EFFECTS OF STRAWBERRIES ON LIPID PROFILES AND BIOMARKERS OF LIPID OXIDATION IN SUBJECTS WITH DYSLIPIDEMIA AND ABDOMINAL ADIPOSITY

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Abstract: Emerging research provides substantial evidence to classify strawberries as a functional food with several preventive and therapeutic health benefits. Strawberries, a rich source of phytochemicals (ellagic acid, anthocyanins, quercetin and catechin) and vitamins (ascorbic acid and folic acid), have been highly ranked among dietary sources of polyphenols and antioxidant capacity. We conducted a 12 week randomized controlled trial to investigate the dose-response effects of freeze-dried strawberries (25 or 50g/day) in lipids and biomarkers of lipid oxidation in obese subjects with dyslipidemia, vs. calorie and fiber-matched controls. Anthropometrics, blood pressure and biochemical variables were measured at screen and 12 weeks of the study. Study findings showed a significant decrease in diastolic blood pressure in the low dose strawberry group from baseline to 12 weeks. In addition, when analyzing the change over the 12 week study period in the low dose strawberry group, diastolic blood pressure significantly decreased when compared to the low dose control. Results showed high dose strawberry supplementation significantly decreased oxidized LDL and malondialdehyde (MDA) versus baseline ($p<0.05$). In addition, high dose strawberry supplementation also revealed a decreasing trend in total and LDL cholesterol ($p=0.07$ and $p=0.006$, respectively). Serum MDA in the high dose strawberry group significantly decreased when compared to high dose controls ($p<0.05$). No effects were noted in glucose, hemoglobin A1c, systolic blood pressure and body weight after strawberry supplementation. Thus, strawberries may selectively lower markers of lipid oxidation and may be incorporated as part of a heart healthy diet in obese subjects with dyslipidemia.

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CHAPTER I

INTRODUCTION

According to the American Heart Association (AHA), cardiovascular disease (CVD) can be defined as hypertension (HTN), coronary heart disease (CHD), including myocardial infarction (MI) and angina pectoris, heart failure, stroke, and congenital cardiovascular defects.[1] In the United States, an estimated 82.6 million people, approximately one in three American adults, have one of more types of CVD: HTN (76.4 million), CHD (16.6 million), MI (7.9 million), angina pectoris (9 million), heart failure (5.7 million), stroke (7 million), and congenital cardiovascular defects (650,000 to 1.3 million).[1] CVD is the leading cause of death in the United States and throughout the world according to the AHA[1]. CVD accounted for 33.6% of all deaths in 2007 and one out of every three deaths in the United States.

Epidemiological studies have found an association between increased consumption of fruits and vegetables and decreased risk of CVD and CVD mortality.[20,24] Specific dietary nutrients abundant in fruits and vegetables may be responsible for certain CVD protective properties. An increased interest in fruits, specifically berries, has lead to the recognition that berries contain high concentrations of fiber and antioxidants, as well as rich sources of polyphenols. Strawberries, rich in the polyphenols anthocyanins, have been linked to a reduced risk of CVD mortality and CVD risk factors.[20] Studies have shown that in subjects with cardiovascular risk factors, supplementation of strawberries in combination with other berries can increase HDL

cholesterol and decrease systolic blood pressure compared to a control group.[28] These limited human studies provide very intriguing results that call for future research and examination of the cardiovascular effects of strawberries in subjects with metabolic risk factors.

The goal of this study was to investigate the effects of freeze-dried strawberries in metabolic risk factors for CVD in a randomized clinical trial. Parameters assessed included measures of adiposity, lipid profile, glucose metabolism, as well as markers of oxidative stress in subjects with dyslipidemia and abdominal adiposity versus age-gendered matched control at baseline and 12 weeks after strawberry supplementation.

The null hypotheses examined in the study are as follows:

- Freeze-dried strawberry supplementation will have no effects in anthropometric measures and blood pressure compared to control
- Freeze-dried strawberry supplementation will have no effects in hemoglobin A1c, glucose, lipid profile, and markers of lipid oxidation compared to control

CHAPTER II

REVIEW OF LITERATURE

Cardiovascular Disease (CVD) Statistics:

The American Heart Association (AHA) Disease and Heart Statistics reported "cardiovascular disease is the leading cause of death in the United States and throughout the world[1], and that approximately 45% of adults in the United States have a total cholesterol (TC) level of 200mg/dl or above, and about 16% have a total cholesterol level above 240mg/dl.[1] They also reported that the mean total serum cholesterol of adults in the United States decreased from 204mg/dl to 199mg/dl in the years 1999 to 2008.[1] Approximately 33% of adults have a high low-density lipoprotein (LDL) cholesterol level (>130mg/dl), while only 16% have a low high-density lipoprotein (HDL) cholesterol level (<40mg/dl).[1]

Heart disease is the number one killer of Oklahomans.[1] According to the National Vital Statistics Report in 2009, more than 1 out of 4 deaths in Oklahoma are due to heart disease and 9,602 Oklahomans died from heart disease in 2007, which is 27.7% of total deaths in Oklahoma.[1] Risk factors for heart disease and stroke in Oklahoma in 2007 included adults who are current smokers (25.5% OK; 17.9% US), adults who did not participate in exercise (47.1% OK; 50.6% US), and adults who are overweight or obese (67.4% OK; 63.4% US).[1] According to the AHA,

obesity has reached epidemic proportions in the United States. In Oklahoma three out of five people are overweight or obese.[1] In 2009-2010, more than one-third (35.7%) of the U.S. adult population were obese.[2] Ninety one percent of Oklahoma's counties have ≥ 20% of their population that is considered obese.[1] Overweight and obesity are associated with many health risks, such as heart disease, high blood pressure, high blood cholesterol, type 2 diabetes, and depression.[1]

According to the Center for Disease Control and Prevention (CDC), in the United States and worldwide, about 90% to 95% of all diagnosed cases of diabetes are type 2 diabetes (T2D).[2] The American Diabetes Association (ADA) also reported that 25.8 million (8.3%) children and adults in the United States have diabetes. Statistics also reported that there are 7 million people who have still not been diagnosed with T2D and 79 million people who are prediabetics.[3] Type 2 diabetes is not an equal opportunity disease, the elderly and different ethnicities are not equally affected.[4] Of the elderly population, 26.9% are reported to have diabetes in the United States.[3] The prevalence of diabetes in non-Hispanic whites 20 years or older is 7.1% of the U.S. population, and the prevalence for other groups of similar age is as follows: 8.4% of Asian Americans, 12.6% of non-Hispanic blacks, 11.8% of Hispanics: 7.6% of Cubans, 13.3% of Mexican Americans, and 13.8% of Puerto Ricans.[3]

According to the AHA, approximately 74 million American adults, or one in three adults (31.3%), have hypertension. In 2008, hypertension was listed as the primary or contributing cause of more than 348,000 deaths in the U.S.[2]

In 1948, the National Heart, Lung, and Blood Institute, previously known as the National Heart Institute knew very little about cardiovascular disease, but they knew that the number of deaths from CVD was steadily increasing. Thus, they recruited many researchers to begin a project known as the Framingham Heart Study.[11] The objective of the study was to distinguish

risk factors, which contribute to CVD. The basic plan of the study was to examine the development of CVD over a long period of time in a large group of participants who had not yet developed or suffered from the symptoms of CVD, including heart attack and stroke.[11] 5,209 men and women with ages ranging from 30 to 62 were recruited for the study that took place in Framingham, MA.[11] The first group recruited began in 1948 with physical exams and lifestyle analysis to aid in recognizing factors associated with CVD development. The participants returned every two years for lab tests and physical exams. In 1971 a second generation of recruits joined the study, this second group consisted of 5, 124 men and women who were the original participants children and their spouses. Many more recruits were added in 1994, 2002 and in 2003.[11] Over the many years of research the Framingham study identified major risk factors of CVD including, high blood pressure, high blood cholesterol, smoking, obesity, diabetes, and physical inactivity, which leads to the scope of the literature review.

Obesity & Abdominal Adiposity:

Obesity is defined as an excess proportion of total body fat. A person is considered obese when his/her weight is ≥ 20% normal body weight.[4] The most common way to measure for obesity is by their body mass index or BMI, using height and weight as measurements. Table 1 represents classifications of weight based on BMI.

Table 1: Classification of Weight/Health status based on BMI

BMI	Weight	Status Health Risk
Below 18.5	Underweight	With < 16 suggests possible eating disorder and other disease risk*
18.5-24.9	Normal	Healthy, low health risk
25.0-29.9	Overweight	Associated with increased risk of disease**
30.0-39.9	Obese	Associated with further increased

		risk of disease
40 and above	Morbidly Obese	Experiencing obesity-related health problems

*Diseases associated with underweight include chronic obstructive pulmonary disease, cancer, and congestive heart failure.

** Diseases associated with overweight and obesity includes diabetes mellitus, CVD, and hypertension.

Source: Nelms, M., Sucher, K.P., Lacey, K., & Roth, S.L. (2011). *Nutrition Therapy & Pathophysiology 2e*. Belmont, CA: Wadsworth Cengage Learning.

"Morbid obesity" means that a person is either 50%-100% over normal weight, more than 100 pounds over normal weight, has a BMI of 40 or higher, or is sufficiently overweight with severe health conditions.[4] Obesity occurs when a person consumes more calories than he or she expends. For most people, obesity results when they consume too much food and are physically inactive, but there are other factors such as age, gender, genetics, environmental factors, physical activity, psychological factors, illness, and medication. Obesity is positively associated with dyslipidemia, hypertension, physical inactivity, and diabetes.[4] Disease risk and obesity can also be measured by waist circumference or abdominal adiposity. A man with a waist circumference ≥ 40 inches and a woman ≥ 35 inches are considered obese and at high risk for diseases such as type 2 diabetes, hypertension, and CVD.[4]

Metabolic Syndrome, although not a disease itself, is a group of risk factors that are closely related to those of obesity.[4] The risk factors include, hypertension, high blood sugar, unhealthy cholesterol levels, and abdominal fat. Not only does metabolic syndrome relate to obesity, but it also plays a role in dyslipidemia and increases the risk for diabetes by five times; it can also double the risk of heart disease, which can lead to heart attacks and stroke.[5]

Dyslipidemia:

Dyslipidemia is a condition marked by abnormal lipid or lipoprotein concentrations within the blood [6]; it refers to a lipid profile that increases the risk of atherosclerotic

development.[4] It has often been associated in persons with type 2 diabetes and atherosclerosis. Atherosclerosis is a chronic inflammatory disease characterized by the formation of plaque within the innermost layer of the arterial wall, substantial cell death and the thickening of the surrounding connective tissues.[7,8] At the site of inflammation there is an accumulation of low-density lipoproteins (LDL), cholesterol, monocytes, macrophages, and fat-laden foam cells; this accumulation is known as plaque formation. Once the accumulation begins LDL particles undergo oxidation, which is mediated by monocytes resulting in the inflammatory response.[9]

Dyslipidemia can also be defined as a condition in which LDL levels and/or triglyceride levels are increased and high-density lipoprotein (HDL) levels are decreased.[6] Table 2 below shows an interpretation of lipid profile values related to a person health status according to the Adult Treatment Panel III (ATP III) guidelines in 2004.[10]

Table 2. ATP III Classification of LDL, Total, and HDL Cholesterol (mg/dL)

LDL Cholesterol		**HDL Cholesterol**	
<100 mg/dL	Optimal	<40 mg/dL	Low; A major risk factor for heart disease
100-129 mg/dL	Near optimal/Above optimal	40-59 mg/dL	Normal
130-159 mg/dL	Borderline high	60 mg/dL and above	High; Protective against heart disease
160-189 mg/dL	High	**Triglycerides**	
190 mg/dL and above	Very high		
Total Cholesterol		<150 mg/dL	Normal
<200 mg/dL	Desirable	150-199 mg/dL	Borderline high
200-239 mg/dL	Borderline high	200-499 mg/dL	High
240 mg/dL and above	High	500 mg/dL or above	Very high

Source: Grundy SM, Cleeman JI, Bairey Merz CN, Brewer HB, Clark LT, Hunninghake DB, Pasternak RC, Smith SC, Stone NJ, for the Coordinating Committee of the National Cholesterol Education Program. Implications of Recent Clinical Trials for the National Cholesterol Education Program Adult Treatment Panel III Guidelines Circulation. Adult Treatment Panel III Guidelines 2004; 110:227-39.

HDL particles are involved in reversing the transport of cholesterol; they transport cholesterol from the different tissues to the liver, which reduces plaque build up.[4] Serum LDL levels are the single strongest indicator of cardiovascular risk; they are also the most involved in the atherosclerotic process.[10] Oxidation of LDL causes them to be altered where they are more likely to be taken up and used as plaque.[10] This development of plaque begins as a fatty and fibrous growth in the lumen of the vascular wall vessels, and overtime it may calcify.[10] This plaque may result in decreased blood flow, which could lead to myocardial infarction, coronary heart disease, peripheral vascular disease, or congestive heart failure.[10]

Dyslipidemia also affects endothelial dysfunction. The endothelium is the single celled lining that covers the internal surface of many blood vessels and body cavities. It plays the role of maintaining homeostasis within the vascular walls. When this homeostatic balance is interrupted, it cause vasoactive factors to come into concern such as, vasomotion, smooth muscle proliferation, thrombosis, inflammation, coagulation, fibrinolysis, and oxidation.[12] Endothelial dysfunction is defined as "the loss of one of more of the vasoactive functions with resultant vascular decomposition."[12] Endothelial dysfunction is extremely important if atherosclerosis is preexisting, because it promotes plaque instability and rupture, which could lead to the development of coronary syndromes.[12]

Arterial stiffness is also an important factor contributing to cardiovascular disease risks. Arterial stiffness is defined as "a hallmark of the aging process and the consequence of many disease states such as diabetes and atherosclerosis… it is also a marker for increased cardiovascular disease risk, including myocardial infarction, heart failure, and total mortality, as

well as stroke, dementia, and renal disease."[13] It develops due to changes involving the structural and cellular elements in the blood vessel.[13] Arterial or vascular stiffness could also increase due to "extrinsic factors" such as hormones, salt, and glucose regulation.[13]

Type 2 Diabetes:

Type 2 diabetes (T2D) was once known as non-insulin dependent diabetes mellitus (NIDDM) or adult-onset diabetes, but NIDDM describes more of a condition rather than characteristics of a disease.[3] T2D is the most commonly diagnosed form of diabetes. In T2D, either the pancreas does not produce enough insulin or the body produces insulin but the body tissues are insulin resistant. This causes an increased need for insulin, so the pancreas must increase production.[3; 4] Insulin is necessary for the body to be able to use glucose for energy. T2D is associated with insulin resistance in the peripheral tissues such as, the skeletal muscle and adipose tissue. The body is resistant to insulin because the synthesis or mobilization of glucose transporters is diminished. In muscle cells and adipocytes the glucose transporters are not able to move into the plasma membrane. People at high risk for developing T2D are those individuals who are obese or overweight, women who have gestational diabetes, those with family members who have T2D and those with metabolic syndrome. Also those individuals who smoke, have inactive lifestyles, or have certain dietary patterns have an increased risk of developing T2D.[4]

Impaired fasting glucose and diabetes are closely associated with the risk of CVD. An impaired fasting glucose is defined as plasma glucose between 100 and 125 mg/dL.[3] There are three blood glucose tests to determine if a person has T2D, they are the fasting plasma glucose (FPG) test, the A1C test, and the oral glucose tolerance test (OGTT). The FPG is a test that measures an individuals blood glucose after at least 8 hours without eating. This test is used to determine if the individual has diabetes or prediabetes. Diabetes is diagnosed if the result is 126 mg/dl or above and prediabetes is diagnosed when the FPG result is 100 to 126mg/dl. The A1C

test measures the average blood glucose level over two or three months. Diagnoses of diabetes is made with values at 6.5% or higher and the A1C for prediabetes is between 5.7% and 6.4%. The OGTT is taken two hours after drinking a measured amount of a glucose solution. If the person is diabetic fasting plasma glucose will be 200 mg/dl or higher and for a prediabetes fasting plasma glucose will be between 140 and 200 mg/dl.

Hypertension:

Hypertension is both a cardiovascular condition and a risk factor for other forms of CVD; it is defined as a chronic elevation in blood pressure (BP).[4] The heart follows a repetitive cycle of contraction or systolic to relaxation or diastolic, termed the cardiac cycle. Systolic BP is when the ventricles are contracting due to the force exerted by the blood on the blood vessel walls. Diastolic BP is simply the relaxation of the ventricles. A measurement of BP is expressed using the reading for systolic pressure as the first (higher) number and the reading for diastolic pressure as the second (lower) number. The classifications of BP are represented in Table 3. Hypertension is referred to as the "silent killer," because there are typically no symptoms. It can cause congestive heart failure, kidney failure, myocardial infarction, stroke, and aneurysms if left untreated.

Table 3: Classification of blood pressure for adults

Blood Pressure	**SBP**	**DBP**
Classification	mmHg	mmHg
Normal	<120	and <80
Prehypertension	120–139	or 80–89
Hypertension, Stage 1	140–159	or 90–99
Hypertension, Stage 2	≥160	or ≥100

SBP, systolic blood pressure; DBP, diastolic blood pressure

Source: The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure.

National High Blood Pressure Education Program.

Bethesda (MD): National Heart, Lung, and Blood Institute (US); 2004 Aug.

There are two types of hypertension, primary and secondary.[4] Primary hypertension is idiopathic, meaning there is no known cause and is about 90% of all diagnosed cases. Lifestyle factors such as diet (excessive sodium intake, low potassium intake, and excessive alcohol intake), lack of exercise, smoking, stress, and obesity may all contribute to the development of primary hypertension. Secondary hypertension is mostly due to other problems already occurring such as renal disease, other CVD, endocrine disorders, and neurogenic disorders.

Oxidative Stress:

Oxidation is the process of removing electrons from an atom or molecule. This can be a destructive change; for example, rusting iron is due to oxidation.[14] Almost every living thing needs oxygen to survive, but in high concentrations oxygen can be toxic and corrosive. Oxidative stress is a controlled metabolic process that can generate harmful products. These harmful products are known as free radicals, which are highly reactive unstable atoms or molecules capable of removing electrons from any other molecules they meet in an effort to achieve stability. During this process they create even more unstable molecules that attack their neighboring cells in a chain-like reaction. Oxidative stress takes place when there is an impaired balance between free radical production and antioxidant capacity, which results in an accumulation of damaging oxidative products: such as reactive oxygen species (ROS) and reactive nitrogen species (RNS).

The primary ROS formed mainly in the mitochondria is known as superoxide anion.[14] This free radical has been implicated in the pathophysiology of many chronic diseases and is the precursor to the chain reaction in the formation of the ROS and lipid radicals. These product indicators include damaged DNA bases, protein oxidation products, and lipid peroxidation

products.[4] Oxidative stress may also be due to environmental factors, such as radiation, toxins in air, food and water, and smoking.[11]

There is a significant increase in oxidative stress in individuals with hyperglycemia and metabolic syndrome. There are two types of oxidative stress: mitochondrial and inflammatory. Mitochondrial oxidative stress is associated mostly with cancer and diabetes, while inflammatory oxidative stress is associated more with atherosclerosis.[14]

The body does have a defense mechanism to form barriers against free radical production. These defense mechanisms include enzymes that break down the reactive form of oxygen, and antioxidants (vitamin C and vitamin E) in the diet, which can diminish free radical production by providing them with electrons to end the chain reactions.[14] These essential enzymes, for instance superoxide dismutase (SOD) and the GSH antioxidant enzyme system, and antioxidants are part of the ROS formation pathway; their goal is to prevent the formation of the damaging free radicals and to keep the beneficial enzyme systems and co-factors functioning. SOD is generating within body cells and functions in reducing the superoxide anion to the less damaging hydrogen peroxide molecule. The pathway then continues with the GSH enzymes, glutathione peroxidase (GPX) and glutathione reductase (Gred). GPX neutralizes hydrogen peroxide to water and molecular oxygen. This reaction uses GSH as a co-factor due to the molecules proton donating qualities. GSH in its reduced form is oxidized to GSSG, which is then reduced back to GSH by the enzyme Gred. Gred uses NADPH as a co-factor to finish out these complementary reactions. These two reactions must happen simultaneously within the body in order to maintain the GSH/GSSG ratio.

The essential vitamins C and E play a major role in functioning within the ROS pathway as antioxidants.[15] They are known to reduce lipid radical formation within the cells and to slow or diminish the lipid peroxidation process. Once the lipid peroxyl radical is formed it could follow

one of two pathways. The most ideal pathway is that the lipid peroxyl radical is reduced by the reduced form of vitamin E resulting in the formation of lipid hydrogen peroxide and vitamin E radical. The lipid hydrogen peroxide can be further reduced to water and molecular oxygen by the GSH enzyme GPX using GSH as its co-factor. The vitamin E radical is reduced by the teamwork of vitamin C and GSH. These vitamins are working together to regenerate each other in order to continue their antioxidant functions.

If the antioxidant capacity is low and does not reduce this radical it follows the lipid peroxidation pathways, which leads to the production of very harmful lipid oxidation products known as malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE). These oxidation products further lead to DNA damage, direct damage to the macronutrients, and decreased cell signaling.[15]

Free radical damaged cells may play a role in heart disease.[11] Oxidation damage is a major contributor to cancer, heart disease and Alzheimer's disease. These toxins seem to accumulate over time into more permanent free radicals, so as a person ages they have higher levels of these toxins that are related to chronic diseases.

Inflammation:

Inflammation is a naturally occurring pathophysiological process within the body. It has been implicated mostly in individuals with chronic disease, but also occurs to some extent in healthy individuals.[16] It is an immune system response to inflammatory stimuli that result in the release of an excess amount of molecules known as primary inflammatory cytokines from monocytes.[16] Monocytes are primary immune cells found in the body that function in the formation and release of primary inflammatory cytokines.[16] Examples of primary inflammatory cytokine molecules include tumor necrosis factor–alpha (TNF-α) and (IL-1β).[16] Inflammatory stimuli are released when there is an increase in inflammatory challenges. There are a wide variety of inflammatory stimuli such as

- Trauma
- Stress
- Any immune challenge, and
- Bacterial, viral or fungal toxins. [16]

There are also stimuli that is mostly related to CVD and risk factors, which include

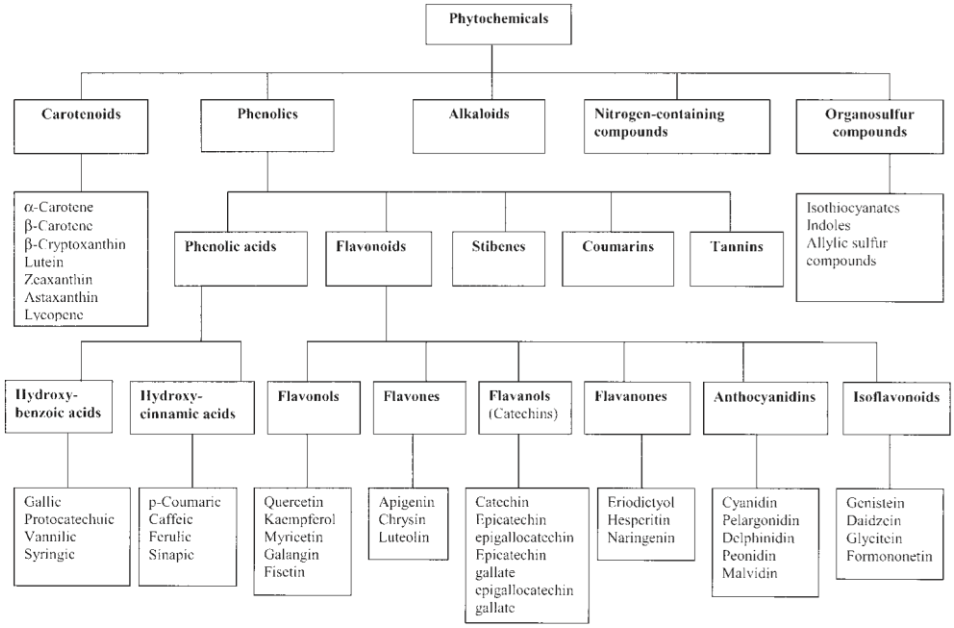
- Diet high in saturated fat
- Obesity
- Hyperglycemia
- Insulin resistance
- Hypertension, and
- Smoking[16]

When the body is in a state of inflammatory stress, brought about by stimuli, the endothelium, or innermost layer of blood vessel tissue, becomes inflamed.[16] The inflamed endothelium along with decreased levels of HDL cholesterol allows monocytes to adhere to the tissue. This process is the initiation of atherosclerosis. Once the endothelium layer becomes inflamed the integrity of the tissue decreases allowing the migration of the monocytes into the intima, where they will reside until they are mature. Monocytes within the intima mature into macrophages, which engulf oxidized lipids leading to the formation of foam cells. Foam cells are still considered to be cells of the immune system; therefore they continue secreting inflammatory cytokines. This continuous secretion leads to the weakening and eventually rupture of the fibrous cap that was formed by smooth muscle cells around the fatty streaks or plaques. Plaques are the final products and are formed from foam cells, lipids, cytokines and some calcium. Thrombotic conditions arise once the fibrous cap ruptures. Once blood comes into contact with the plaques blood clots are formed which restricts blood flow. This can result in a heart attack, stroke or other major condition depending on where the blood clot is formed in the body.

Phytochemicals:

The term "phyto" simply means plant, therefore when discussing phytochemicals a simple enough definition would be chemical compounds produced from plants.[8] Phytochemicals can also be defined as dietary bioactive compounds and functional foods, but are not considered to be a class of nutrients. According to the International Food Information Council, functional foods are defined as foods that provide health benefits beyond basic nutrition. Diets rich in fruits, vegetables, whole grains, nuts and legumes provide the added health benefits that phytochemicals offer. They also function as antioxidants by scavenging free radical, such as superoxide anion and hydroxyl radical.[15] This mechanism leads to an increase in the plasma antioxidant status. Another function of phytochemicals as antioxidants is that they become concentrated within the lipids resulting in a decrease in the oxidation of LDL. Through increased consumption of exogenous antioxidants the body is able to up regulate endogenous antioxidant defense mechanisms. This allows for the regeneration of the GSH enzyme system as well as many other beneficial factors within the body. Phytochemicals break down into many different subgroups and those break down into even more subclasses making this area of science a very broad and continually researched topic. Phytochemical subgroups are shown in Figure 1, but are not limited to:

Figure 1: Classification of dietary phytochemicals



Source: Liu, Journal of Nutrition. 134: 3479S-3485S; 2004

The two phytochemicals that have the most relevance to this review are carotenoids and phenolics, specifically flavonoids.[8] Other classes of phytochemicals not listed in figure 1 include:

- Chlorophyll and chlorophyllin,
- Curcumin,
- Fiber,
- Garlic,
- Indole-3-carbinol,
- Lignans (phytoestrogens),
- Phytosterols,
- Resveratrol, and
- Soy isoflavones (phytoestrogens)[8]

Carotenoids account for the yellow, red and orange colors in plants. Fruits and vegetables provide sufficient amounts of carotenoids in the human diet. The most common carotenoids are categorized into two broad groups:[8]

- Carotenes
  - α-carotene
  - β-carotene
  - Lycopene

- Xanthophylls
  - β-cryptoxanthin
  - Lutein
  - Zexanthin[8]

Alpha-carotene, beta-carotene and beta-cryptoxanthin are known as "provitamin A carotenoids."[8] This means that they can easily convert to retinol by the body, while the other carotenoids cannot. Vitamin A is an essential vitamin for normal human growth and development. Vitamin A plays a large role in vision, inadequate amounts of vitamin A could lead to a condition known as "night blindness." Carotenoids also function as antioxidants to scavenge molecular oxygen before it becomes oxidized.

Flavonoids are a class of widely consumed plant-based phytochemicals. Numerous studies have shown that they significantly decrease risk factors for many chronic diseases.[17] Flavonoids are mostly found in fruits, vegetables, nuts, cocoa, as well as in tea and red wine. Flavonoids are bioactive polyphenols that cannot be broken down in a human's digestive system. They function as potent antioxidants to reduce the likelihood of free radical formation; they are also anti-inflammatory, they reduce oxidized-LDL, and regulate endothelial nitric oxide (eNOS) synthesis. Table 4 shows the flavonoid classes, the subclasses or compounds that make them up and some common sources found in the diet.

Table 4: Flavonoid Classes, Compounds, and Sources

Flavonoid Class	**Respective Compounds**	**Common Plant Sources**
Anthocyanidins	Cyanidin, delphinidin, malvindin,	Blueberries, red wine, strawberries

	pelargondin, peonidin, petunidin	
Flavan-3-ols	Epicatechin, epicatechin 3-gallate, epigallocatechin, epigallocatechin gallate, catechin, gallocatechin	Apples, black tea, blueberries, chocolate, red wine
Flavanones	Eridictyol, hesperetin, naringenin	Citrus fruit and juices, herbal tea
Flavones	Apigenin, luteolin	Celery, garlic, green peppers, herbal tea
Flavonols	Isorhamnetin, kaempferol, myricetin, quercetin	Blueberries, garlic, kale, onions, spinach, tea, broccoli, red wine, cherry tomatoes
Proanthocyanidins	Monomers, dimers, trimers, 4-6mers, 7-10mers, polymers	Apples, black tea, blueberries, chocolate, mixed nuts, peanuts, red wine, strawberries, walnuts
Isoflavones	Daidzen, genistein, glycitein, coumestrol, formononetin, biochanin A	Soy products, peanuts
Total Flavonoids		
>50 mg/100g		Apples, black tea, blueberries, chocolate, garlic powder, grapefruits, grapes, herbal tea, mixed nuts, oranges, pears, red wine, strawberries, walnuts
>50 mg/serving[1]		Apples, black tea, blueberries, chocolate, grapefruit juice, grapefruit, grapes, herbal tea, oranges, pears, red wine, strawberries

[1]Serving sizes that are predefined on the food-frequency questionnaire are available from www.cancer.org and are generally similar to those of the USDA Nutrient Database of Common Reference (http://www.ars.usda.gov).

Source: McCullough ML, Peterson JJ, Patel R, Jacques PF, Shah R, & Dwyer JT. (2012). Flavonoid intake and cardiovascular disease mortality in a prospective cohort of US adults[1-4]. *American Journal of Clinical Nutrition,* 95: 454-464.

Berry phytochemicals:

Berry phytochemicals include the subgroup of flavonoids called anthocyanins.[18] These are the red, blue and purple colors that constitute the berry pigments. Berries are a commonly consumed fruit in the U.S. and worldwide. Subgroups of anthocyanins are listed in Table 4, which also provides common dietary plant sources. Anthocyanins offer many health benefits.

They have strong antioxidant properties, which reduce or inhibit reactive oxygen species.[17] Dietary sources of anthocyanin-rich foods consist of blackberries, blueberries, red grapes, raspberries, strawberries, plums, and red wine. Intake of anthocyanins has been estimated to exceed 200 mg/day.[18] Studies have also reported that even with relatively low intakes of anthocyanins there is a reduced risk for chronic disease. The following table (table 5) gives the flavonoid content, calories and fiber content for the different fruits associated with increased cardiovascular health.

TABLE 5: Fruit	**Total anthocyanidin content (mg)***	**Total flavan-3-ols (mg)†**	**Total flavonols (mg)‡**	**Calories (kcal)**	**Fiber (g)**	**Vitamin C (mg)**	**Vitamin E (mg)**
Blackberry	90.46	42.5	2.49	43	5.3	21	1.17
Blueberry	**163.52**	**51.71**	**9.72**	**57**	**2.4**	**9.7**	**0.57**
Bilberry	430.91	4.13	NF	NF	NF	NF	NF
Chokeberry, raw	437.22	NF	8.90	NF	NF	NF	NF
Cranberry juice (unsweetened)	NF	0.92	20.82	46	0.1	9.3	1.20
Cranberry juice cocktail	0.46	0.19	1.79	54	NF	42.3	0.22
Cranberry (dried, sweetened)	**0.72**	**NF**	**6.91**	**NF**	**NF**	**NF**	**NF**
Cranberry sauce (canned, sweetened)	0.14	NF	5.11	151	1.0	2.0	0.83
Currant, black, raw	272.44	1.17	12.69	63	NF	181	1.0
Mulberries, raw	NF	NF	2.47	43	1.7	36.4	0.87
Black raspberry	324.02	NF	NF	NF	NF	NF	NF
Red raspberry (raw)	38.68	6.63	1.32	52	6.5	26.2	0.87
Strawberry	**33.63**	**4.51**	**1.6**	**32**	**2.0**	**58.5**	**0.29**

Basu, A., Rhone, M., & Lyons, T. J. (2010). Berries: Emerging impact on cardiovascular health. *Nutr Rev, 68*(3), 168-177.

Berries contain large amounts of anthocyanins and make it fairly easy to consume the recommended daily amounts. Although strawberries do not contain as much anthocyanin content as other berries, they have a very high vitamin C content, which acts synergistically with other antioxidants including polyphenols.[18] Berries have many beneficial effects in the physiological system. They are antioxidants, anti-inflammatory, anti-hyperlipidemic, anti-hypertensive, as well as anti-proliferative. Strawberries are considered one of the most commonly consumed fruits in the U.S. along with blueberries and cranberries.[19] Blueberries contain significant amounts of folic acid, fiber, and vitamin C, which explain their high antioxidant activity within cells. Multiple studies have shown that blueberries play a role in lowering lipid oxidation as well as decreasing harmful DNA damaging molecules such as MDA. Cranberry phenolic content depends on the form that is consumed. Cranberries are not usually consumed fresh due to their bitter, tangy taste. They are more commonly consumed as a sweetened juice. Unsweetened cranberry juice is a good source of total flavonols, better than strawberries and blueberries combined.

Fruit & Vegetable Consumption:

According to the AHA, Americans are advised to consume at least 2½ cups of vegetables and 2 cups of fruits every day, along with grains, dairy and protein.[1] These recommendations are set to be sure that Americans are consuming daily vitamins and minerals from food that will benefit their health and help with weight management, but do Americans really follow these recommendations? A balanced diet high in fruits and vegetables has proven to be beneficial in reducing the risks for many chronic diseases, including CVD, stroke, and cancer.[20] Cities and states have implemented more community and local approaches in order to increase fruit and

vegetable consumption. They have advertised and improved local farmers markets and programs aiming to improve overall consumption of local produce. Americans should focus on increasing their daily consumption of fruits and vegetables, especially dark green, orange, and red vegetables. This is crucial for present and future dietitians to help Americans comprehend and implement the new recommendations into their daily lives in order to better their patient's health.

Diets high in fruit and vegetable consumption have been found to be associated with a reduced risk of CVD morbidity and mortality.[21] Two prospective cohorts by Hung et al.[21], evaluated the relationship between fruit and vegetable consumption and the incidence of chronic diseases in healthy healthcare professionals. Fruit and vegetable intake was associated with a modestly significant decrease in the development of chronic diseases (P=0.07). Of the specific fruit and vegetable groups, only green leafy vegetables showed a statistically significant association with lower risk among the participants (P=0.01). Researchers concluded that the moderate significant decrease is primarily geared toward a decreased risk of CVD and not for cancer. A similar study by Liu et al.[22] focused on fruit and vegetable intake and CVD risk in female health professionals. They observed a significant decrease in CVD risk in participants who consumed fruits and vegetables in the highest quintiles as well as a lower risk of MI. Consuming 5 or more servings of fruits and vegetables per day leads to better outcome measures and a healthier lifestyle.

Strawberries:

Strawberries are a popular fruit in the United States. They are the fourth highest in production behind grapes, oranges and apples, and they are fifth highest in fruit consumption behind bananas, apples, oranges, and grapes.[23] Strawberries may be consumed in many different ways including fresh, frozen, in jams or jellies, and also, freeze-dried, which are usually added to cereals and juices. They are high in vitamins, phytochemicals, and phytosterols. As mentioned

previously, strawberries are excellent sources of antioxidants. They contain significant amounts of phytochemicals, including over 40 phenolic compounds. The vibrant red color that strawberries exhibit is due to anthocyanins, which make up about 13% of the flavonoid capacity along with other flavonoids such as 24% ascorbic acid and 19% ellagitannins that increase the antioxidant capacity.[24]

There have been many human studies investigating the effects of strawberries and cardiovascular health or risk of chronic disease in humans, specifically in CVD, CVD risk factors, and mortality caused by CVD. In the Women's Health Study, conducted by Sesso et al.[24] the prospective association of strawberry intake with CVD risk in 38,176 women as well as the cross-sectional association of strawberry intake with lipids and CRP in 26,966 women for an average of 10.9 years were examined. Strawberry intake was assessed at baseline using a 131 item semiquantitative food frequency questionnaire, as well as other factors including self-reported lifestyle, clinical, and dietary factors. Categories for strawberry consumption were divided into four groups: none, 1-3 servings/month, 1 serving/week, and ≥ 2 servings/week. Blood taken at baseline was assayed for lipid parameters and CRP. This study defined total CVD as confirmed myocardial infarction, stroke, coronary artery bypass graft surgery, percutaneous transluminal coronary angioplasty, and CVD mortality.

Strawberry intake was strongly and significantly associated with varying baseline characteristics.[24] Women, who consumed greater amounts of strawberries tended to be older, exercised more often, did not smoke or smoked less, and were currently using post-menopausal hormones ($p< 0.00001$). In the prospective study, higher intakes of strawberries were not associated with risk of CVD ($p=0.28$) as well as for specific cardiovascular endpoints (all $p>0.05$). However, there was a suggestion of a lower risk of CVD mortality when strawberries were consumed ≥ 1 serving/week, but evidence was limited due to a low case count. In the cross-sectional study, total cholesterol and LDL cholesterol were moderately lower in the groups that

consumed higher amounts of strawberries, despite that there was not a relationship between strawberry intake and the relative risk of higher plasma lipids levels ($p>0.05$). Furthermore, after adjusting for lifestyle, clinical, and dietary factors, CRP had a borderline significant 14% lower likelihood of a value ≥ 3 mg/L in women who consumed ≥ 2 servings of strawberries per week (P value trend=0.012). This could mean that there is a possible decrease in an inflammatory response associated with higher consumption of strawberries. The study concluded that strawberry intake was not associated with the risk of a CVD event, lipid parameters, or CRP in women, though increased strawberry consumption may slightly reduced the likelihood of having elevated CRP levels.

In the Iowa Women's Health Study, Mink et al.[20] evaluated the prospective association between flavonoid intake and CVD mortality in 34,489 post-menopausal women. Results from this study concluded that they found a significant inverse relationship between consumption of strawberries and CVD mortality after 16 years of follow-up. Yu et al.[25] reported significant lipid profile improvement with purified anthocyanin supplementation for 12 weeks in subjects with dyslipidemia versus a control. Jenkins et al.[26] showed antioxidant effects in 28 hyperlipidemic subjects complying to a portfolio diet for 2.5 years after one-month strawberry consumption in reducing oxidative damage to LDL. Other studies have reported a positive association between cardiovascular health and strawberry anthocyanin intakes.

Clinical Studies:

Table 6 summarizes the human intervention studies solely focused on strawberry intake. It gives the basics of their research as well as methods to explain how they found their results.

Table 6: Summary of Human Intervention Studies using fresh or processed strawberry products

Source	Duration	Study design	Study subjects	Control	Strawberry intervention	Significant findings ($p<0.05$)
Cao et al. in 1998[27]	Postprandial	Controlled trial	Healthy female subjects (n=8)	Coconut drink	240g strawberries	Increase in vitamin C and antioxidant capacity
Paiva et al. in 1998[28]	Postprandial	Controlled trial	Healthy elderly women (n=7)	378mL coconut drink	240g strawberries	Decrease plasma carotenoids
Chung et al. in 2002[29]	Four days	Three consecutive control days followed by experimental agents on the fourth day	Healthy volunteers (n=40)	Control diet: low in NDMA, nitrate, amine, sulfur compounds, ascorbic acid and phenolic-compound-containing food items	Experimental diet containing whole strawberries (300g),	Decrease in NDMA excretion following whole strawberry intake
Prior et al. in 2007[30]	Postprandial	Randomized cross-over multicentered trial	Healthy women (n=7)	None	Seascape' strawberries (300g)	Increase in postprandial whole plasma antioxidant capacity in strawberry group
Erlund et al. in 2008[31]	8 weeks	Randomized, single-blind, placebo-controlled, trial	Subjects with cardiovascular risk factors (n=72)	One of four control products each day to match the energy intake in the berry group; 2dL sugar-water, 100g sweet semolina porridge, 100g sweet rice porridge, or 40g marmalade sweets	2 portions of berries daily; whole bilberries (100g) and a nectar of 50g crushed lingonberries every other day; black currant or strawberry puree and cold-pressed chokeberry and raspberry juice on alternating days	Inhibition of platelet function; Increase in HDL-cholesterol; Decrease in systolic BP in berry group
Jenkins et al. in	Ten weeks	Randomized cross-over study with 2-	Hyperlipidemic	Oat bran bread (65g/day)	Fresh strawberries	Decrease in oxidative damage to LDL

2008[26]		week washout phase	subjects;(n=28)		(454g/day)	in strawberry group; maintained Decrease in LDL-cholesterol as result of previous dietary regimen; Increase dietary palatability
Basu et al. in 2009[32]	Four weeks	Baseline and Post-intervention effects	Women with metabolic syndrome (n=16)	None	50g of freeze-dried strawberry powder as beverage	Decrease in total and LDL-cholesterol and lipid peroxidation
Basu et al. in 2010[33]	Eight weeks	Randomized controlled trial	Men and women with metabolic syndrome (n=27)	Four cups water	50g of freeze-dried strawberry powder as beverage	Decrease in total and LDL-cholesterol, small LDL particles, and vascular cell adhesion molecule
Torronen et al. in 2010[34]	Postprandial	Randomized controlled cross-over trial	Healthy men and women (n=12)	Control meal: 250 mL water, 35g sucrose, 4.5g glucose, 5.1g fructose	Mixed berry puree (150g) consisting of black currants, bilberries, European cranberries and strawberries, with 35g sucrose	Decrease postprandial glucose at 15 and 30 min and at 150 min in berry versus control group; smaller peak Increase in glucose from baseline in berry group
Henning et al. in 2010[35]	Three weeks	Baseline and Post-intervention effects	Healthy female volunteers (n=21)	None	250g frozen strawberries daily for 3 weeks	Increase in serum antioxidant capacity
Burton-Freeman et al. in 2010[36]	Postprandial and twelve weeks	Randomized placebo-controlled crossover trial	Hyperlipidemic men and women (n=24)	Placebo beverage matched for calories, carbohydrates and sugars	10g freeze-dried strawberries ~ 110g/day of fresh strawberries	Postprandial lipemia and oxidized LDL were significantly decrease following high-fat meal

						challenge with strawberries
Zunino et al. in 2011[37]	7 weeks	Double-blind, randomized, cross-over trial	healthy subjects (n=20)	Two servings of strawberry flavoring and red food color in yogurt, cream cheese, or water-based, sweetened beverage	Two servings of strawberry powder in yogurt, cream cheese, or water-based, sweetened beverage	Decrease plasma concen-trations of cholesterol and small HDL-cholesterol particles, and increase LDL particle size in obese subjects

Strawberries and antioxidant capacity

Henning et al.[35] conducted a crossover intervention study in 2010 focused on the impact of strawberry consumption on antioxidant capacity. During this 3 week study, 250 grams of frozen strawberries were consumed daily at breakfast, by 21 healthy female participants. The authors state that 2,5-Dimethyl-4-hydroxy-3-[^{2}H]furaone (DMHF) is an important flavor constituent that gives strawberries their distinct aroma. DMHF is widely used industrially in food and beverages as a flavoring agent due to its pleasant taste and odor; it has also been found to be highly bioavailable after consumption of at least one large dose of strawberries, about a 2.5 to 3 kg dose. It is believed that this component of strawberries may be responsible for the modest increases in antioxidant defense following interventions.

They chose to measure oxidative damage to lipids in serum and not in isolated LDL fractions because most of the phytochemicals in strawberries are assumed to be in the aqueous phase of serum.[35] In this study, each 250g serving of frozen strawberries provided at least 164.5mg of Pg-3-glucoside, 3.7mg of ellagic acid and 3mg of DMHF daily. Urinary concentrations of DMHF were significantly increased at the end of the strawberry intervention in 19 of the 21 participants and returned to baseline level at the end of the washout period ($p <$

0.05). DMHF urinary concentrations were found at its highest in the 0-12 hour urine sample and were decreased in the 12-24 hour urine sample. Lipid peroxidation lag time increased from baseline in both fasting (25.4 ± 21.0 to 31.5 ± 22.9) and non-fasting (25.0 ± 19.6 to 31.1 ± 24.6) serum ($p < 0.05$). LDL oxidation lag phase returned to baseline levels after a washout period of three weeks. Lipid peroxidation lag time increased by 20% indicating that daily consumption of strawberries resulted in a slightly significant increase in antioxidant capacity in healthy female participants ($p < 0.01$). Henning et al.[35] suggested that consumption of 250g of strawberries everyday for 3 weeks decreased oxidative damage likely from the increase seen in antioxidant capacity in serum, which has potential to improve the body's defense against chronic disease.

Strawberries, lipids, and blood pressure

In an 8 week single-blind, randomized, placebo-controlled intervention trial, Erlund et al.[31], which took place in 2008, aimed to investigate the effects of berry consumption on HDL cholesterol, platelet function and blood pressure (BP) in 72 middle-aged unmedicated subjects with CVD risk factors. Subjects were randomized into either the berry group (n=35) or the control group (n=36). Subjects in the berry group consumed 2 portions of berries daily, 100g whole bilberries and 50 g crushed lingonberries every other day and 100 g of black currant or strawberry puree and 0.7 mL of cold-pressed chokeberry and raspberry juice on the alternative days. The control group consumed 1 of 4 different calorie-control products each day. Researchers found that systolic blood pressure (SBP) in the berry group was significantly different from control. SBP significantly decreased by 1.5 mmHg in the treatment group and slightly increased by 0.5 mmHg in the control group ($P = 0.050$). On the other hand, diastolic blood pressure (DBP) did not change in the berry group, but slightly increased by 0.9 mmHg in the control group ($P = 0.044$). In the subgroup analysis conducted on SBP, the difference between groups was significant only in the highest tertile. Within the highest tertile, the mean decreases in SBP were 7.3 and 0.2 mmHg in the berry and control groups, respectfully ($P = 0.024$). These findings

suggested that berry supplementation had a greater influence on systolic blood pressure in higher ranges.

In addition, researchers found that total serum cholesterol and triacylglycerol were unaffected by the berry intervention.[31] Despite that, serum HDL-cholesterol concentrations increased significantly more in the berry group by 5.2% (0.08 mmol/L) than in the control group (0.6%; 0.01 mmol/L) (P = 0.006). Researchers put these findings into perspective by reporting that for each 1% increase in HDL cholesterol there is a 1% decrease in the cardiovascular event rate.

Platelet function was measured using a platelet function analyzer studying closing time when collagen and ADP were used as platelet activators.[31] A short closing time may be a novel risk factor for acute coronary syndromes. Erlund et al.[31] found that berry consumption significantly inhibited platelet function, as measured by CADP-CT (P = 0.018). Supplementation with berries was found to significantly increase collagen and ADP closing time by 11% (8.4 seconds) compared to a decrease of 1.4% (1.2 seconds) in the control group. Favorable changes in platelet function were demonstrated by an increase in platelet aggregation time this indicates that berries increase cardiovascular health by reducing the risk of clotting.

The results of this study implied that the consumption of moderate amounts of berries resulted in positive changes in blood pressure, HDL cholesterol, and platelet function, which leads to the conclusion that regular consumption of multiple types of berries may play a role in the prevention of CVD versus just consuming one type of berry.[31]

Strawberries, lipid profile & lipid peroxidation

Many studies have taken place focusing on the effects of antioxidants (mainly strawberries) and CVD risk.[32,33] Some factors of CVD that saw a decrease in patients with metabolic syndrome with the addition of strawberries were elevated blood pressure,

hyperglycemia, dyslipidemia, and inflammation. Dietary intervention by increasing strawberry intake has been shown to decrease markers of lipid profile in several clinical studies.

In a study conducted by Basu et al.[32] in 2009, 16 women with metabolic syndrome consumed 50g of freeze-dried strawberry powder for 4 weeks. Again, this study tested the hypothesis that FSP will lower fasting lipids and biomarkers of oxidative stress and inflammation at 4 weeks compared to baseline. The study concluded that total cholesterol (-5%) and LDL cholesterol levels (-6%) significantly decreased at 4 weeks versus baseline ($P < 0.05$), as well as lipid peroxidation in the form of malondialdehyde and hydroxynonenal (-14%, $p < 0.01$). Oxidized LDL cholesterol showed a decreasing trend in the 4 weeks ($P = 0.123$) and no significant differences on any other measurements were seen. Limitations are the same for both studies, but this study had no control. Researchers concluded that short-term supplementation of freeze-dried strawberries appeared to have hypocholesterolemic effects as well as significantly decreased lipid peroxidation in women with metabolic syndrome.

In a randomized controlled trial conducted by Basu et al.[33] in 2010, researchers hypothesized that freeze-dried strawberry powder (FSP) supplementation would improve clinical features of metabolic syndrome associated with atherosclerotic markers of CVD. The primary objective was to assess changes in lipid profiles including lipoprotein particle size and concentrations along with other biomarkers after FSP supplementation or control treatment for 8 weeks in obese subjects with metabolic syndrome. Twenty seven subjects with an average BMI of 37.5 and average age of 47 years old completed the study. The subjects were randomized into the control group (4 cups of water a day) or the strawberry group (2 cups strawberry beverage + 2 cups of water a day) for 8 weeks. The intervention was a 50g FSP beverage, which is equal to approximately 500g of fresh strawberries or about 3 cups of sliced strawberries.

Strawberry supplementation significantly decreased total and LDL cholesterol levels in subjects with metabolic syndrome.[33] The study concluded that the strawberry supplementation caused a 10% decrease in total cholesterol (5.8 ± 0.2 to 5.2 ± 0.2 mmol/L), as well as an 11% decrease in LDL cholesterol (3.5 ± 0.2 to 3.1 to 0.1 mmol/L) at the end of 8 weeks compared to the control ($P < 0.05$). Supplementation of FSP did not affect triglycerides, HDL cholesterol, or VLDL cholesterol. Limitations of the study included the small sample size as well as the absence of a dose-response study design. The study also lacked a matched energy and fiber control group, which may be more appropriate for future studies. These findings correlate with the hypothesis that freeze-dried strawberry supplementation decreases atherosclerotic risk factors, such as total and LDL cholesterol in subjects with metabolic syndrome.

Strawberries and postprandial metabolism:

Strawberries and postprandial antioxidant capacity

In a study by Cao et al.[27] in 1998, seven healthy elderly women were recruited to investigate the effects of strawberries, spinach, red wine or vitamin C on serum total antioxidant capacity. Total antioxidant capacity was determined using different methods including: oxygen radical absorbance capacity (ORAC) assay, Trolox equivalent antioxidant capacity (TEAC) assay and ferric reducing ability of plasma (FRAP) assay. An increase in the area under the curve (AUC) in serum and urinary ORAC and FRAP demonstrated an increase in total antioxidant capacity when 240g of strawberries were consumed. The increases in the AUC for $ORAC_{PCA}$ and FRAP were 13% and 10%, respectively, compared to control. Urinary ORAC excretion after the consumption of strawberries showed an increase of 9.6% and was significantly higher than the control ($p < 0.05$). The increases in serum and urinary antioxidant capacity following the consumption of strawberries indicated a direct absorption and/or increased production of antioxidants. The contribution of vitamin C to the increased serum $ORAC_{PCA}$ and FRAP was only 8-14% and the

contribution of urate to the increased serum $ORAC_{PCA}$ and FRAP was 39-42% following consumption of the strawberry drink. It is clear that other antioxidant phenolic compounds found in strawberries contribute to the other half of the increased serum antioxidant capacity following strawberry consumption.

In addition, continued analysis in 1998 by Paiva et al.[28], examined the postprandial plasma responses of carotenoids following consumption of strawberries, red wine, vitamin C or spinach in the same seven elderly women in the study by Cao et al.[27] Out of the five beverages studied only the spinach beverage had a significant source of carotenoid content. The control intervention had no significant difference in carotenoid levels from baseline, but the strawberry beverage had lower postprandial carotenoid concentrations from baseline. However, for most carotenoids, strawberry feeding resulted in significantly lower carotenoids values from baseline at 11 and 15 hours postprandial ($p < 0.05$). Strawberry intervention also significantly lowered LDL cholesterol by 14.1% after 1 hour from baseline ($p < 0.05$). Paiva et al.[28] hypothesized that strawberries may have played a role in interfering with the secretion of carotenoids into the circulation based on the findings from this study.

Strawberries and postprandial lipid metabolism

In a randomized, single-blind, placebo-controlled, 12 week crossover trial by Burton-Freeman et al.[36] in 2010, the postprandial effects of strawberry supplementation on in response to a high fat meal, about 28% fat, in 24 participants with dyslipidemia were investigated. Participants, first, at baseline, consumed a 10 g freeze-dried strawberry or a macronutrient matched controlled beverage and a high fat meal and second, after 6 weeks of consuming a strawberry or control beverage daily, both groups ingested a high fat meal with a control beverage only, without a strawberry beverage. The authors found that acute and chronic strawberry supplementation helps to protect a postprandial rise in serum levels after a high fat meal.

Results indicate that there was a significant increase in triglycerides, HDL cholesterol, and oxidized LDL in the postprandial state under both the acute and chronic experimental conditions ($P < 0.0001$).[36] However, after consuming strawberry beverages with a high fat meal, there was a significant reduction in postprandial triglycerides ($P = 0.005$), HDL cholesterol ($p = 0.003$), and oxidized LDL ($P = 0.0008$) compared to the control. Reduction of triglycerides may be beneficial in decreasing CVD risk. The results of this study supported the hypothesis that strawberry phenolic compounds decrease postprandial lipemia and LDL oxidation with a high fat meal. Triglyceride concentrations significantly increased from baseline after both strawberry and control meals, reaching its peak concentration after 4 hours. However, triglyceride concentrations were found to be significantly lower after consumption of strawberries. The study also showed that daily strawberry consumption in the form of a beverage for 6 weeks had a persistent beneficial effect on postprandial lipemia.

Conclusion:

Cardiovascular risk factors including: obesity, metabolic syndrome, hypertension, dyslipidemia, and, diabetes are all strongly associated with atherosclerotic CVD and CVD mortality. Dietary management of these specific and serious conditions can prevent or reverse the severity and even the condition itself. Several human studies have been conducted to investigate the cardioprotective benefits of strawberries in healthy subjects. Strawberries are a good source of polyphenolic anthocyanins, micronutrients, vitamin C, and fiber. They have been shown to increase antioxidant capacity as well as to increase HDL cholesterol and decrease systolic blood pressure. To date, there have been no reported studies investigating the long-term, dose-response strawberry supplementation in subjects with CVD or CVD risk factors, which therefore leads us to the scope of our study.

CHAPTER III

MATERIALS AND METHODS

Participants

Following approval from the Institutional Review Board (IRB) for both Oklahoma State University (OSU) and University of Oklahoma (OU), men and women with abdominal adiposity and dyslipidemia were recruited from the Clinical Research Center (CRC) at the University of Oklahoma and at the Department of Nutritional Sciences (NSCI) at Oklahoma State University through flyers and campus e-mail advertisements. Participants had an initial telephone screen and then were scheduled for a screening visit. Subjects were qualified through inclusion and exclusion criteria. After qualification, 40 subjects were randomized to match for age and gender. Eleven subjects were enrolled in each the high dose strawberry intervention and the high dose control groups and 9 subjects were enrolled in the low dose strawberry intervention and low dose control groups. Those in strawberry intervention groups consumed freeze-dried strawberry powder (FSP).

Inclusion criteria

Subjects were included based on inclusion criteria:

- Abdominal adiposity (men >40 inches, women >35 inches), dyslipidemia (two of four criteria: fasting total cholesterol >200 mg/dL, triglycerides >150 mg/dL, low density lipoprotein (LDL) –cholesterol >100 mg/dL, or high density lipoprotein (HDL)-cholesterol (men <40 mg/dL, women <50 mg/dL), normal liver, kidney, and thyroid function tests, stable multivitamin/mineral supplements or prescription medications (except hypolipidemic, hypoglycemic, and steroid agents), males and females, as well as any ethnic group.

Exclusion criteria

Subjects were excluded based on exclusion criteria:

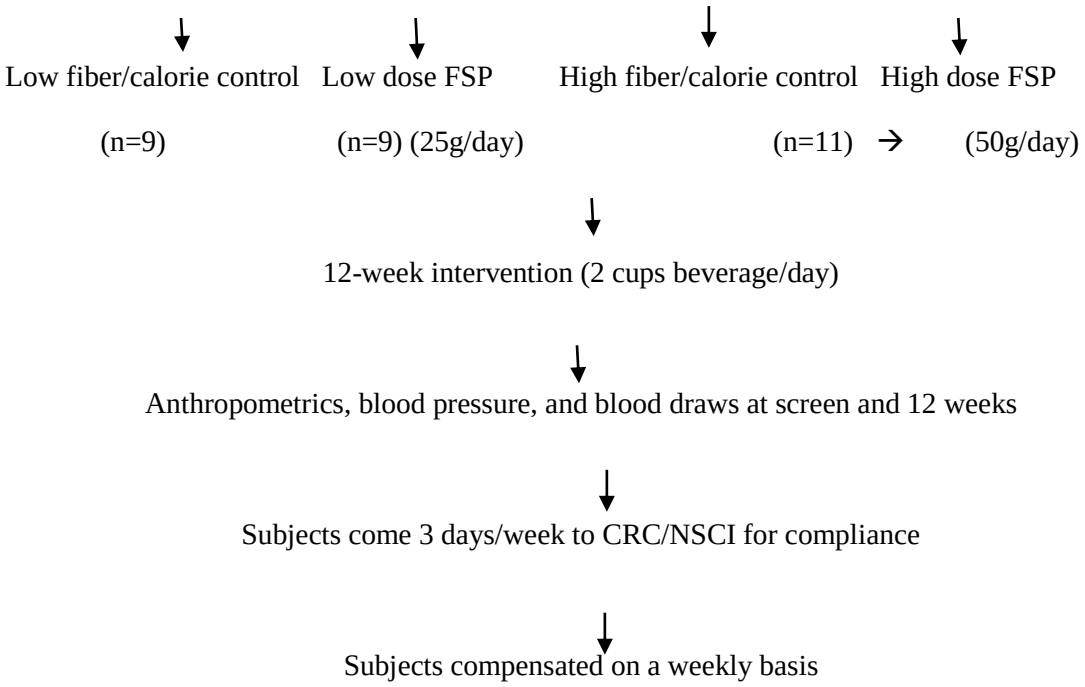
- Pre-existing disease (e.g. anemia, cancer, heart disease, diabetes [fasting glucose ≥126 mg/dL]), liver or renal disorders, pregnancy and lactation, consumption of mega doses of antioxidants/fish oil supplements (>1 g/day), consumption of hypolipidemia, hypoglycemic, and steroid medications, abnormal hemoglobin (Hb) (normal range: 12.0-18.0 g/dL), white blood cell (WBC) (normal range: 4.0-11.0 K/mm3), or platelets (140-440 K/mm3), hypo/hyperthyroidism (normal range for thyroid stimulating hormone: 0.35-4.940 uiu/mL), abnormal liver enzymes (normal range for aspartate transaminase (AST): 7-40 units/L; alanine transaminase (ALT): 10-45 units/L), abnormal kidney function (normal creatinine: females: 0.7-1.2 mg/dL; males: 0.8-1.2 mg/dL; normal blood urea nitrogen (BUN): 1-59 years: 7-18 mg/dL; >59 years: 8-21 mg/dL), as well as smoking and drinking alcohol (>1 oz/day).

Research design

Subjects identified with abdominal adiposity and dyslipidemia

↓

Block Randomization (n= 40)



Intervention and compliance

Subjects in the low or high dose intervention or control groups consumed 2 cups of beverage per day for 12 weeks. In the low dose intervention group, each cup contained 12.5g freeze-dried strawberry powder (FSP) blended in 1 cup water with added splenda (optional). In the high dose intervention group each cup contained 25g FSP that was blended in 1 cup of water with the option of added splenda. The matched controls consumed 2 cups of the control beverage per day. Each cup contained 2g fiber (Fiberfast) and 2 tsp sugar blended in 1 cup water in the low fiber/calorie control group, or 4g fiber (Fiberfast) and 4 tsp sugar blended in 1 cup water in the high fiber/calorie control group. The control beverage also contained a total of 4 tsp artificial strawberry flavor, specifically strawberry flavored Koolaid©, as well as 4 drops of red food coloring blended in to 2 cups of water per day. The low-dose FSP and matched control group consumed equal amounts of fiber (4g) and calories (75 kcal) per day, while the high dose FSP and matched controls consumed 8g and 150 kcal per day for 12 weeks. The nutrition composition of the FSP and control intervention beverages is listed in Table 7.

TABLE 7: Composition of freeze-dried strawberries and control beverages

Nutritive value	Freeze-dried strawberries (25g)[1]	Freeze-dried strawberries (50g)[1]	Control (25g)[2]	Control (50g)[2]
Calories (kcal)	75	150	80	160
Protein (g)	1.75	3.5	-	-
Fat (g)	0.25	0.5	-	-
Carbohydrates (g)	25	50	20	40
Dietary fiber (g)	4	8	4	8
Ash (g)	1.60	3.17	-	-
Vitamin C (mg)	54.5	109	1.5	3
Total Phenolics (mg)[3]	1003	2006	-	-
Total Anthocyanins (mg)[4]	77	154	-	-
Ellagic acid (mg)	20.5	41	-	-
Phytosterols (mg)	25	50	-	-

[1] Ten percent fresh weight; California Strawberry Commission (Watsonville, CA, USA).

[2] Fiberfast

[3] Expressed as milligram gallic acid equivalents

[4] Expressed as milligram cyaniding-3-glucoside equivalents

Source: CSC

All subjects made mandatory visits to the research facilities 2-3 days/week to ensure compliance. The remaining quantities of beverages were provided in containers for later consumption. Height, weight, blood pressure, and waist circumference were measured by trained personnel at the Clinical Research Center (CRC) at the University of Oklahoma Health Sciences Center (OUHSC) and at Nutritional Sciences department at Oklahoma State University (OSU) at screen, 6 weeks, and at 12 weeks of the study. Blood draws were performed by trained nurses at CRC and by trained phlebotomists at Stillwater Medical Center in Stillwater.

Dietary analyses

All subjects were asked to follow their usual diet and lifestyle but refrain from other sources of berries and related products while on the study. Subjects also maintained 3-day food (2 weekday and 1 weekend) records at screen, 6 and 12 weeks of the study. Three day averages of micro- and macronutrient intakes were calculated using Food Processor (ESHA Research, Salem, OR). If a participant consumed a food that was not in the database, an online search of the nutrient composition was made or a food with very similar nutrient composition was chosen.

Clinical variables

Fasting blood samples, after each draw were immediately sent to OU Medical Center laboratory or the Stillwater Medical Center laboratory for comprehensive metabolic panel (CMP) including glucose, insulin, glycated hemoglobin, lipid panel, electrolytes, liver, kidney, thyroid tests, and complete blood count at each visit. Remaining plasma and serum samples were stored at -80^{o}C for future analyses.

Biomarkers of oxidative stress

Plasma concentrations of oxidized-LDL were measured using enzyme-linked immunosorbent assay (ELISA) kits (Mercodia, Uppsala, Sweden) according to the manufacturer's instructions. Lipid peroxidation was measured in serum as malondialdehyde (MDA) using a colorimetric assay according to the manufacturer's protocol (LPO-586TM, Oxis Health Products, Inc., Portland, OR). All samples were assayed in triplicates within our established inter assay variations of 10% for each variable.

Statistical analyses

For all measures, descriptive statistics were calculated and graphs drawn to look for outliers; no data points were determined to be outliers. Data are presented as means ± standard deviation in the tables. Our primary comparisons for analysis were between (1) the high dose strawberry and high dose control group and (2) the low dose strawberry and low dose control group.

Within group comparisons at baseline and 12 weeks for each of the 4 groups were conducted using a paired t test. Changes in measurements over the 12-wk study period were assessed by calculating the difference between the pre- and postintervention measurements in each group; the differences in high dose strawberry versus high dose control and the differences in low dose strawberry versus low dose control. Differences were calculated by subtracting baseline values minus 12 week values for each group. For each variable, significance was

assessed by comparing the change over the 12-wk study period between high dose strawberry versus high dose control as well as between low dose strawberry versus the low dose control using Student's *t* tests. The sample included a total of 40 total participants (n=11 in both high dose groups & n=9 in both low dose groups). Multiple hypothesis testing was not accounted for, but results were reviewed for consistencies. All statistical tests were 2-tailed with significance level set at 0.05. SPSS for Windows (version 15.0, SPSS, 2006) was used for the statistical calculations.

CHAPTER IV

RESULTS

A total of 40 participants met the inclusion and exclusion criteria and completed the study. Randomization took place as follows: high dose strawberry group (n=11), high dose control group (n=11), low dose strawberry group (n=9), low dose control group (n=9). All of the subjects who were enrolled completed the 12 week study. Additionally, all of the subjects in all 4 groups adhered to the intervention and compliance was assessed by mandatory visits (2 times per week) for monitored drink consumption and recording of any left over amounts of beverages. There were no complaints from the participants about adverse effects of strawberry and/or fiber supplementation.

Baseline characteristics

Table 8 shows all of the baseline characteristics of the participants. Baseline values of hemoglobin A1c and total cholesterol in the low dose strawberry group were significantly different versus control (p=0.04 and p=0.01, respectively). Although not significant, LDL cholesterol tended to be lower in the low dose strawberry group versus control (p=0.08). No other differences were seen in any other parameters.

In the high dose strawberry group, baseline values of waist circumference and diastolic blood pressure were significantly different versus control (p=0.04 and p=0.05, respectively). There were no other differences observed in any other variables.

Anthropometric measures and blood pressure

Anthropometric (BMI and waist circumference) and blood pressure measures showed no significant difference in the low dose control group at 12 weeks versus baseline (Table 9). However, low dose strawberry supplementation significantly decreased diastolic blood pressure by 3.3% at 12 weeks versus baseline (p=0.02). When analyzing the change over the 12 week period in the low dose strawberry group, diastolic blood pressure significantly decreased by an average of 2.67 mmHg when compared to low dose control (2.22 mmHg)(p=0.04).

Anthropometrics and blood pressure measures were not significantly different in the high dose control group at 12 weeks versus baseline. Similarly, no significant differences in anthropometric measures and blood pressure at 12 weeks versus baseline in the high dose strawberry group were seen. Also, the 12 week change showed no significant differences between the high dose control and high dose strawberry groups.

Glucose and hemoglobin A1c

Fasting glucose showed a decreasing trend of 7.7% (p=0.07) in the low dose control group and no change in hemoglobin A1c at 12 weeks versus baseline. Low dose strawberry supplementation did not affect fasting glucose or hemoglobin A1c levels in the subjects at 12 weeks versus baseline. No changes were observed over the 12 week period in the low dose strawberry group when compared to the low dose control group.

Hemoglobin A1c significantly decreased by 3.4% in the high dose control group at 12 weeks versus baseline (p=0.02). In contrast, there was no significant difference in fasting glucose

at 12 weeks versus baseline. No significant differences for hemoglobin A1c and fasting glucose were found in the high dose strawberry group at 12 weeks versus baseline. Finally, the 12 week change showed no significant difference between the high dose control and high dose strawberry groups.

Lipid profile

The low dose control group showed no significant differences in total cholesterol, LDL cholesterol, HDL cholesterol, VLDL cholesterol, or triglyceride levels at 12 weeks versus baseline. Likewise, the low dose strawberry group showed no significance in lipid profiles at 12 weeks versus baseline. When analyzing the change over the 12 week period, the decrease in HDL cholesterol in the low dose strawberry group was significantly lower (0.11 mg/dL) when compared to the low dose controls (0.78 mg/dL, $p=0.03$).

Total, LDL, HDL, and VLDL cholesterol, along with triglycerides, showed no significant decreases in the high dose control group at 12 weeks versus baseline. However, high dose strawberry supplementation showed decreasing trends in total cholesterol and LDL cholesterol at 12 weeks versus baseline ($p=0.07$ and $p=0.06$, respectively). No changes were observed over the 12 week period in the high dose strawberry group when compared to the high dose control group in these lipid profiles.

Biomarkers of lipid oxidation

Oxidized LDL cholesterol and serum MDA significantly decreased by 6.6% and 11.1% respectively, in the low dose control group at 12 weeks versus baseline ($p<0.05$ and $p=0.01$, respectively). However, low dose strawberry supplementation did not affect ox-LDL or serum MDA at 12 weeks versus baseline. When evaluating the 12 week change, the decrease in serum MDA in the low dose strawberry group was significantly lower (0.12 μM) when compared to the low dose control group (0.28 μM, $p=0.03$).

Biomarkers of lipid oxidation such as, ox-LDL and serum MDA, decreased significantly in the high dose control group at 12 weeks versus baseline as follows: oxidized LDL cholesterol decreased by 5.8% ($p=0.03$) and serum MDA decreased by 11.5% ($p<0.05$). In our study, high dose strawberry supplementation also significantly decreased ox-LDL by 11.2% and serum MDA by 26.7% ($p<0.05$). When assessing the 12 week change, we found serum MDA in the high dose strawberry group to be significantly decreased by an average of 0.79 μM when compared to high dose controls (0.35 μM, $p=0.03$).

TABLE 8: Baseline characteristics of participants

	Desirable Ranges[4]	LDC	LDFSP (25g)	HDC	HDFSP (50g)
n		9	9	11	11
Age (years)		44.9 ± 9.3	49.2 ± 8.9	48.6 ± 10.5	46.5 ± 10.7
Male/Female (n/n)		2/7	2/7	3/8	4/7
BMI (wt/m^2)	18.5-24.9	37.5 ± 5.1	35.5 ± 4.9	34.8 ± 5.7	40.1 ± 7.2
Waist (in.)	Men <40 Women <35	43.2 ± 3.7	41.5 ± 3.5	41.9 ± 2.9	46.0 ± 5.0*
SBP (mmHg)	<120	128 ± 12.4	125.0 ± 11.3	131.3 ± 12.1	136.5 ± 10.1
DBP (mmHg)	<80	87.7 ± 6.1	80.6 ± 7.6	82.9 ± 3.96	86.5 ± 9.0*
Glucose (mg/dL)	90-130	103.7 ± 12.7	97.1 ± 11.8	91.5 ± 16.7	92.7 ± 15.5
HbA1c (%)	4.5-6	5.9 ± 0.8	5.6 ± 0.3*	5.9 ± 0.6	5.8 ± 0.5
BUN (mg/dL)	6-20	13.7 ± 3.0	12.6 ± 1.9	15.5 ± 3.4	15.3 ± 4.5
Total Protein (gm/dL)	6-8.3	6.9 ± 0.4	6.9 ± 0.2	7.1 ± 0.4	7.0 ± 0.4
Total Cholesterol (mg/dL)	<200	207.2 ± 22.1	204.9 ± 51.4*	213.5 ± 36.9	215.3 ± 27.9
LDL (mg/dL)	<100	133.1 ± 18.4	121.6 ± 44.0	132 ± 33.2	138.5 ± 28.7
HDL (mg/dL)	Men >40 Women >50	49.9 ± 12.7	47.8 ± 19.4	45.2 ± 9.6	45.4 ± 17.1
VLDL (mg/dL)	5-40[3]	22.4 ± 8.2	34.0 ± 18.0	34.5 ± 14.8	37.1 ± 14.1
Triglycerides (mg/dL)	<150	110.4 ± 44.8	170.3 ± 90.6	172.8 ± 73.7	185.5 ± 71.5
ALT (U/L)	7-56	31.9 ± 14.1	31.8 ± 13.3	28.7 ± 7.2	24.7 ± 6.2
AST (U/L)	5-40	24.9 ± 6.7	29 ± 12.2	24.8 ± 5.8	23.2 ± 3.5
WBC (mcL)	4,500-10,000	6.8 ± 1.4	7.2 ± 0.9	6.9 ± 1.7	6.9 ± 2.0
RBC (cells/mcL)	4-6	4.5 ± 0.4	4.5 ± 0.5	4.7 ± 0.3	4.5 ± 0.5
Hemoglobin (gm/dL)	12-17	13.3 ± 1.3	13.8 ± 1.4	14.2 ± 1.3	13.7 ± 1.3
Hematocrit (%)	36-50	39.4 ± 3.6	40.9 ± 4.2	42.1 ± 3.8	40.8 ± 4.5

* Significantly different versus control at baseline (0 weeks) ($p<0.05$)

Data are means ± standard deviations

Abbreviations: LDC, low dose control; LDFSP, low dose freeze-dried strawberry powder; HDC, high dose control; HDFSP, high dose freeze dried strawberry powder; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1c, hemoglobin A1c; BUN, blood urea nitrogen; LDL, low density lipoprotein; HDL, high density lipoprotein; VLDL, very low density lipoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; WBC, white blood cell; RBC, red blood cell

TABLE 9. Anthopometric measures, blood pressure, glucose control, lipid panel, and biomarkers of lipid oxidation after 12 week supplementation with FSP or control treatment

	Desirable Range[4]	Low-Dose Control (n=9)		Low-Dose FSP (n=9)		High-Dose Control (n=11)		High-Dose FSP (n=11)	
		0 week	12 weeks	0 week	12 weeks	0 week	12 weeks	0 week	12 weeks
BMI (kg/m^2)	18.5-24.9	37.5 ± 5.1	37.2 ± 5.2	35.5 ± 4.9	35.5 ± 4.8	34.8 ± 5.7	34.7 ± 5.7	40.1 ± 7.2	39.8 ± 7.8
Waist (inches)	Men <40 Women <35	43.2 ± 3.7	42.9 ± 3.2	41.5 ± 3.5	41.5 ± 3.8	41.9 ± 2.9	42.3 ± 3.4	46.0 ± 5.0	45.9 ± 4.9
SBP (mmHg)	<120	128.3 ± 12.4	130.3 ± 8.7	125.0 ± 11.3	126.1 ± 14.6	131.3 ± 12.1	131.6 ± 8.3	136.5 ± 10.1	134.1 ± 12.3
DBP (mmHg)	<80	87.7 ± 6.1	85.4 ± 3.5	80.6 ± 7.6	77.9 ± 8.5*	82.9 ± 3.96	84.6 ± 4.7	86.5 ± 9.0	82.8 ± 8.4
Glucose (mg/dL)	90-130	103.7 ± 12.7	95.7 ± 8.8	97.1 ± 11.8	92.7 ± 14.9	91.5 ± 16.7	93.5 ± 18.5	92.7 ± 15.5	91.2 ± 14.8
HbA1c (%)	4.5-6	5.9 ± 0.8	5.9 ± 0.6	5.6 ± 0.3	5.6 ± 0.4	5.9 ± 0.6	5.7 ± 0.4*	5.8 ± 0.5	5.7 ± 0.7
TC (mg/dL)	<200	207.2 ± 22.1	202.0 ± 40.4	204.9 ± 51.4	208.1 ± 58.9	213.5 ± 36.9	214.1 ± 37.7	215.3 ±27.96	193.8 ± 22.96
LDL (mg/dL)	<100	133.1 ± 18.4	131.8 ± 25.6	121.6 ± 44.0	127.2 ± 57.5	132.0 ± 33.2	129.2 ± 31.3	138.5 ± 28.7	114.2 ± 26.6
HDL (mg/dL)	Men >40 Women >50	49.9 ± 12.7	49.1 ± 17.5	47.8 ± 19.4	47.7 ± 21.2	45.2 ± 9.6	48.0 ± 10.5	45.4 ± 17.1	44.6 ± 11.4
VLDL (mg/dL)	5-40[3]	22.4 ± 8.2	23.4 ± 12.9	34.0 ± 18.0	29.4 ± 12.1	34.5 ± 14.8	36.4 ± 13.8	37.1 ± 14.1	36.6 ± 10.3
TG (mg/dL)	<150	110.4 ± 44.8	115.7 ± 65.8	170.3 ± 90.6	147.1 ± 60.7	172.8 ± 73.7	173.5 ± 56.9	185.5 ± 71.5	180.6 ± 53.1
OX-LDL (U/L)		136.2 ± 7.4	127.2 ± 7.6*	127.8 ± 19.9	119.9 ± 17.8	128.5 ± 16.3	121.1 ± 17.2*	137.1 ± 13.8	121.7 ± 9.2*
MDA (uM)		2.7 ± 0.7	2.4 ± 0.6*	2.4 ± 0.6	2.3 ± 0.5	2.6 ± 0.5	2.3 ± 0.4*	3.0 ± 0.6	2.2 ± 0.4*

*Significantly decreased at 12 weeks compared to baseline ($p<0.05$)

Data are means ± standard deviations

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1c, hemoglobin A1c; TC, total cholesterol; LDL, low density lipoprotein; HDL, high density lipoprotein; VLDL, very low density lipoprotein; TG, triglycerides; ox-LDL, oxidized low density lipoprotein; MDA, malondialdehyde

TABLE 10. Change in variables after 12 week supplementation of FSP or control

	Δ (0-12 wk)			
	LDC (n=9)	**LDFSP (n=9)**	**HDC (n=11)**	**HDFSP (n=11)**
BMI (kg/m^2)	0.29 ± 1.34	0.02 ± 3.76	0.02 ± 6.76	0.27 ± 2.89
Waist (inches)	0.28 ± 0.75	-0.03 ± 2.12	-0.41 ± 4.62	0.10 ± 3.84
SBP (mmHg)	-2.00 ± 8.96	-1.11 ± 8.02	-0.36 ± 16.48	2.45 ± 17.47
DBP (mmHg)	2.22 ± 5.12	2.67 ± 2.65*	-1.73 ± 5.64	3.72 ± 13.93
Glucose (mg/dL)	9.11 ± 13.07	4.44 ± 8.65	-1.91 ± 10.89	1.55 ± 10.46
HbA1c (%)	0.04 ± 0.35	0.05 ± 0.28	0.22 ± 0.27	0.08 ± 0.53
TC (mg/dL)	5.22 ± 26.99	-3.22 ± 54.89	-0.55 ± 52.22	21.45 ± 35.57
LDL (mg/dL)	1.33 ± 23.62	-5.67 ± 45.14	2.82 ± 42.78	24.27 ± 38.35
HDL (mg/dL)	0.78 ± 7.03	0.11 ± 4.01†	-2.82 ± 14.16	0.73 ± 10.61
VLDL (mg/dL)	-1.00 ± 9.15	4.56 ± 14.81	-1.82 ± 18.47	0.45 ± 10.72
TG (mg/dL)	-5.22 ± 45.97	23.22 ± 74.74	-0.64 ± 85.60	4.91 ± 54.60
OX-LDL (U/L)	9.00 ± 4.18	7.89 ± 20.09	7.45 ± 9.36	15.36 ± 11.13
MDA (uM)	0.28 ± 0.22	0.12 ± 0.59†	0.35 ± 0.28	0.79 ± 0.55*

* Significantly decreased compared to control ($p<0.05$)

† Decrease was significantly lower compared to control ($p<0.05$)

Data are means ± standard deviations

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1c, hemoglobin A1c; TC, total cholesterol; LDL, low density lipoprotein; HDL, high density lipoprotein; VLDL, very low density lipoprotein; TG, triglycerides; ox-LDL, oxidized low density lipoprotein; MDA, malondialdehyde

CHAPTER V

DISCUSSION

General findings

Diastolic blood pressure significantly decreased in the low dose strawberry group from baseline to 12 weeks. When analyzing the change over the 12 week period in the low dose strawberry group, diastolic blood pressure significantly decreased when compared to the low dose control.

High dose strawberry supplementation significantly decreased biomarkers of lipid oxidation, oxidized-LDL and serum malondialdehyde (MDA), at 12 weeks versus baseline.

In addition, high dose strawberry supplementation also revealed a decreasing trend in total and LDL cholesterol. When assessing the 12 week change in the high dose strawberry group, serum MDA significantly decreased when compared to the high dose controls.

Freeze-dried strawberries

In our study, subjects in the low dose strawberry group were supplemented with 25 grams freeze-dried strawberries, equivalent to 250 grams of fresh strawberries, or approximately 1.5 cups of sliced strawberries daily for 12 weeks. Subjects in the high dose strawberry group consumed 50 grams freeze-dried strawberries, equivalent to 500 grams of fresh or 3 cups of sliced strawberries daily for 12 weeks.

The freeze-dried strawberry powder (FSP) was obtained from the California Strawberry Commission (Watsonville, CA) and was approximately 10% fresh weight of strawberries and had no added ingredients. The FSP doses administered were within the current American Heart Association recommendations of at least 5 cups of fruits and vegetables per day for adults and provided a concentrated source of strawberry polyphenols, fiber, and micronutrients.[1] Furthermore, the strawberry doses selected were based on previous clinical trials that have shown health benefits in healthy volunteers or those with CVD risk factors when supplemented with fresh strawberry doses ranging from 100 to 500 grams. [26,32,33,35]

Fresh strawberries are processed to various products, such as frozen and dried fruits, jams, jellies, puréed, and juice concentrates, in order to enhance shelf life. Freeze-drying is a drying technique that significantly retains polyphenol and anthocyanin content of strawberries when compared to other methods such as, microwave, convection, and vaccum-drying.[38] A study conducted by Marques et al.[39] reported that freeze-dried strawberries have higher total antioxidant activities when compared to fresh or frozen berries. In our study, freeze-dried strawberries were well tolerated by participants; FSP also provided a palatable and convenient source of delivery of berry nutrients and polyphenols in order to obtain the health benefits of daily fruit consumption.

Strawberries and anthropometric and blood pressure measures

Anthropometric measures (body mass index (BMI) and waist circumference) did not significantly change from baseline to 12 weeks as a result of strawberry intervention, thus our study findings did not support our hypothesis. Similarly, other research studies using similar doses did not show any significant changes in BMI or waist circumference after long term strawberry intervention. [26, 31-33]

However, we saw a significant decrease in diastolic blood pressure (DBP) in the low dose strawberry group versus the low dose control group whereas there was no change in systolic

blood pressure (SBP). In contrast to these null findings in our study, two studies that analyzed the effects of berry consumption in blood pressure showed a significant decrease in SBP: one was conducted in middle-aged subjects with cardiovascular risk factors consuming a mixed berry intervention[31] and the other was conducted in healthy men consuming cranberry juice.[40] It is possible that there are many different factors contributing to these differences. First, in the study conducted by Erlund et al.[31] , the intervention consisted of 100 grams mixed berries plus 1 small glass of mixed berry drink each day whereas our study intervention was only one single type of berry and a lower dosage in both intervention groups. Using mixed berries may have led to higher and different profiles of polyphenols and micronutrients compared to our intervention, which may have a positive impact in blood pressure. Secondly, the sample size in the study by Erlund et al.[31] was double the number in our sample size. In addition, although in both studies subjects were prehypertensive, subjects in our study had much higher BMI values compared to the previous study. Since obesity is positively associated with hypertension,[4] a greater dose of strawberry intervention and/or longer study duration might produce greater effects in blood pressure than what was observed in the present study in obese subjects.

Strawberries, glucose and hemoglobin A1c

In our study, glucose and hemoglobin A1c concentrations were not significantly affected by low or high dose strawberry supplementation. In an acute postprandial study conducted by Törrönon et al.[34], berry purée (150 gram mix of bilberries, blackcurrants, cranberries, and strawberries, and sweetened with 35 grams sucrose) supplementation did not show a statistically significant difference in the postprandial area under the glucose response curve, but results indicated a decreased glucose response due to berry consumption. The duration of the study by Törrönon and colleagues was very short compared to our study; the sample size was also considerably smaller and the baseline characteristics of the subjects were relatively normal, all of these factors could contribute to the differences versus our findings.

Strawberries and lipid profile

There were no statistically significant differences in lipid profiles at 12 weeks versus baseline in the low or high dose strawberry groups. Similarly, when the 12 week change was analyzed no statistical significance was shown for either intervention group. Despite the fact that total and LDL cholesterol did not show a statistically significant decrease at 12 weeks versus baseline, both showed a decreasing trend in the high dose strawberry group, which may have a positive biological relevance. The desirable range for TC is less than 200 mg/dL [4] and although not significant, the mean TC reduced to levels below the desirable range after high dose strawberry intervention. The ideal range in LDL cholesterol is less than 100 mg/dL [4] and after high dose strawberry supplementation mean LDL reduced to levels closer to the desired range. These findings in total and LDL cholesterol suggest that a larger sample size, a longer duration and/or a more concentrated source of purified strawberry anthocyanins might exert more significant changes in these lipid parameters than what was observed in the current study.

Conflicting results related to the effects of high and low dose strawberry intervention in lipid profile have been reported in other clinical trials. In two clinical studies by Basu et al.[32,33], serum total and LDL-cholesterol levels significantly decreased from baseline and no significance was found in other lipid profile variables. The previous study by Basu et al.[32] did not have a control group so the results should be interpreted with caution compared to other studies that reported significance with respect to a control group. In addition, another clinical study by Basu and colleagues[33] reported a significant decrease in total and LDL cholesterol in patients consuming high doses of strawberry supplementation (50g) for 8 weeks when compared to a non-intervention control group. In our study, total and LDL cholesterol showed a mean decreasing trend from baseline after 12 weeks of high dose strawberry supplementation. The baseline characteristics of the participants in all three studies were relatively similar and participants in all three studies had two out the three criteria that classifies metabolic syndrome.[5] Other similarities

include, the doses of intervention and form of intervention were the same throughout (50g FSP). Our 12 week study duration was longer than both of the previous clinical studies.

However, in a study by Erlund et al.[31] berry intervention did not significantly decrease TC, but it did significantly increase HDL cholesterol concentrations compared to control. Supplementing mixed berries could be the explanation behind the significant increase in HDL, which is not seen in other studies using a single variety of berry intervention. In another study by Jenkins et al.[26], strawberry supplementation (454 g/d) did not show any significant differences in lipid profile parameters compared to a oat bran bread/fiber control. The strawberry intervention in this study was different from ours; participants consumed 1 pound of fresh strawberries per 2000 kcals as part of their daily meal. Similarities between the study by Jenkins et al.[26] and our study include, doses were similar in both, subject were hyperlipiemic, and the controls both consisted of fiber. Larger clinical trials may be helpful in interpreting the effects of high and low dose strawberry supplementation in lipid profiles.

Strawberries and biomarkers of lipid oxidation

The most notable finding of our study was the significant decrease in serum malondialdehyde (MDA) in the high dose strawberry group compared to high dose controls. Also, we observed a statistically significant decrease in oxidized-LDL (ox-LDL) and serum MDA in the high dose strawberry group at 12 weeks versus baseline. Oxidized LDL is a key factor in the development and progression of atherosclerosis.[10] It has been suggested that ox-LDL enhances monocyte accumulation in the subintimal space as well as increasing transition from macrophages to foam cells.[10] A significant decrease in ox-LDL might slow the progression of atherosclerosis.[10] MDA plays a key role in the cellular and DNA damage, so similarly decreases in serum MDA may help lessen or alleviate oxidative stress.[15]

The reduction in ox-LDL and MDA and the increase in antioxidant capacity by freeze-dried strawberry supplementation, which was observed in our study, are consistent with previous study results.[26, 32,35] In a study by Basu et al.[32], MDA significantly decreased in women with metabolic syndrome at 4 weeks versus baseline. In addition, ox-LDL showed a decreasing trend at 4 weeks. Subjects in the study by Basu and colleagues consumed a daily dose of 50g freeze-dried strawberry powder throughout the 4 week study duration. While the doses consumed are in comparison with the present study, the study duration was about one-third of our study, which is a possible reason as to why our results showed a significant decrease in ox-LDL from baseline and a significant between group difference in MDA. The previous study did not have a control group and the duration was only 4 weeks, which could be a factor as to why significance was not found in ox-LDL.

In a study conducted by Jenkins and colleagues[26], 28 hyperlipidemic subjects were supplemented with the same amount of strawberries and fiber-calorie matched control used in the current study. Following a 4 week intervention, the authors concluded that strawberry supplementation significantly decreased oxidative damage to LDL cholesterol, while also maintaining a decrease in lipid profile variables. Consumption of flavonoid rich strawberries, which have a high polyphenolic content, have also been shown to reduce ox-LDL and MDA concentrations, but increase antioxidant capacity.[32,35] A study conducted by Henning et al.[35] was designed to determine the effects of a 3 week 250g strawberry intervention in oxidative DNA damage and antioxidant capacity. The authors concluded that LDL oxidation lag time increased by 20% and antioxidant capacity demonstrated a modest, but significant increase. Evaluating lipid oxidation lag time has been shown to be a reliable marker to analyze the antioxidant activities of different dietary compounds. This study was only 3 weeks compared to our current 12 week study and doses of strawberry intervention were equal to our low dose strawberry groups supplementation. Oxidative stress and inflammation pathways can function synergistically, so a

decrease in markers of inflammation could possibly decrease markers of oxidative stress and vice versa. This may be an explanation as to why the fiber control also showed a significant decrease in ox-LDL and MDA at 12 weeks versus baseline. Thus, the polyphenols and fiber in the strawberries might exert a synergistic effect in producing greater decreases in biomarkers of lipid oxidation compared to fiber controls. This remains to be confirmed in future research.

Limitations

Major limitations in this current study include the small sample size as well as the duration of the study. The small sample size can also be linked to the large standard deviations, because a smaller sample size can lead to an increased variability between participants.

In the current study we did not control for subjects physical activity and we did not ask participants to make any dietary changes throughout the study duration, which could be a reason we did not find any significant differences in BMI or waist circumference.

Another limitation could be that a fiber-containing beverage was used in the control groups versus an inactive control beverage. The control beverage was matched for fiber, which has some known effects in lipids. However, we addressed the research question if strawberry polyphenols affect metabolic variables and lipid oxidation beyond the known effects of fruit fibers.

In the current study we measured compliance by participants coming in to our office to collect their respective beverages twice per week. We did not measure urinary anthocyanins, which is probably a better measure of compliance because of the feasibility and availability of the resources.

Conclusion

In our study, high dose freeze-dried strawberry supplementation significantly decreased MDA compared to high dose controls. Also, high dose freeze-dried strawberry supplementation along with low and high dose controls significantly decreased biomarkers of lipid oxidation, oxidized-LDL and malondialdehyde (MDA), at 12 weeks versus baseline. Diastolic blood pressure significantly decreased in the low dose strawberry group (1) at 12 weeks versus baseline and (2) compared to low dose controls.

Strawberries were found to exert cardioprotective effects in subjects with abdominal adiposity and dyslipidemia in the current study. The AHA recommends eating 8 or more fruits and vegetable servings per day and an average adult who consumes about 2,000 kcals/day should aim for about 4.5 servings per day.[1] Therefore, increased berry consumption should be encouraged as part of a heart healthy diet. Furthermore, it should be promoted in populations with low fruit and vegetable intake and in those at a high risk of cardiovascular disease. Future research should be focused on increasing the sample size as well as study duration. Further studies comparing commercially available strawberry products to freeze-dried strawberry powder (used for research purposes only) may help confirm their effects in lipid profile and biomarkers of lipid oxidation in subjects at risk for cardiovascular disease.

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APPENDICES

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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.

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What Is Involved In The Study?

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

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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

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IRB No.:15109

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

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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

VITA

Catherine Kelly Curd

Candidate for the Degree of

Master of Science

Thesis: EFFECTS OF STRAWBERRIES ON LIPID PROFILES AND BIOMARKERS OF LIPID OXIDATION IN SUBJECTS WITH DYSLIPIDEMIA AND ABDOMINAL ADIPOSITY

Major Field: Nutritional Sciences

Biographical:

Education:

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

Completed the requirements for the Bachelor of Science in Dietetics and Nutrition at University of Mississippi, Oxford, Mississippi in 2011.

Professional Memberships:
American Dietetic Association 2009-2011
Academy of Nutrition and Dietetics 2011-2013
American Society for Nutrition 2012-2013