

THE EFFECT OF FREEZE-DRIED STRAWBERRY  
POWDER ON LIPID PROFILE AND MARKERS  
OF OXIDATIVE STRESS IN WOMEN WITH  
METABOLIC SYNDROME

By

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## CHAPTER I

### INTRODUCTION

#### Obesity and Metabolic Syndrome

Obesity is now considered a public health epidemic in the United States and throughout the world. The term overweight is usually defined as body mass index (BMI) greater than  $25 \text{ kg/m}^2$  and obesity is defined as BMI greater than  $30 \text{ kg/m}^2$ . BMI takes into account an individual's weight based on their height (1). The rates of overweight and obesity in the United States are increasing rapidly. Data collected through the CDC's Behavioral Risk Factor Surveillance System (BRFSS) showed that in 2007 all but one state in the U.S. had an obesity rate above 20% (2). In 1990, the rates were much lower; the prevalence of obesity in all states was below 15% and ten states had a prevalence of below 10% (3). Globally, the prevalence of obesity has also been increasing at an alarming rate. In 2005, the World Health Organization estimated that 400 million adults were obese (1), not taking into account the growing numbers of children that are obese or overweight as well.

Obesity occurs primarily as a result of an imbalance in calories consumed and calories expended (4). This is often a consequence of the combination of over consumption of energy dense foods, which are high in calories and often low in nutrients, and low physical activity. Some medications, however, can also cause weight gain (4).



Underlying factors of the development and increased rates of obesity include social interactions, socioeconomic status, and culture, which may have a determining effect on energy intake and physical activity levels (4). Obesity itself poses health risks and also places individuals at risk for other chronic diseases including cardiovascular diseases, type 2 diabetes mellitus, osteoarthritis, and some cancers (1). As the prevalence of obesity increases, the prevalence of these chronic diseases is also on the rise.

Chronic diseases such as type 2 diabetes and cardiovascular diseases have become increasingly prevalent in the United States as well as around the globe. A constellation of metabolic risk factors, which has been found to put individuals at greater risk for developing cardiovascular disease and type 2 diabetes, has been collectively termed metabolic syndrome (5). A diagnosis of metabolic syndrome requires that an individual meet three of the following five diagnostic criteria: a large waist circumference ( $\geq 35$  inches in women or  $\geq 40$  inches in men), elevated serum triglycerides ( $\geq 150$  mg/dl), low serum high density lipoprotein, or HDL ( $\leq 50$  in women or  $\leq 40$  in men), elevated blood pressure ( $\geq 130$  mg/dl systolic or  $\geq 85$  diastolic), or elevated fasting glucose ( $\geq 100$  mg/dL) (5).

It has been indicated that chronic disease may be prevented by a diet high in fruits and vegetables, and specifically by the action of the non-nutrient compounds present in the fruits and vegetables, called phytochemicals (6). This may be due, in part, to the antioxidant content and function of these phytochemicals. Epidemiological evidence has revealed that a healthy diet high in fruits and vegetables is associated with lower prevalence of cardiovascular risk factors such as those found in metabolic syndrome (7)

and may be beneficial for the prevention of cardiovascular disease (8, 9). Many individuals, however, do not consume the recommended amounts of fruits and vegetables in their daily diet. The Dietary Guidelines for Americans recommend that adults consume 5-13 servings (about 2 ½ to 6 ½ cups) of fruits and vegetables per day with 2-4 servings coming from fruit and 3-8 servings coming from vegetables depending on the individuals caloric intake (10).

### Oxidative Stress

Large amounts of dietary antioxidants can combat oxidative stress. Oxidative stress is due to the imbalance of free radical production and antioxidant status in the body and can lead to oxidative damage (11). Free radicals are molecules containing unpaired electrons, which causes them to be highly reactive (12). Because of the reactive nature of free radicals, if there is excess free radical production and inadequate antioxidants to neutralize them, the free radicals can then cause oxidative damage to cells and tissues of the body (11). Forms of oxidative stress include the oxidative modification of LDL in the blood as well as lipid peroxidation of the polyunsaturated fats in cell membranes (13, 14). Antioxidants are substances that scavenge and neutralize free radicals (12), such as reactive oxygen species (ROS), to prevent the oxidative damage to tissues and cells they cause (15). Many dietary substances can function as antioxidants including vitamins A and E, some minerals, and the phytochemicals found in plant foods.

### Biomarkers of Oxidative Stress

Oxidative stress in the body can be measured by specific biomarkers, such as oxidized low density lipoprotein (LDL), malondialdehyde (MDA) and 4-hydroxyalkenals (HAE), and myeloperoxidase (MPO), which were utilized in the current study. Oxidized LDL is utilized as a measure of oxidative stress because it has been determined to be contributing factor in the development of atherosclerosis (13). MDA is an aldehyde formed as a product of peroxidized polyunsaturated fatty acids and it is often used to measure lipid peroxidation as an indicator of oxidative stress in the body, as lipid peroxidation is one type of damage caused by the action of free radicals (14). MPO is an enzyme released during inflammation to catalyze the formation of reactive species which can also cause oxidative damage and lead to the development of atherosclerosis (16).

Metabolic syndrome, as well as obesity itself, is associated with increased oxidative stress. Among participants in the Cardiovascular Risk Development in Young Adults Study, oxidized LDL was significantly positively associated with BMI and with features of metabolic syndrome including elevated waist circumference, blood glucose, and blood pressure (17). Oxidized LDL was also found to be negatively associated with an HDL cholesterol level, which is important, as low HDL is also a feature of metabolic syndrome. Recent research has also indicated that increased MDA levels are associated with obesity and metabolic syndrome. A study by Demircan et al. (18) revealed that individuals newly diagnosed with metabolic syndrome had significantly higher levels of plasma MDA compared with healthy controls. In addition, individuals with metabolic syndrome had significantly higher waist circumferences than the healthy controls (18).

In adults free of cardiovascular disease, MPO levels above the median levels were reported to be associated with higher BMI, increased LDL, higher blood pressure, and lower HDL when compared to below median MPO levels. Higher MPO levels were also found to be associated with increased likelihood of CVD event occurrence (19).

### Phytochemicals

Phytochemicals, literally “plant chemicals,” are bioactive compounds found in plants which are not nutrients but may confer additional health benefits over that of the nutrients present in foods (20). These phytochemicals can be divided into several categories including carotenoids, phenolics, alkaloids, nitrogen-containing compounds, and organosulphur compounds (20). One of the largest categories of phytochemicals is phenolic compounds. Phenolic compounds are plant metabolites that are very sensitive and vulnerable to change because they are easily oxidized (21). The structure of phenolic compounds includes one or more aromatic rings with attached hydroxyl groups. They are found in many fruits and vegetables but are especially abundant in berry fruits (22).

### Strawberry Phytochemicals

Strawberries are excellent sources of phytochemicals, especially phenolic compounds. Strawberries obtained from the California Strawberry Commission were analyzed and found to contain tannins (gallotannin, ellagitannin, and ellagic acid), anthocyanins (pelargonidin and cyanidin), flavonol glycosides (quercetin and kaempferol glycosides), flavonols (catechin), and hydroxycinnamic acid (22). In another study, Zhang et al. (23) isolated and identified strawberry phenolics as cyanidin, pelargonidin, kaempferol,

quercetin, 3,4,5-trihydroxyphenyl-acrylic acid, glucose ester of (*E*)-*p*coumaric acid, and ellagic acid. The most abundant phenolic compounds present in strawberries are the anthocyanins and ellagitannins (24). Anthocyanins are a part of a subgroup of polyphenols called flavonoids (25, 26). Anthocyanins are usually found as glycosides; the three common aglycone types, called anthocyanidins, are cyanidin, delphinidin, and pelargonidin (27). Ellagitannins are derivatives of ellagic acid (25). In the presence of an acid or base, ellagitannins are hydrolyzed to hexahydroxydiphenic acid which is then converted to ellagic acid (25, 28).

Many fruits and vegetables contain high amounts of antioxidants. The major antioxidant in strawberries is ascorbic acid, or vitamin C, however, polyphenols are also significant contributing sources of the antioxidant capacity of the fruit, the largest of which are anthocyanins and ellagitannins (24). The antioxidant capacity and content of fruits and vegetables can be assessed by various methods.

Hypotheses.

For the present study, it was our hypothesis that:

1. Freeze-dried strawberry powder will reduce oxidative stress in subjects with metabolic syndrome.
2. Freeze-dried strawberry powder will improve lipid profiles in subjects with metabolic syndrome.
3. Freeze-dried strawberry powder supplementation will not cause changes in safety parameters including kidney and liver function and the complete blood count in subjects with metabolic syndrome.

## CHAPTER II

### REVIEW OF LITERATURE

#### Antioxidant Properties of Strawberries

The oxygen radical absorbance capacity (ORAC) can be used to evaluate the antioxidant function in foods as a measure of the inhibition of free radicals (15). Among commonly eaten fruits examined in the study by Wang et al. (15), strawberries were found to have the highest ORAC activity on the basis of the wet weight (edible portion) of the fruit as well as by dry weight. Other fruits tested were orange, apple, pink grapefruit, plum, red grape, white grape, kiwi fruit, banana, tomato, pear, and, honeydew melon. Although some of the fruits measured had relatively high vitamin C content, the estimated contribution of vitamin C to the total ORAC is low, being generally less than 15% of total ORAC activity of the fruits. These fruits also contain flavonoids which have been shown to possess antioxidant activity and may contribute a large portion of the fruits' total ORAC activity (15).

Cellular antioxidant activity (CAA), which is a measure of cytotoxicity, is another measure of antioxidant capacity. This method was used in a study by Wolfe et al. (12), in evaluating the CAA of 25 commonly eaten fruits in the United States. Among those fruits, the common supermarket strawberry had the third highest CAA value, following blueberries and cranberries. In addition, the blueberries, cranberries, and strawberries did

not have significantly different ORAC scores, indicating that the fruits are similarly effective in their ability to scavenge peroxy radicals. Strawberries contained a moderately high phenolic content with 235 mg +/- 6 mg of phenolics (GAE) per 100 g fruit. The quality of the antioxidants in strawberries was also found to be high, when calculated from the amount of antioxidants present and their effectiveness in neutralizing free radicals. Based on the USDA food consumption data, it was determined that strawberries are one of the largest contributors of fruit phenolics in the typical American diet, after apples, oranges, and grapes (12).

In a sample of 1113 foods provided by the USDA National Food and Nutrient Analysis Program, the ferric reducing ability of plasma (FRAP) was used to determine the antioxidant capacity and content of each food (28). These foods were sampled so as to be nationally representative of foods eaten across the US and included a variety of food groups including fruits, vegetables, grains, nuts, meats, dairy, sweets, and others. Of all foods examined, strawberries were determined to have the third highest antioxidant content per serving (about 3.6 mmol antioxidants per 1 cup, or 166 grams, sliced strawberries). They were also among the top 50 foods having the highest antioxidant content per 100 grams (28).

The antioxidant capacity of fruits and vegetables, along with their high nutrient content, are important for human health and may reduce the risk of chronic disease. Many studies have been conducted to determine the relationship of fruit and vegetable intake and chronic disease as well as the mechanisms behind their effect on human health. Research



has also begun to examine the role of specific phytochemicals in health and chronic disease. Because strawberries are widely consumed and have been found to possess high antioxidant activity, they may be especially beneficial for improving the health of individuals in the United States.

#### Bioavailability of Anthocyanins

Anthocyanins are the one of the most abundant phytochemicals found in strawberries (24). The absorption, metabolism, and specific functions of anthocyanins in the body, though, are not fully understood. Although anthocyanin metabolism in the human body has not been studied extensively, a few details on their metabolism are available. For one, anthocyanins have been found to be metabolized rapidly into phenolic acids under simulated physiological conditions. Anthocyanins may demonstrate low bioavailability because of their instability and metabolism in the body, where pH and temperature are the major contributing factors to their degradation (27).

The metabolism of both of the major anthocyanins present in strawberries, cyanidin and pelargonidin, has been experimentally evaluated. In intestinal epithelial cell culture studies, cyanidin-3-glucoside (C3G) and cyanidin were shown to metabolize quickly to protocatechuic acid (PCA) and phloroglucinaldehyde (PGA). The same degradation most likely occurs in the human intestine and these degradation products may be further metabolized by intestinal epithelial cells (30).

The metabolism of pelargonidin, the second major strawberry anthocyanin, has been studied in rats as well as humans. After oral administration in rats, aglycone pelargonidin and its metabolites were detected in plasma after 2 hours but none were detected at 18 hours. The major form identified in the plasma was pelargonidin gluconuride. At 2 hours, this metabolite was also the major form detected in the kidney and liver while aglycone pelargonidin was found in the brain and lungs (31).

In humans, after consumption of a meal including thawed, deep-frozen strawberries, urine samples were collected 6 times during the following 24 hours (32). In the strawberries used, pelargonidin-3-glucoside was identified as the major anthocyanin present. No other polyphenol rich foods were consumed in the meal prior to or at any time during the 24 hour study. Several forms of pelargonidin metabolites were identified in the urine including pelargonidin monoglucuronide, pelargonidin-3-glucoside, pelargonidin sulfate, and aglycone pelargonidin. The majority of anthocyanins and metabolites excreted were pelargonidin monoglucuronide, with a small amount excreted as pelargonidin-3-glucoside. Of the anthocyanins and metabolites excreted, two-thirds was excreted within the first 4 hours and the amount of total excreted anthocyanins was 1.8% of the ingested amount from the strawberries (32), suggesting that strawberry anthocyanins are poorly bioavailable and undergo rapid plasma clearance.

## **Human Studies**

### Epidemiological Studies

It is evident from a large body of research that fruits and vegetables are an important part of a healthy diet, and may reduce the risk of chronic diseases. The Swedish INTERGENE research program (7) was conducted to examine the relationship between food patterns and risk factors for cardiovascular disease, especially those associated with metabolic syndrome, in adult men and women. High intake of fruits and vegetables was determined to be an important part of a healthy diet pattern which also consisted of higher intakes of low fat dairy, high fiber bread, fish, tea, nuts, and legumes along with lower intakes of coffee, soft drinks, sugar, pastry, fast food, meat, and full or medium fat milk products. This healthy diet pattern was found to be associated with a significantly lower BMI and waist to hip ratio than the other patterns, and a higher HDL than the traditional diet. However, another variable influencing the cardiovascular risk was that individuals in the healthy diet group smoked less and exercised more than the rest of the sample population (7).

A study by Esmailzadeh et al. (33), evaluated the relationship between fruit and vegetable intake and the prevalence of metabolic syndrome in 486 adult women. Fruit and vegetable consumption was measured through food frequency questionnaires. The highest quintiles of both fruit and vegetable intake were associated with reduced risk of metabolic syndrome. Individuals within the highest quintile of fruit intake, with mean consumption of  $362 \pm 6$  grams of fruit per day, had a 34% lower risk of metabolic

syndrome. Individuals within highest quintile of vegetable intake, with a mean intake of  $307 \pm 9$  grams per day, had a 30% lower chance of having metabolic syndrome (33).

In the EPIC Norfolk Cohort Study (8), an inverse relationship was found between fruit and vegetable intake, assessed by a 7 day food diary, and the occurrence of ischemic heart disease in 12,474 adult men and women. Ischemic heart disease is the result of a lack of blood flow, and therefore lack of oxygen, delivered to the heart causing damage to the heart muscle. The researchers also noted an inverse relationship with ischemic heart disease and plasma ascorbic acid level, which was used as a biomarker of fruit and vegetable intake (8).

Results from food frequency questionnaires collected as components of the Nurses' Health Study and the Health Professionals' Follow-up Study revealed that a high intake of fruits and vegetables, especially green leafy vegetables, is associated with a slightly reduced risk of cardiovascular disease (34). In the same populations, both fruit and vegetable intake separately reduced the risk of coronary heart disease. This was especially true for consumption of green leafy vegetables, cruciferous vegetables and fruits and vegetables that are high in vitamin C (9).

Consumption of fruits and vegetables may also lower the risk of acute myocardial infarction, or heart attack. The INTERHEART Study (35) was structured to identify the relationship between dietary patterns and acute myocardial infarction among 52 countries in various regions around the world. In this study, a prudent diet was identified to be

high in fruits and vegetables. This diet was found to significantly reduce the risk of acute myocardial infarction when compared to western or oriental diets. A Western diet was characterized by greater consumption of fried foods, salty snacks and meats while the Oriental diet was characterized by high intakes of tofu and soy sauce (35).

Low fruit and vegetable intake is a national problem as well as a global problem. In a study conducted by the World Health Organization (WHO), it was determined that increasing fruit and vegetable consumption to an adequate level could reduce ischemic heart disease by 31% and ischemic stroke by 19% worldwide (36). Specific phytochemicals in fruits and vegetables have been identified to be important elements in a healthy diet. Flavonoids, in particular, are related to a reduced risk of cardiovascular disease. Two studies, the Women's Health Study and the Iowa Women's Health Study, examined this relationship. Food frequency questionnaires were completed by 38,445 women participating in the Women's Health Study, from which their flavonoid intake was calculated. This study by Sesso et al. (37) did not show an association of flavonoid intake with reduced CVD risk; however, strawberry intake was not included in the assessment as a measure of flavonoid consumption. In a more recent study, the specific effect of strawberry intake on CVD risk was examined (38). In a subset of 26,966 women from the Women's Health Study (38,176 total women), the association of strawberry intake with blood lipids and C-reactive protein (CRP) was assessed. Although little association was found for the intake of strawberries and risk of CVD, it was found that women with strawberry intake of  $\geq 2$  servings were less likely to have an elevated C-

reactive protein level, which is a risk factor for CVD. This was strengthened when adjusted for clinical, lifestyle, and dietary factors (38).

Although, strawberry intake may not be associated with the reduction of CVD occurrence, it may reduce the risk CVD mortality. In the Iowa Women's Health Study, including 34,489 women, the relationship of flavonoid intake and CVD mortality was examined. (39). Data collected through food frequency questionnaires revealed that anthocyanin intake, when compared with no anthocyanin intake, was significantly associated with reduced CHD and CVD mortality as well as total mortality. Specifically, strawberries were found to be associated with a significant reduction in CVD mortality with consumption of one serving per week or more as compared to consuming none (39).

While consumption of all fruits and vegetables is related to increased health, strawberries may confer specific benefits for CVD risk factors. It is evident from these studies that flavonoid intake, from sources such as strawberries may reduce CVD risk and that strawberry intake itself may have beneficial effects on CVD risk factors. These observational data suggest the need for interventional studies to investigate the specific physiological function of strawberries in human health and disease. The effects of diets high in strawberries on features of metabolic syndrome have not yet been investigated.

### Clinical Studies

Existing studies have examined the effects of particular phytochemicals, such as those found in strawberries, on human health. The effects of anthocyanins on oxidative stress

were measured in a study conducted by Weisel et al. (40), using an anthocyanin rich juice, consisting of red grape juice, blackberry juice, sour cherry juice, black currant juice, and chokeberry juice. Healthy males were given the anthocyanin rich juice or a control juice, in which the phenolic compounds had been removed, for a period of 4 weeks. The participants consumed 700 ml of the juice daily which contained 197.9 mg/L anthocyanins. Following supplementation, the amount of total DNA damage, as determined by Comet assay, was significantly reduced by the anthocyanin rich juice. MDA levels, however, remained unchanged (40).

Not enough is known about the impact of strawberries alone on human health and disease. This is partly due to the fact that there is very limited controlled research specifically studying on the effect of strawberry intake on health and disease in humans. In a study by Naemura et al. (41), fresh strawberries were found to have an anti-thrombotic effect. Thrombosis refers to the formation of blood clots within the vessels, causing a loss of blood flow and reduced oxygen supply to the area. Healthy male and female subjects were given two oral doses of filtered strawberry juice (7.7 ml/kg body weight) 30 minutes apart. Blood samples drawn before and after filtered strawberry juice supplementation revealed that relative values of occlusion time were significantly reduced by the strawberries (41).

Strawberries may also confer health benefits through their antioxidant activity. A study conducted by Jenkins et al. (42) measured the effect of dietary supplementation with fresh strawberries on blood lipids. During the one month intervention, 30 hyperlipidemic

subjects received 1 lb (454 g) of fresh strawberries per 2000 calories daily. All subjects had been recruited from a study in which they consumed a cholesterol lowering diet for the previous 2.5 years. This cholesterol lowering dietary pattern was low in fat and included plant sterols, fiber, soy protein, and almonds. Strawberry supplementation significantly reduced thiobarbituric acid reactive substances (TBARS) levels in LDL ( $p = 0.016$ ) indicating a reduction in oxidative damage to LDL. No changes were observed in the cholesterol or lipoprotein levels, probably as a result of the subjects consuming a cholesterol lowering diet previous to this study, however, changes seen with that diet were maintained with strawberry supplementation (42).

There is little information on strawberry supplementation in humans, however, anthocyanins and strawberries may have an effect on CVD risk factors and oxidative stress, as evidenced in epidemiological and mechanistic data. No research has been conducted thus far to examine the effect of strawberry supplements in individuals with metabolic syndrome, which has been associated with a higher risk of CVD and higher levels of oxidative stress. Therefore, clinical trials are warranted to determine whether strawberries have beneficial effects on risk factors of CVD and oxidative stress in this population.

### Mechanistic Studies

The effects of phenolic compounds, such as anthocyanins, and whole strawberries on oxidative stress, has been most extensively studied through animal models and cell culture studies. Anthocyanins extracted from mulberries have been found to reduce lipid



peroxidation and oxidation of LDL in vivo by Liu et al. (43). LDL was isolated from human blood samples and oxidation was induced by  $\text{CuSO}_4$  in the presence of mulberry water extracts or mulberry anthocyanin-rich extracts. These were found to contain cyanidine-3-glucoside, cyanadine-3-rutinoside, pelargonadin-3-glucoside, and pelargonadin-3-rutinoside. Both mulberry extracts were able to significantly reduce the induced oxidation of the LDL. TBARS production was assessed as measure of lipid peroxidation after the reaction of the LDL with thiobarbituric acid. The extracts were also able to significantly reduce TBARS in the samples. The anthocyanin-rich extracts were evaluated for anthocyanins and found to have a higher anthocyanin content of 0.89 mg/mg compared to 0.23 mg/mg in the mulberry water extracts. As a result, the anthocyanin rich extract had a greater effect than that of the mulberry water extract in most experiments performed (43).

In a study conducted by Pajk et al. (44), pigs were used as a model for the effectiveness of apples, tomatoes, and strawberries in reducing oxidative stress due to lipid peroxidation. Oxidative stress was induced by supplementing the pig's diet with linseed oil, containing a large amount of polyunsaturated fatty acids. The sample of 48 pigs was divided into six experimental groups in which they received the standard diet plus maize starch, linseed oil, apples, strawberries, tomatoes, or a combination of the three fruits. Pigs were given the experimental diets for 22 days and oxidative stress was measured from fasting blood samples and 48 hour urine samples which were collected at the beginning and end of the experimental phase. The linseed oil significantly raised MDA levels in the blood and caused a significantly higher MDA excretion rate. All diets

containing fruits significantly reduced blood levels of MDA. Oxidative stress was most reduced by the mixed fruit group. Also, compared with each other group, animals in the strawberry group had a significantly higher plasma total antioxidant status (TAS) (44).

Mateos et al. (14) utilized rats as a model for hypercholesterolemia to measure the effect of phenolics from fruit and cocoa on oxidative stress. Rats were divided into eight dietary groups: normo-cholesterolemic and hypercholesterolemic and within those groups, rats received a control diet or a diet supplemented with either 12% freeze-dried strawberry or plum powder, or 16.5% cocoa fiber. Each supplemented diet provided 3 g of polyphenols per kilogram of the diet and the diets were fed to the rats for a period of three weeks. After rats were sacrificed, MDA levels were measured in the both the plasma and liver homogenates. For most groups, serum MDA levels were increased in the hypercholesterolemic rats. In the plasma, MDA levels were significantly reduced by strawberry, plum and cocoa supplementation in both the normocholesterolemic and hypercholesterolemic groups. In liver homogenates, all three supplemented diets significantly decreased MDA levels, and in the hypercholesterolemic strawberry diet group, MDA was decreased to below that of the normocholesterolemic strawberry diet group (14).

Strawberries may also be beneficial for the lipid profile. Strawberry anthocyanins have been shown to decrease cholesterol in mice (45). For mice fed a high fat diet (45% calories from fat) plus 10% calories from strawberry powder, body weight gain was not altered from that of the high fat control and strawberry powder did not have an effect on

the elevated cholesterol seen in the high fat diet group. However, feeding an equivalent amount of purified anthocyanins from strawberries in drinking water, as part of a 60% fat diet, was effective in reducing cholesterol levels to closer to that of the low fat diet control (45).

The evaluated antioxidant properties of strawberries as well as the mechanistic data indicating their effect in cell culture and in animals provides evidence that strawberries may have beneficial effects on oxidative stress and hyperlipidemia in humans. However, the data for the effects of strawberry consumption is limited. Because strawberries are commonly consumed, are one of the largest contributors of phenolic compounds to the American diet, and have high antioxidant activity (12), they may have potential to serve as a functional food providing beneficial effects for metabolic risk factors associated with the development of cardiovascular disease.

## CHAPTER III

### METHODS

#### Institutional Review Board Approval

The strawberry supplementation study was designed as a four week clinical trial to observe the effect of freeze dried strawberry powder on the lipid profile and biomarkers of oxidative stress in human subjects with metabolic syndrome. As such, the study was conducted according to the guidelines laid down in the Declaration of Helsinki.

Approval for all procedures was gained from the Institutional Review Board (IRB) at Oklahoma State University. In addition, investigators and research assistants completed IRB training through the Collaborative Institutional Training Initiative (CITI) prior to their involvement with the study. All participants provided a signed informed consent prior to being enrolled in the study.

#### Subjects

Subjects were recruited at the Oklahoma State University campus through the use of flyers posted at various locations on campus including the HES building and the Seretean Wellness Center as well as through e-mail advertisements sent out by the university.

Subjects included in the study were adult obese women (BMI>30) with dyslipidemia.

Participants were also required to meet three of the following criteria: a waist circumference  $\geq 35$  inches, HDL  $\leq 50$  mg/dL, triglycerides  $\geq 150$  mg/dL, or cholesterol  $\geq$

200 mg/dL. Individuals were excluded if they were on medications for chronic diseases (including cardiovascular disease and diabetes), were pregnant or lactating, currently using tobacco, consuming alcohol in amounts greater than 1 oz/day, taking antioxidant or fish oil supplements (>1 g/day), or had abnormal lab values as reflected in the complete blood count or tests associated with thyroid, liver, or kidney function.

Following recruitment, individuals came to HES 416 for an initial screening visit which included a blood draw and blood pressure, waist circumference, weight, and height measurements. During the screening visit, participants were informed of the purpose of the study, the procedures as well as any risks or benefits associated with the study and the individuals read and signed an informed consent participation agreement. After signing the informed consent, the anthropometric and blood pressure measurements and blood draws were conducted. Based on the screening results and inclusion criteria, participants were included or excluded from the study and notified by phone of their participation status.

### Study Design

This was a clinical trial including a four week intervention period consisting of daily supplementation with 50 grams of freeze-dried strawberry powder. The freeze-dried strawberry powder was kindly donated by the California Strawberry Commission (California, USA). Participants received the supplement in the form of drinks prepared from the freeze-dried strawberry powder. Each serving of the strawberry drink contained 1 cup water, 1 teaspoon vanilla extract, and 1 teaspoon Splenda® blended together with

25 grams strawberry powder. Participants were asked to consume two servings of the drink daily. In order to allow full absorption of the strawberry powder, participants were asked to consume the strawberry drinks in the morning and evening at least 6 hours apart and at least 1.5 hours before or after meals or snacks. It was also asked that they refrain from consuming other berries, which have high phytochemical or antioxidant content and might interfere with the effect of the strawberry powder.

During the supplementation period, participants were also asked to complete a three day food record each week. Participants were instructed on the use of the food record and on accurately recording food portions. For each food, participants recorded the amount of food eaten, the type of meal or snack during which the food was eaten (breakfast, morning snack, lunch, afternoon snack, dinner, evening snack), and the location at which the food was consumed (home, restaurant, with friends, or at work). To ensure accurate analysis, participants were asked to record names of restaurants and menu items eaten and to include nutrition labels for packaged foods and recipes for home prepared foods when possible.

Participants made three weekly visits to 416 HES, Oklahoma State University, in order to receive their supply of the strawberry supplement. To ensure compliance, participants consumed their first cup of the strawberry drink in the presence of the research staff during the visit and were asked to consume the second cup at least 6 hours later in the day. They were provided with the remaining supply of the drink in plastic containers.

Participants were also asked to keep the strawberry drink under refrigeration and to avoid exposing it to direct light or heat to maintain its nutritional value.

For the duration of the study, participants were asked to maintain their usual diet, physical activity, and lifestyle. At the end of the four week intervention, anthropometrics and blood pressure were again measured and a final blood sample was taken. All blood samples were drawn by a certified phlebotomist. Subjects were compensated thirty dollars weekly for their participation.

### Materials

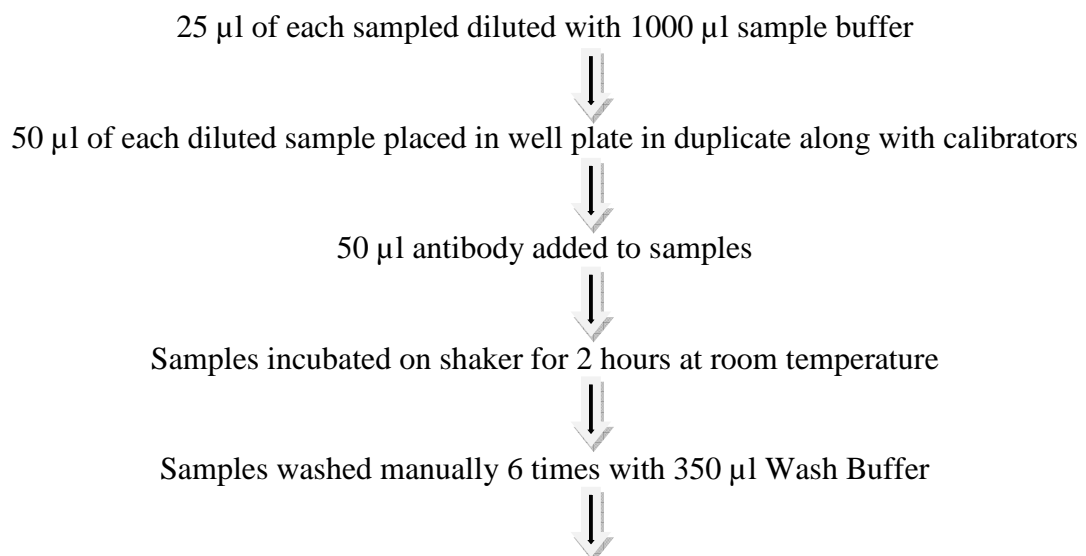
Blood pressure was obtained by a portable blood pressure machine with arm cuff or portable wrist cuff. The balance beam scale (Continental Scale Corporation, Chicago, IL), located in 307 HES, was used to determine body weight. All strawberry drinks were prepared in a blender in HES facilities and provided in plastic bottles for the participants. Food safety practices were followed in the preparation and storage of the drinks.

Fasting blood samples of about 45 mL were collected in SST tubes and in tubes containing the anticoagulant EDTA. Serum and plasma samples were stored at -80°F for future analysis. Plasma and serum were separated by centrifugation at 3000 rpm for 10 minutes at 4°C with the Centrifuge 5810 R (Eppendorf, Hamburg, Germany). Laboratory tests to evaluate serum glucose, lipids, albumin, hemoglobin and hematocrit, and liver, renal, and thyroid function were conducted at Stillwater Medical Center (Stillwater, OK).

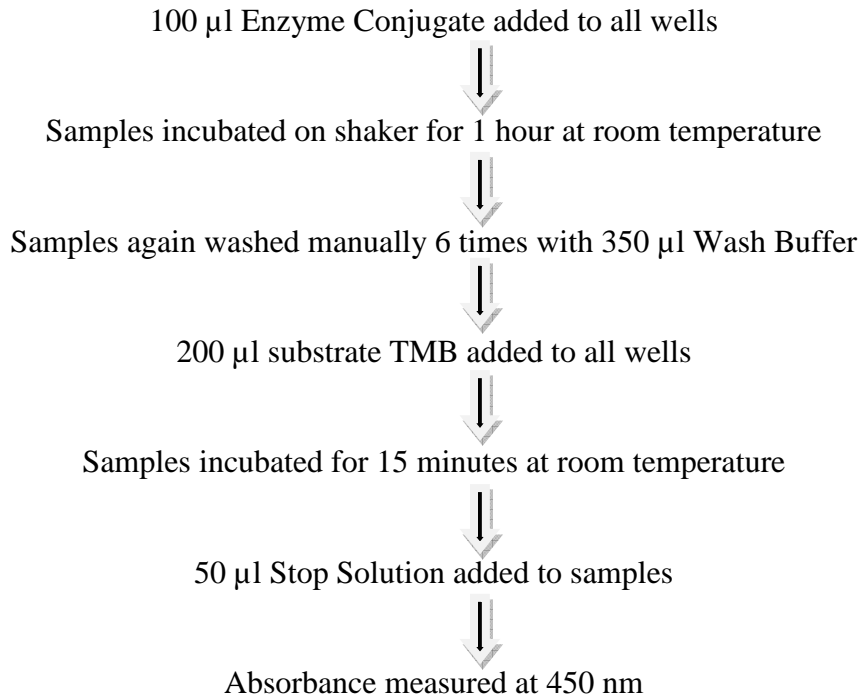
Remaining serum and plasma were stored at  $-80^{\circ}\text{C}$  for analysis of biomarkers of oxidative stress.

### Biomarkers of Oxidative Stress

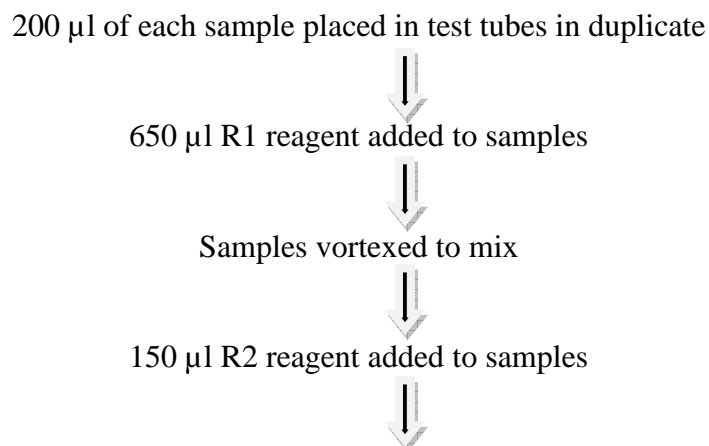
Screening and 4-week blood samples were analyzed for biomarkers of oxidative stress. Three serum biomarkers of oxidative stress were identified and measured in the blood samples provided by each participant: oxidized LDL, malondialdehyde (MDA) and 4-hydroxyalkenals (HAE), and myeloperoxidase (MPO). Using an Oxidized LDL Competitive ELISA (Merckodia, Uppsala, Sweden), serum oxidized LDL levels were measured in duplicate with the use of a murine monoclonal antibody, mAb-4E6 as follows. In this method, oxidized LDL in the blood sample competes for binding with the biotin-labeled antibodies. The biotin-labeled antibodies are then identified by HRP-conjugated streptavidin. The bound conjugate is detected through its reaction with 3,3',5,5'-tetramethylbenzidine (TMB). After a stop solution is administered, the sample is read spectrophotometrically.

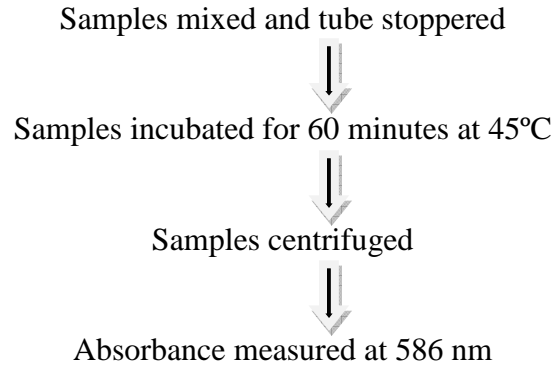




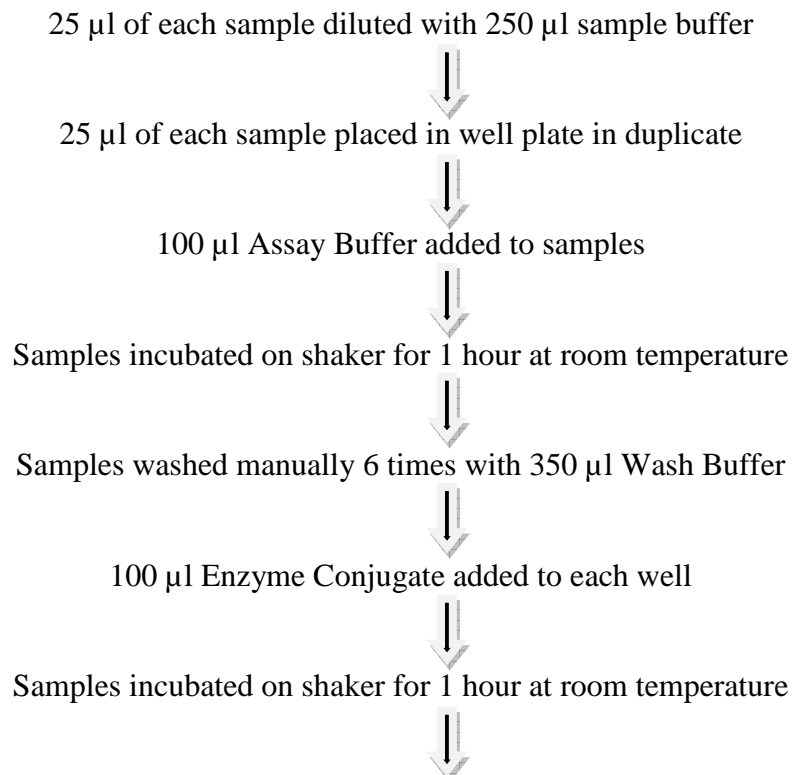


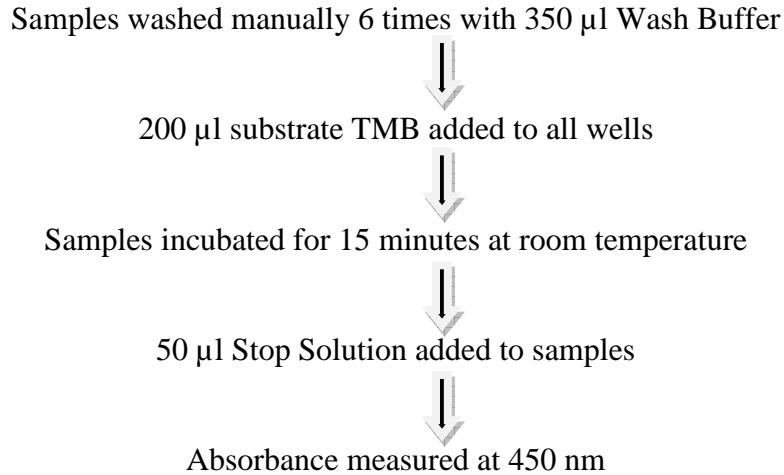
MDA & HNE in serum were assessed using the Bioxtch® LPO-586 assay (OxisResearch™, Foster City, CA) according to the following procedure. The basis of this assay is the reaction of MDA and 4-hydroxyalkenals with N-methyl-2-phenylindole, which is a chromogenic reagent. Methanesulfonic acid is the acid solvent for the reaction.





MPO levels were measured in duplicate using procedures outlined in the Mercodia MPO ELISA kit (Mercodia, Uppsala Sweden). This technique uses two anti-MPO antibodies which react with MPO in the blood samples. First, the samples react with anti-MPO antibodies which are bound to the microtitration wells. Secondly, after washing, peroxidase conjugated anti-MPO antibodies are added. The conjugate bound to the MDO is then able to be detected by TMB.





### Dietary Analyses

The food records turned in by participants were used to account for dietary habits and changes in diet in study results. Food records from the first and fourth weeks of intervention were entered and analyzed using ESHA Food Processor 9.1.0 (ESHA Research Inc., Salem, OR). Dietary energy, protein, carbohydrate, fat, and selected vitamins and minerals were included in the analyses.

### Statistical Analyses

Pre and post-intervention anthropometric and blood values were compared and evaluated by performing paired t-tests. Outcome measures analyzed were changes in body weight, waist circumference, blood pressure, lipid profile levels, blood glucose, and liver, kidney, and thyroid function. All data analyses were performed using SPSS 16.0 (SPSS Inc., Chicago, IL). Statistical significance was established at  $p < 0.05$  (two-sided test).

## CHAPTER IV

### FINDINGS

#### Nutrient Content of Freeze-dried Strawberry Powder

Composition analysis was conducted to determine the nutritional content of the freeze-dried strawberry powder (FSP) as a dietary supplement in the study (**Table 1**). Analysis revealed that 50 grams of the FSP, the amount given to participants daily, provided an additional 150 calories to their daily dietary intake. The 50 grams provided to participants was equivalent to about 500 grams of fresh strawberries. The macronutrient composition of the powder was 87.7% carbohydrate, 9.3% protein, and 3.0% fat. The FSP was found to be high total phenolics as well as anthocyanins. It is also a good source of Vitamin C (145% RDA for females) and fiber (8 grams per day).

#### Baseline Characteristics

Baseline characteristics of participants of the participants are outlined in **Table 2**. Although the study was open to recruitment of both men and women, only women volunteered and were enrolled in the study. In total, 16 adult women, ranging in age from 39 to 71 years (mean of  $51.4 \pm 9.1$ ), were eligible and agreed to participate in the study. Eight of the 16 participants were currently taking a dietary supplement at the screening visit and continued to do so for the duration of the supplementation. None of

the participants reported the use of aspirin prior to the study. Aspirin is a blood thinner commonly used as a preventative medication for heart attack. Fruit and vegetable consumption was analyzed in ESHA Food Processor. A serving of fruit was the equivalent of ½ cup chopped fruit, 1 medium fruit, or ¾ cup fruit juice and a vegetable serving the equivalent of 1 cup leafy vegetables, ½ cup non-leafy vegetable, or ¾ cup vegetable juice. Mean fruit intake was 1.9 servings per day while mean vegetable intake was 2.6 servings per day, which falls below current recommendations for both fruit and vegetable intake. Intakes for most participants actually fell below this level. The mean is elevated due two participants whose fruit and vegetable intake was much higher at 12.1 and 21.8 servings per day.

#### Anthropometrics, Blood Pressure, Lipid Profile, and Safety Parameters

All participants were classified by BMI as overweight or obese and 14 of the 16 participants had a BMI greater than 30 kg/m<sup>2</sup> at baseline. All participants presented with waist circumference greater than 35 inches and dyslipidemia. Although, the FSP provided calories to the dietary intake, mean body weight, BMI, and waist circumference remained constant throughout the intervention. No changes were seen in blood pressure or fasting glucose. FSP supplementation did affect dyslipidemia, reducing total cholesterol and LDL cholesterol by 5.1% and 7.6% respectively. Other lipoprotein levels and triglyceride levels were unchanged (**Table 3**). Kidney and liver function were unaffected by strawberry supplementation, as safety parameter values remained within normal ranges after FSP supplementation and no significant changes were seen in the complete blood count (**Table 4**).

### Dietary Analysis

Average total caloric intake remained constant over the supplementation period (**Table 4**). The FSP supplement was not included as part of the dietary analysis, so as to reflect the participants' normal diet patterns. At baseline, macronutrient composition was 47.6% carbohydrate, 15.6% protein, and 36.0% fat of total calories on average. In addition to macronutrient content, several vitamins and minerals were used as dietary markers for consistency of the participants' diets. Mean zinc intake slightly increased over the 4 week study ( $p=0.50$ ) but intake of all other nutrients was not significantly different at baseline and 4 weeks. Overall, fiber intake was relatively low, excluding the strawberry drink, which provided a significant source of dietary fiber. Mean cholesterol intake was moderate at an average of 221 mg/day and 261 mg/day at baseline and 4 weeks of intervention, respectively, which is within the recommendation for dietary cholesterol at less than 300 mg/day.

### Biomarkers of Oxidative Stress

Three measures of oxidative stress were used in the evaluation of the antioxidant properties of FSP in the body (**Figure 1**). A trend for slight decrease in oxidized LDL was observed ( $p = 0.123$ ). MDA and HNE were significantly reduced by 15.6% ( $p < 0.001$ ). No change was detected in MPO levels.

**Table 1.** Composition of freeze-dried strawberry powder<sup>1</sup>

Component	Amount
Carbohydrates (g)	32.83
Protein (g)	3.48
Fat (g)	0.5
Calories (kcal)	149.6
Moisture (%)	19.45
Ash (g)	3.17
Vitamin C (mg)	109.0
Total Phenolics (mg)*	2005.5
Total Anthocyanins (mg)**	154.0
Phytosterols (mg)	50.5
Total Dietary Fiber (%)	15.9

<sup>1</sup>Data presented per 50 grams (10% fresh weight). Source: California Strawberry Commission (Watsonville, CA, USA).

\*Expressed as mg gallic acid equivalents.

\*\*Expressed as mg cyanidin-3-glucoside equivalents.

**Table 2.** Baseline Characteristics

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Characteristic (n=16)	
Gender	
Female (%)	16 (100%)
Age (mean, SD)	51.4, 9.2
Supplement Use (%)	
Vitamin/mineral (%)	7 (43.8%)
Herb or Botanical (%)	0 (0%)
Unspecified/Other (%)	1(6.3%)
Aspirin Use (%)	0 (0%)
Fruit and Vegetable Intake, servings per day (mean, SD)	4.6, 5.7

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**Table 3.** Effects of freeze-dried strawberry powder supplementation on anthropometrics, blood pressure, and fasting glucose, and fasting lipids

	Baseline (n=16)		Post-Intervention (n=16)	
	Mean	SE	Mean	SE
Body Weight (kg)	102.0	5.6	102.6	5.7
BMI (kg/m <sup>2</sup> )	38.6	2.3	38.78	2.3
Waist Circumference (in)	43.0	1.5	44.0	1.5
Systolic Blood Pressure (mm Hg)	135.0	3.3	134.5	3.5
Diastolic Blood Pressure (mm Hg)	88.0	2.6	87.5	2.5
Blood Glucose (mg/dL)	94.1	3.0	93.4	2.5
Total cholesterol (mg/dL)	205.6	7.2	195.2	6.2*
LDL (mg/dL)	124.3	6.4	114.9	4.8*
HDL (mg/dL)	47.8	1.9	46.7	1.5
VLDL (mg/dL)	38.7	7.2	32.3	3.1
Triglycerides (mg/dL)	150.75	68.82	161.38	62.18

\*significantly different from baseline (p<0.05)

**Table 4.** Effects of freeze-dried strawberry powder supplementation on safety parameters

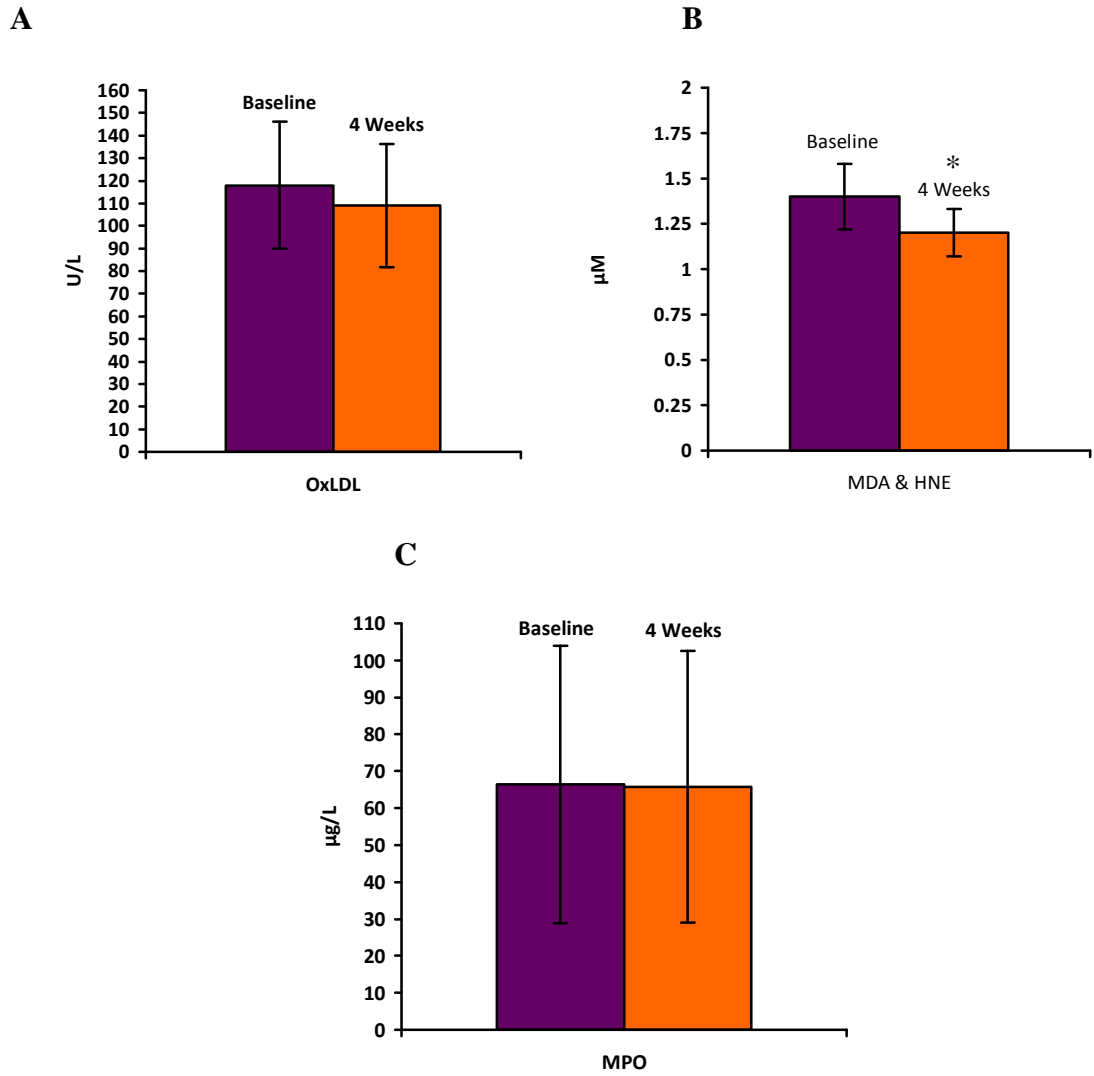
	Baseline (n=16)		Post-Intervention (n=16)		Normal Range
	Mean	SE	Mean	SE	
BUN (mg/dL)	13.25	.906	12.56	.801	7-18
Creatinine (mg/dL)	0.881	0.0306	0.900	0.0274	0.7-1.2
Total Protein (g/dL)	6.925	0.1002	6.988	0.1056	6.3-8.2
Albumin (g/dL)	4.119	.2257	4.138	0.0554	3.9-5.0
Aspartate Aminotransferase (U/L)	25.69	1.779	26.25	1.740	14-36
Alanine Aminotransferase (U/L)	29.81	3.031	31.50	2.907	7-56
Alkaline Phosphatase (U/L)	88.81	5.187	87.69	5.115	38-126
Bilirubin (mg/dL)	0.450	0.365	0.431	0.373	0.2-1.3
Thyroxine (T4) ( $\mu\text{g/dL}$ )	7.3656	0.34978	7.1294	0.31180	5.53-11.0
White Blood Cells ( $\text{K/mm}^3$ )	7.288	0.4918	7.250	0.5063	4.5-10.8
Red Blood Cells ( $\text{M/mm}^3$ )	4.6088	0.07559	4.6375	0.6657	4.20-5.40
Hemoglobin (g/dL)	13.738	0.2158	13.738	0.2260	12.0-14.0
Hematocrit (%)	40.119	0.7069	40.188	0.6334	35.0-49.0
Mean Corpuscular Volume (fl)	87.069	0.7593	86.700	0.7784	80.8-94.0
Platelet count ( $\text{K/mm}^3$ )	268.38	15.762	272.94	18.432	130-400
Neutrophils (%)	59.520	1.9221	60.013	2.0074	37.0-73.0
Lymphocytes (%)	29.747	1.7126	29.247	1.9348	20.0-55.0
Monocytes (%)	7.307	0.5945	7.180	6.066	2.0-12.0

**Table 5.** Dietary analysis of participant’s intake from week 1 and week 4 of intervention.<sup>1</sup>

Nutrient	Week 1 (n=14)		Week 4 (n=14)	
	Mean	SE	Mean	SE
Energy (kcal)	1738.3	103.32	1790.3	136.40
Protein (g)	67.9	5.68	76.1	6.05
Carbohydrate (g)	206.8	16.55	203.8	16.99
Fiber (g)	14.0	1.33	14.78	1.60
Total fat (g)	69.6	4.70	75.0	7.39
Saturated fat (g)	24.0	1.99	26.5	2.37
Monounsaturated fat (g)	15.9	2.15	19.0	2.84
Polyunsaturated fat (g)	7.1	1.02	9.2	1.99
Cholesterol (mg)	221.0	34.98	261.2	30.69
Carotenoids (RE)	258.4	75.19	291.2	53.87
Vitamin C (mg)	68.9	22.11	69.6	14.0
Vitamin E (mg)	3.7	0.74	4.6	1.13
Iron (mg)	12.1	1.26	13.2	1.21
Copper (mg)	0.6	0.09	0.8	0.11
Zinc (mg)	7.1	1.11	9.9	1.44

<sup>1</sup>Data was collected from 3 day food records

**Figure 1.** Effects of freeze-dried strawberry powder (FSP) supplementation on biomarkers of oxidative stress.



(A) Oxidized LDL, (B), Malondialdehyde and 4-Hydroxynonenal and (C) Myeloperoxidase at baseline and 4 weeks of FSP supplementation, (n = 16) Presented as mean ± SD.

\*Significantly different from baseline at  $p < 0.05$

## CHAPTER V

### CONCLUSION

Not only is cardiovascular disease a public health epidemic, the rising costs of treatment for CVD and metabolic disorders poses challenges for affected individuals as well as the health care system. Modulation of CVD risk factors by functional foods, such as fruits and vegetables, may serve to reduce occurrence of CVD as well as treatment costs. Many animal and cell culture studies have already shown the antioxidant and anti-hyperlipidemic properties of strawberries and anthocyanins (14, 43-45). In the present study, we have shown the antioxidant and anti-hyperlipidemic effects of freeze-dried strawberry powder in women with metabolic syndrome. To our knowledge, this is the first human study reporting the impact of freeze-dried strawberry powder supplementation on cardiovascular risk factors in this population. Dyslipidemia is significantly associated with atherosclerosis and the occurrence of CVD events, such as myocardial infarction and stroke. In fact, individuals with dyslipidemia may be twice as likely to have atherosclerosis compared to individuals with blood lipids within normal ranges (46). The increased risk of cardiovascular disease seen in metabolic syndrome is most likely related to the feature of dyslipidemia but may also be associated to the increased levels of oxidative stress present in individuals with metabolic syndrome (47).

The previous study by Jenkins et al. (42) utilized fresh strawberry supplementation, and found no significant effects on cholesterol levels. It is of note, however, that the participants had also been following a cholesterol lowering diet for the previous 2.5 years. This research did support our findings that strawberry antioxidants may reduce oxidative stress, as a reduction in oxidative damage to LDL, measured by TBARS in the LDL fraction, was reported (42). Conversely, anthocyanin supplements have been shown to improve the lipid ratio in a study conducted by Qin et al. (48). Participants consumed capsules containing 320 mg anthocyanins per day, which was slightly double the amount of anthocyanins received from the daily freeze-dried strawberry powder supplement in our study. In the 120 dyslipidemic adults, anthocyanins purified from bilberry and black currant were effective in reducing LDL cholesterol levels by 13.6% compared with 0.6% in the control group ( $p < 0.001$ ) and increasing HDL cholesterol levels by 13.7% compared with a 2.8% increase in the control group ( $p < 0.001$ ).

Participants in our study were asked to maintain current dietary intake for the duration of supplementation with the freeze-dried strawberry powder. The strawberry powder used was high in phenolic compounds, as well as vitamin C and fiber which may have contributed to the antioxidant function and cholesterol lowering properties seen in this trial. The strawberry powder also contributed an additional 150 calories to the participant's daily intake, which may have reduced intake of other foods normally consumed. The total caloric intake remained relatively constant throughout the study and body weights did not change significantly as a result of the strawberry supplementation. The 50 grams of the freeze-dried strawberry powder that the participants in our study

received daily, was the equivalent of about 500 grams (3.5 cups) whole fresh strawberries. This amount of strawberry powder, blended with water into drink form, was well tolerated by participants and had no effect on safety parameter values.

Due to the fact that this was a small pilot study, the results from our research may not be widely generalized. Participation was limited to 16 individuals and the experimental phase was of relatively short duration at 4 weeks. A study of larger size or longer duration may have revealed even more positive effects on the cholesterol levels and oxidative stress of the participants. Supplementation data was not compared to that of a control group, as it would have been difficult to develop a control strawberry drink that was comparable in taste and texture to the original, antioxidant rich drink prepared from the freeze-dried strawberry powder. In addition, food records were self-reported and although compliance procedures were established and followed, it was not possible to monitor consumption of each serving of the drink for all participants.

The strawberry powder used for supplementation in the present study is not commercially available. Although a large consumption of fresh strawberries would be needed to replicate the amount supplemented in our study, anthocyanins and other antioxidants are present in many fruits and vegetables. In general, most individuals in the United States must increase the daily consumption of fruits and vegetables to meet current recommendations. As part of a healthy diet, high consumption of strawberries and other berry fruits high in anthocyanins, may be effective in reducing cholesterol and oxidative

stress. These effects may be beneficial in the prevention of metabolic syndrome and cardiovascular diseases.



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## APPENDICES

**Appendix A.** IRB Approval

**Appendix B.** Screening Questionnaire

**Appendix C.** Informed Consent Form

**Appendix D.** Food Diary Form

## Appendix A. IRB Approval

### Oklahoma State University Institutional Review Board

Date: Tuesday, July 22, 2008

IRB Application No HE0845

Proposal Title: The Effects of Freeze-Dried Strawberry Drink Consumption on Biomarkers of Lipid Peroxidation and Inflammation in Obese Dyslipidemic Subjects

Reviewed and Processed as: Expedited

**Status Recommended by Reviewer(s): Approved Protocol Expires: 7/21/2009**

Principal Investigator(s):

Arpita Basu

416 HES

Stillwater, OK 74078

Nancy Betts

301 HES

Stillwater, OK 74078

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The IRB application referenced above has been approved. It is the judgment of the reviewers that the rights and welfare of individuals who may be asked to participate in this study will be respected, and that the research will be conducted in a manner consistent with the IRB requirements as outlined in section 45 CFR 46.

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

As Principal Investigator, it is your responsibility to do the following:

1. Conduct this study exactly as it has been approved. Any modifications to the research protocol must be submitted with the appropriate signatures for IRB approval.
2. Submit a request for continuation if the study extends beyond the approval period of one calendar year. This continuation must receive IRB review and approval before the research can continue.
3. Report any adverse events to the IRB Chair promptly. Adverse events are those which are unanticipated and impact the subjects during the course of this research; and
4. Notify the IRB office in writing when your research project is complete.

Please note that approved protocols are subject to monitoring by the IRB and that the IRB office has the authority to inspect research records associated with this protocol at any time. If you have questions about the IRB procedures or need any assistance from the Board, please contact Beth McTernan in 219 Cordell North (phone: 405-744-5700, beth.mcternan@okstate.edu).

Sincerely,



Shelia Kennison, Chair  
Institutional Review Board

## Appendix B. Screening Questionnaire

Day/ Date of Appointment: \_\_\_\_\_ Time: \_\_\_\_\_

### SCREENING QUESTIONNAIRE FOR STRAWBERRY STUDY

NAME: \_\_\_\_\_

ADDRESS: \_\_\_\_\_

PHONE (WORK): \_\_\_\_\_

PHONE (HOME): \_\_\_\_\_

AGE: \_\_\_\_\_ DATE OF BIRTH: \_\_\_\_\_ GENDER: \_\_\_\_\_

### SCREENING QUESTIONS:

- Do you currently take any cholesterol/triglyceride lowering medications? YES NO
- Are you pregnant or lactating? YES NO N/A
- Do you smoke? YES NO
- Do you currently take vitamins or nutritional supplements? YES NO  
What are they? \_\_\_\_\_
- Have you taken antioxidant supplements regularly in the past 3-6 months? YES NO
- Do you take more than 1 g/day of fish oil capsules? YES NO
- Do you exercise  $\geq 60$  min/day? YES NO
- Do you drink more than 1 oz of alcohol/day?  
(1 oz alcohol = 2 beers or 10 oz of wine or 2 1/2 oz liquor) YES NO
- Do you have diabetes?  
We will confirm with fasting blood glucose YES NO UNSURE
- Do you have hypo/hyperthyroidism?  
We will check TSH YES NO UNSURE

Day/ Date of Appointment: \_\_\_\_\_ Time: \_\_\_\_\_

Do you have any gastrointestinal problems? YES NO

Do you have anemia? YES NO

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

Do you have high blood pressure?  
If controlled, what medications does the patient take?  
\_\_\_\_\_

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

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

Do you take aspirin? How often? Dose? \_\_\_\_\_  
\_\_\_\_\_

Is the subject ELIGIBLE based on the questionnaire? YES NO

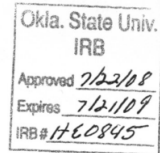
**ELIGIBILITY REQUIRES THE FOLLOWING FEATURES:**

(Check all that apply):

1. \_\_\_\_ Waist circumference (Male  $\geq$  40 inches) (Value: \_\_\_\_)  
(Female  $\geq$  35 inches)
2. \_\_\_\_ HDL Cholesterol (Male  $\leq$  40 mg/dL) (Value: \_\_\_\_)  
(Female  $\leq$  50 mg/dL)
3. \_\_\_\_ Triglycerides ( $\geq$  150 mg/dL) (Value: \_\_\_\_)
4. \_\_\_\_ Cholesterol ( $\geq$  200 mg/dL) (Value: \_\_\_\_)



## Appendix C. Informed Consent Form



### INFORMED CONSENT DOCUMENT

Project Title: Effects of freeze-dried whole Strawberry drink consumption on biomarkers of lipid peroxidation and inflammation in obese subjects with high blood lipids.

Investigators:

Arpita Basu, PhD, RD  
Nancy Betts, PhD, RD

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

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

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

In order to qualify you should not have any other serious health problems or take medicines to lower your lipids or glucose levels. If you qualify, you will be asked to take 2 cups of strawberry drink per day, for a period of 4 weeks. A cup of the drink will be made of 25g of strawberry powder, one cup of water, one teaspoon of splenda, ½ teaspoon of vanilla essence, and ¼ cup of ice (optional). You will be taking two cups of this drink every day. This is a 4-week study and you will be coming to the Department of Nutritional Sciences or Seretean Wellness Center 3 days/week to get your strawberry drink, for blood draws, as well as for screening and biweekly visits, each for about half an hour, as stated below-

1. Screening- You will be asked to sign the consent form, and we will measure your height, weight, blood pressure, and waist. You will be asked to fast the previous night and about 3-4 tablespoons of blood will be taken for measuring your blood sugar, lipids, blood cell counts, and do some tests to find out how well your liver and kidney are working. If you qualify, we will let you know over the telephone and ask you to come back for the drink. You will also be asked to record your food intake before you start the study and will also be told how to do it.
2. 2 weeks- You will return at 2 weeks to turn in 3-day food records and for a brief talk on how you are doing in the study.
3. 4 weeks- This will be your final visit and you will give us your 3-day food records. You will be asked to fast the previous night and about 3-4 tablespoons of blood will be taken for measuring your blood sugar, lipids, and do some tests to find out how well the cells in your body are working. We will also measure your waist, body weight and blood pressure.

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

Risks of Participation: You may experience slight pain during the blood draw. But, a trained nurse

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 IRB  
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 Expires 7/21/09  
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will be there for any help. Also, you will not be allowed to participate in the study if you have any blood-related health problems. You may experience slight stomach problems like flatulence if you are not used to taking 2 cups of strawberry drink per day. But, these symptoms may soon go away.

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

**Confidentiality:** The records of this study will be kept private. Any written results will discuss group findings and will not include information that will identify you. Research records will be stored securely and only researchers and individuals responsible for research oversight will have access to the records. It is possible that the consent process and data collection will be observed by research oversight staff responsible for safeguarding the rights and wellbeing of people who participate in research.

**Compensation:** You will be compensated for \$30 at the end of each week throughout the 4 week study. This payment will be made to you in cash and you will need to sign a form as a receipt.

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

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

**Signatures:**

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

\_\_\_\_\_  
 Signature of Participant

\_\_\_\_\_  
 Date

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

\_\_\_\_\_  
 Signature of Researcher

\_\_\_\_\_  
 Date

## Appendix D. Food Diary Form

### Oklahoma State University Nutritional Sciences Strawberry Study

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

**When you come back, please bring in any bottles/packages of dietary or nutritional supplements you have taken within the past week.**

This would include any pills, powders, capsules, oils, tablets, or liquid vitamin/mineral supplements, herbal supplements, herbal teas or tinctures or any other type of dietary supplement you have taken.

#### GENERAL INSTRUCTIONS FOR RECORDING FOOD INTAKE

1. Please record on the Food Diary Form the place (home, home of a friend, restaurant) of each meal and snack.
2. Record one food item per line on the Food Diary Form. Space is provided on both sides of the form. Be sure to include gum, candy and beverages.
3. Record the amount and food item on the Food Diary Form using common household measurements, for example: Tablespoons, cups, package size etc.
4. Remember to record everything you possibly can about a food. The more detail you include the better.
5. When you record an item, please note if it was baked, boiled, broiled, fried, or roasted. This is extremely important, especially for meats.
6. Record any additions to a food item. This **would** include sugar, relish, margarine, butter, catsup, pickles, mayonnaise, mustard, gravies, cream, etc., which were served with the food.
7. When eating out, record the menu item and amount eaten. Refer to Hints for Eating Out.
8. List the method of mixing a package mix if it is different from the directions given on the package. You may record this on a Recipe Form.
9. Use the Recipe Form to record any homemade items you have prepared. Measure each ingredient and record the method of preparation on the bottom of that form.
10. If you have any questions, please call Arpita Basu at 405-447-7723.

## HINTS FOR EATING OUT

1. Record the name of the restaurant.
2. Quiz the wait staff regarding portion sizes.
3. Record amounts in standard household measurements, ie: teaspoons (ts), tablespoons (Tb), ounces, cups, etc.
4. For items such as bacon, rolls, and cucumbers, record the number of each item eaten.  
For example:     3 small white rolls  
                  4 cucumber slices  
                  2 medium bacon slices
5. For meats, record the dimensions of the cooked meat. Do not include the bone.  
For example: 2 slices of roast beef 4" x 3" x 1/4". State the weight of the meat if it is mentioned on the menu.
6. Refer to the Food Description Flow Charts to describe your food.
7. For national fast food restaurants, (i.e. McDonald's, Arby's, Burger King), record the name of the sandwich/item you ate (i.e. Big Mac, Whopper).

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Nutritional Sciences  
Strawberry Study  
Food Diary

Name: \_\_\_\_\_ ID# \_\_\_\_\_ Protocol No: \_\_\_\_\_

Date of Record: \_\_\_\_\_ Day of Week: \_\_\_\_\_

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

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

- \*Meal Codes: Breakfast - BR  
Morning Snack - MS  
Lunch - LU  
Afternoon Snack - AS  
Supper - SU  
Evening Snack - ES
- \*Place Codes: Home - HO  
Restaurant - RE (Please Specify name of Restaurant)  
Friends - FR  
Work - W



## VITA

Marci Paige Wilkinson

Candidate for the Degree of

Master of Science

Thesis: THE EFFECT OF FREEZE-DRIED STRAWBERRY POWDER ON LIPID PROFILE AND MARKERS OF OXIDATIVE STRESS IN WOMEN WITH METABOLIC SYNDROME

Major Field: Nutritional Sciences

### Biographical:

Personal Data: Born in Elkhart, Kansas, on May 6, 1985, the daughter of James and Cheryl Wilkinson.

Education: Graduated from Stillwater High School, Stillwater, OK in 2003; received Bachelor of Science degree in Nutritional Science with an option in Dietetics from Oklahoma State University, Stillwater, Oklahoma in December 2007. Completed the requirements for the Master of Science in Nutritional Sciences at Oklahoma State University, Stillwater, Oklahoma in December, 2009.

Experience: Employed by Oklahoma State University, Department of Human Development and Family Sciences as an undergraduate research assistant, 2004-2007; Dietetic Intern with the University of Houston, 2008; Employed by Oklahoma State University, Department of Nutritional Sciences as a graduate research assistant and teaching assistant, 2008 to present.

Professional Memberships: American Dietetic Association, American Society for Nutrition

Name: Marci Wilkinson

Date of Degree: December, 2009

Institution: Oklahoma State University

Location: Stillwater, Oklahoma

Title of Study: THE EFFECT OF FREEZE-DRIED STRAWBERRY POWDER ON  
LIPID PROFILE AND MARKERS OF OXIDATIVE STRESS IN  
WOMEN WITH METABOLIC SYNDROME

Pages in Study: 55

Candidate for the Degree of Master of Science

Major Field: Nutritional Sciences

Scope and Method of Study: Oxidative stress is linked to metabolic and cardiovascular diseases. Strawberries contain nutrients and phytochemicals that possess high antioxidant capacity and strawberry intake has been shown to reduce the risk of CVD mortality in women. In this feeding study, the hypothesis that freeze-dried strawberry powder will reduce oxidative stress and improve the lipid profile was examined. Females (n=16) with metabolic syndrome were given 50 g of freeze-dried strawberry powder each day for four weeks. The freeze-dried strawberry powder was prepared by blending it into a drink with water, vanilla extract, and Splenda®. Women consumed two cups of the drink daily, each cup containing 25 grams of the strawberry powder. Fasting blood draws, anthropometrics, and blood pressure were collected at screen and 4 weeks of the study for analysis.

Findings and Conclusions: The strawberry drink was well tolerated by the participants. Compared with baseline levels, the freeze-dried strawberry powder significantly reduced total cholesterol ( $205.56 \pm 7.19$  vs.  $195.25 \pm 6.23$ ,  $p = 0.017$ ) and LDL cholesterol ( $124.27 \pm 6.37$  vs.  $114.93 \pm 4.84$ ,  $p = 0.038$ ) after the 4 week supplementation. As measures of oxidative stress, malondialdehyde (MDA) and 4-hydroxynonenals (HNE) were significantly decreased as compared with baseline ( $1.19 \pm 0.03$  vs.  $1.41 \pm 0.04$ ,  $p < 0.001$ ) and oxidized LDL showed a decreasing trend ( $p = 0.123$ ). Thus, freeze-dried strawberry powder was shown to have a hypocholesteremic effect and to reduce oxidative stress in women with metabolic syndrome.

ADVISER'S APPROVAL: Dr. Arpita Basu

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