOSE
PROTECTED HEALTH INFORMATION FOR RESEARCH

An additional Informed Consent Document for Research Participation may also be required. Form 2 must be used for research involving psychotherapy notes.

Title of Research Project: **Chronic effects of freeze-dried strawberry beverage on cardiovascular risk factors in subjects with abdominal adiposity and dyslipidemia.**

Leader of Research Team: **Timothy J Lyons, MD, FRCP**

Address: **Diabetes & Endocrinology, OUHSC, WP1345, 920 Stanton L. Young Blvd. Oklahoma City, OK 73104**

Phone Number: **405-271-5896**

If you decide to join this research project, University of Oklahoma Health Sciences Center (OUHSC) researchers may use or share (disclose) information about you that is considered to be protected health information for their research. Protected health information will be called private information in this Authorization.

Private Information To Be Used or Shared. Federal law requires that researchers get your permission (authorization) to use or share your private information. If you give permission, the researchers may use or share with the people identified in this Authorization any private information related to this research from your medical records and from any test results. Information, used or shared, may include all information relating to any tests, procedures, surveys, or interviews as outlined in the consent form, medical records and charts, name, address, telephone number, date of birth, race, and government-issued identification number.

Purposes for Using or Sharing Private Information. If you give permission, the researchers may use your private information to design future research projects on the basis of the results from the present study.

Other Use and Sharing of Private Information. If you give permission, the researchers may also use your private information to develop new procedures or commercial products. They may share your private information with the research sponsor, the OUHSC Institutional Review Board, auditors and inspectors who check the research, and government agencies such as the Food and Drug Administration (FDA) and the Department of Health and Human Services (HHS). The researchers may also share your private information with other researchers for future research projects.

Confidentiality. Although the researchers may report their findings in scientific journals or meetings, they will not identify you in their reports. The researchers will try to keep your information confidential, but confidentiality is not guaranteed. Any person or organization receiving the information

based on this authorization could re-release the information to others and federal law would no longer protect it.

YOU MUST UNDERSTAND THAT YOUR PROTECTED HEALTH INFORMATION MAY INCLUDE INFORMATION REGARDING ANY CONDITIONS CONSIDERED AS A COMMUNICABLE OR VENEREAL DISEASE WHICH MAY INCLUDE, BUT ARE NOT LIMITED TO, DISEASES SUCH AS HEPATITIS, SYPHILIS, GONORRHEA, AND HUMAN IMMUNODEFICIENCY VIRUS ALSO KNOWN AS ACQUIRED IMMUNE DEFICIENCY SYNDROME (AIDS).

Voluntary Choice. The choice to give OUHSC researchers permission to use or share your private information for their research is voluntary. It is completely up to you. No one can force you to give permission. However, you must give permission for OUHSC researchers to use or share your private health information if you want to participate in the research and if you revoke your authorization, you can no longer participate in this study.

Refusing to give permission will not affect your ability to get routine treatment or health care from OUHSC.

Revoking Permission. If you give the OUHSC researchers permission to use or share your private information, you have a right to revoke your permission whenever you want. However, revoking your permission will not apply to information that the researchers have already used, relied on, or shared.

End of Permission. Unless you revoke it, permission for OUHSC researchers to use or share your private information for their research will never end. You may revoke your permission at any time by writing to:

Privacy Official
University of Oklahoma Health Sciences Center
PO Box 26901, Oklahoma City, OK 73190
If you have questions call: (405) 271-2511

Okla. State Univ.
IRB
Approved 4/21/11
Expires 12/31/11
IRB # HE 1015

IRB Office Use Only – Version 010605

APPROVED
JAN 0 3 2011
OUHSC IRB

APPROVAL EXPIRES
DEC 3 1 2011
OUHSC IRB

Giving Permission. By signing this form, you give OUHSC and OUHSC's researchers led by Dr. Timothy Lyons, permission to share your private information for the research project called Chronic effects of freeze-dried strawberry beverage on cardiovascular risk factors in subjects with abdominal adiposity and dyslipidemia.

Patient/Subject Name: _____

_____ _____
Signature of Patient-Subject or Parent if subject is a child — Date

Or

_____ _____
Signature of Legal Representative** — Date

**If signed by a Legal Representative of the Patient-Subject, provide a description of the relationship to the Patient-Subject and the Authority to Act as Legal Representative:

_____
OUHSC may ask you to produce evidence of your relationship.

A signed copy of this form must be given to the Patient-Subject or the Legal Representative at the time this signed form is provided to the researcher or his representative.

IRB No.: 15109

Okla. State Univ. IRB
Approved 1/21/11
Expires 12/31/11
IRB # ME1015

APPROVED
JAN 03 2011
OUHSC IRB

APPROVAL EXPIRES
DEC 31 2011
OUHSC IRB

Appendix E. Food diary form

Oklahoma State University
Nutritional Sciences
Strawberry 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
Strawberry 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

Appendix F. Participant recruitment flyer

Volunteers Needed

For a Study related to:

The Health Effects of Strawberry Drink.

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

- A waist circumference of greater than 40 inches in men or 35 inches in women

And any 2 of the following-

- HDL less than 40mg/dL in men or less than 50mg/dL in women
- Triglycerides greater than 150 mg/dL
- Total cholesterol greater than 200 mg/dL
- LDL cholesterol greater than 100 mg/dL

Following an initial telephone questionnaire, eligible participants will be scheduled for a screening blood draw for final qualification and enrollment in the study and 3 follow-up visits. There are three blood draws at screening, 6 and 12 weeks of the study if you qualify based on the screening blood reports.

There is no charge to participate in the study. Study participants will receive compensation for each follow-up visit. Visits will take place locally at the Department of Nutritional Sciences, OSU

For more information, please contact Dr. Arpita Basu at arpita.basu@okstate.edu
Or (405) 744-4437

Oklahoma State University IRB # HE-10-15

VITA

Bethany Lyn Hamilton

Candidate for the Degree of

Master of Science

Thesis: METABOLIC RISK FACTORS FOR CARDIOVASCULAR DISEASE IN OKLAHOMA ADULTS WITH ABDOMINAL ADIPOSITY AND DYSLIPIDEMIA: A CROSS-SECTIONAL STUDY.

Major Field: Nutritional Science

Biographical:

Education:

Completed the requirements for the Master of Science in Nutritional Science at Oklahoma State University, Stillwater, Oklahoma in December, 2013.

Completed the requirements for the Bachelor of Science in Nutritional Science at Oklahoma State University, Stillwater, Oklahoma, USA in 2010.

Experience: Dietetic Intern with Oklahoma State University, 2013. Employed by Oklahoma State University, Department of Nutritional Sciences as a graduate research assistant, 2011.

Professional Memberships: Academy of Nutrition and Dietetics, Oklahoma Academy of Nutrition and Dietetics, Diabetes Care and Education Professionals.