THE EFFECTS OF DIETARY FREEZE-DRIED STRAWBERRIES ON PLASMA TRACE ELEMENTS AND ANTIOXIDANTS IN ADULTS WITH OBESITY AND ABOVE OPTIMAL SERUM LIPIDS

By

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Dietary flavonoid intake, especially berry flavonoids, has been associated with antioxidant functions and metal chelation in mechanistic studies. We examined the hypothesis that freeze-dried strawberries (FDS) improve antioxidant biomarkers in adults with abdominal adiposity and elevated serum lipids. In a randomized dose-response controlled trial, 60 volunteers [5 men and 55 women; age: 49 ± 10 years; BMI: 36 ± 5 kg/m^2 (mean±SD)] were assigned to one of the following arms: low dose FDS (LD-FDS) (25g/d), low dose control (LD-C), high dose FDS (HD-FDS) (50g/d), and high dose control (HD-C) beverages for 12 weeks. Control beverages were matched for calories and total fiber. Serum levels of trace elements and whole blood glutathione, and catalase activity were examined at screening (0 week) and after 12 weeks intervention. At 12 weeks, glutathione levels were higher in HD-FDS vs. LD-FDS, as well as vs. HD-C, and catalase activity was lower in HD-FDS vs. baseline (all $P<0.05$). No differences were noted in serum trace elements following FDS intervention. Thus, dietary strawberries may selectively modulate antioxidant biomarkers that influence risk factors of chronic diseases.

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CHAPTER I

INTRODUCTION

Cardiovascular disease (CVD) remains a significant public health concern and the leading cause of mortality in the US[1]. Several of the risk factors underlying CVD are modifiable by dietary and lifestyle factors, including those related to overweight/obesity and dyslipidemia[2]. Obesity and dyslipidemia have also been associated with elevated oxidative stress, reduced antioxidant status and consequently the initiation and progression of CVD[3-6].

Oxidative stress identifies a biological condition when the production of harmful reactive oxygen species (ROS) and the concentrations of intracellular and extracellular antioxidants are under imbalance[7]. An antioxidant is any substance that can delay or prevent oxidation of a particular substrate. Antioxidants help protect against oxidative stress[7].

Trace elements are vital for biochemical reactions necessary for cell survival and human health, though they constitute only <0.01% of our total body weight[8]. Trace minerals like selenium and zinc are essential in reducing oxidative stress, and in performing antioxidant functions, while iron and copper can behave as an oxidant[8]. Studies have shown typically higher levels of iron and copper, and lower levels of zinc and selenium in populations with obesity and dyslipidemia[9-12]. Cells protect themselves against oxidative stress by endogenous enzymatic antioxidants,

including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX), in a highly regulated endogenous defense system. These enzymes are important in reducing oxidative stress[13]. Trace elements are an integral component of antioxidant defense enzymes, such as the role of selenium in GPX, and that of copper and zinc in the antioxidant function of SOD[8,14-16].

Phytochemicals are plant-derived compounds that have gained attention for their protective effects against chronic diseases like CVD, cancer, osteoporosis, neurodegenerative diseases, and diabetes mellitus[17]. Strawberries are a popular berry fruit consumed in the US and previous clinical and observational studies show the role of strawberries in reducing CVD risks[18-22]. However, only a few studies have examined the role of dietary strawberries in modulating biomarkers of oxidative stress/antioxidant status, especially in participants with CVD risk factors, and this constitutes the scope of our current investigation.

Purpose

The purpose of this study is to identify the dose-dependent effects of a 12-week dietary strawberry supplementation on plasma trace elements and selected endogenous antioxidants in subjects with obesity and dyslipidemia.

Hypotheses

1. Consumption of freeze-dried strawberries (low vs. high dose) will affect plasma levels of trace elements, especially lower plasma iron, in obese individuals with above optimal serum lipids at 12 weeks when compared to baseline and their respective control groups.

2. Consumption of freeze-dried strawberries (low vs. high dose) will increase plasma levels of endogenous antioxidant catalase activity in obese individuals with above optimal serum lipids at 12 weeks when compared to baseline and their respective control groups.
3. Consumption of freeze-dried strawberries (low vs. high dose) will increase whole blood levels of endogenous antioxidant glutathione in obese individuals with above optimal serum lipids at 12 weeks when compared to baseline and their respective control groups.

CHAPTER II

REVIEW OF LITERATURE

A. Cardiovascular disease, dyslipidemia, and obesity and associations with trace elements and antioxidant status

i. Cardiovascular disease

Cardiovascular disease (CVD) tragically takes a toll on much of the world's population and remains the leading cause of mortality in the U.S.[1] Several of the risk factors underlying CVD are modifiable by dietary and lifestyle practices. These modifiable risk factors include overweight/obesity, especially abdominal obesity; dyslipidemia (high triglycerides, low HDL cholesterol, high LDL cholesterol, and high total cholesterol) and smoking[2]. Globally, the prevalence of overweight and obesity has reached what we know to be an all-time high: over 1.6 billion adults (20 years or older) are overweight and approximately 500 million within that category are also classified as obese[2]. In the US, 35.9% of adults over the age of 20 years are obese, while those who are overweight and including obesity make up 69.2% of adults aged 20+ years[23]. Dyslipidemia and hyperlipidemia, as strong CVD risk factors are prevalent in the developed countries, including the US[2]. Nearly one in six of Americans over 20 years of age have

high serum cholesterol levels – particularly high levels of serum LDL cholesterol – which is a major carrier of cholesterol and contributes to CVD[24]. High levels of HDL cholesterol and low levels of LDL cholesterol are desirable for cardiovascular health[8].

ii. **Dyslipidemia**

Dyslipidemia is characterized by four types of lipid disorders: high triglycerides, high total cholesterol, high LDL cholesterol, and low HDL cholesterol concentrations[25]. One in three US adults have elevated cholesterol levels, while less than half of this population seeks treatment to manage the condition. The American Heart Association (AHA) recommends optimal total serum cholesterol as below 200 mg/dL, with LDL cholesterol levels less than 100 mg/dL and HDL cholesterol levels greater than 60 mg/dL. The AHA also recommends optimal triglyceride levels as below 150 mg/dL[26].

Several aspects of research have established the positive relationship between elevated lipids and CVD[27,28,29]. Dietary intakes of total fat, saturated fatty acids, cholesterol, and *trans* fats have been shown to have a positive correlation with CVD risks, mostly due to the hypercholesterolemic effect, and increases in LDL: HDL cholesterol ratios. Lipids such as monounsaturated fatty acids, polyunsaturated fatty acids, and omega-3 fatty acids show an inverse correlation with CVD risks[30]. Dietary and lifestyle factors like weight loss, reduction in fat intake, increased fiber intake, and increased physical activity have been shown to reduce CVD risks and improve cardiovascular health.

iii. **Obesity**

Obesity is a strong CVD risk factor and its incidence is observed similarly in younger and older age groups. More notably, significant evidence has shown that an obese child is likely to grow into an obese adult[31-33]. For this reason, obesity is a significant public health concern in the US. The morbidity related to the next generation of adults who are obese is predicted to become worse

than it is now[33]. Body mass index (BMI), generally used as an indicator of obesity, is a calculation using an individual's height and weight to determine their weight classification. A BMI value 25 kg/m^2 or higher categorizes the individual as overweight, while a BMI 30 kg/m^2 or higher indicates that the individual is obese. Hip-to-waist ratio is also important in determining metabolic health by one's size and weight[34].

Increased amounts of adipose tissue in obese subjects lead to increased non-esterified fatty acids, glycerol, hormones (such as leptin and insulin), and pro-inflammatory cytokines when compared to the non-obese individual[35]. Obesity and weight gain result in increased oxidative stress, often resulting from the dysregulation of adipokine secretion that occurs in adipose tissue[5]. This dysregulation of adipokine secretion over time can lead to chronic conditions related to obesity, including insulin resistance, type 2 diabetes mellitus, atherosclerosis, and CVD[36,37].

The abnormal adipokine secretion in obesity affects the functions of other tissues, like the liver, muscle, central nervous system, and vasculatures[38]. Identifying the effects of oxidative stress in obesity is important because adipokine secretion influences many vital metabolic processes, such as impaired carbohydrate and lipid metabolism, energy expenditure, inflammation, endothelial function, and blood coagulation. All of these conditions contribute to elevated CVD risks[39].

iv. Oxidative stress

Oxidative stress identifies a biological condition when the production of ROS and the amounts of intracellular and extracellular antioxidants are under imbalance. While production of ROS is a normal metabolic process in living cells, the overproduction of ROS could lead to deleterious effects. Excessive oxidants mediate injury to cells or tissues and may lead to cell death by mechanisms of apoptosis and necrosis[7]. This damage may be caused by normal cellular metabolism, and by factors such as oxygen, light, free radicals, and metal ions, as well as the

aging process[40]. Endogenous oxidants like superoxide anion radical (O_2^-), hydrogen peroxide non-radical (H_2O_2), and hydroxyl radical ($^{\bullet}OH$) are products of normal aerobic respiration by mitochondria. Yet, chronic infections by bacteria, viruses, and parasites result in chronic inflammation, and thus lead to higher levels of these ROS[40].

Oxidative stress and obesity

Obesity has been associated with elevated oxidative stress and is a well-established CVD risk factor[3,4]. Fat accumulation has been shown to be correlated with systemic oxidative stress in both animal and human models of oxidative stress[5]. In studies involving healthy obese subjects with no pronounced health abnormalities other than high BMI, findings have shown a positive correlation between BMI and oxidative stress[41-44]. Animal models of oxidative stress have also produced identical results correlating adiposity with lipid peroxidation[45,46].

Since obesity increases the mechanical and metabolic burden on the myocardium, myocardial oxygen consumption is increased and production of ROS is also increased due to increased mitochondrial respiration[47]. A study evaluating abdominal obesity in pigs fed a high-fat diet reported that early phases of abdominal obesity resulted in increased incidence of coronary endothelial dysfunction, as well as vascular oxidative stress, hypertension, and mild abnormalities of lipid profiles compared to controls. However, no systemic inflammation or oxidative stress was observed, indicating that early obesity-related abnormalities are localized in the vasculature and contributes to increased risk for CVD[48].

Oxidative stress related to obesity is also likely to be induced by other possible conditions associated with obesity, including dyslipidemia , hypertension, poor diet, hyperglycemia, low physical activity, and smoking[49].

Oxidative stress and dyslipidemia

Dyslipidemia has been shown to elevate oxidative damage, resulting in increased oxidative stress[50]. LDL cholesterol contains a large amount of polyunsaturated fatty acids which is susceptible to becoming oxidized lipid radicals[6]. ROS promptly inactivates vascular nitric oxide (necessary for inhibition of platelet function and is a vasorelaxant), and can increase risks for atherosclerosis and stroke. Fibrinogen activity is enhanced by ROS and reactive nitrogen species (RNS), resulting in accelerated clot formation and thrombosis[38,51]. Oxidation of LDL cholesterol promotes monocyte activation leading to transformation into foam cells, which are mediators of the chronic process of atherosclerosis [52].

v. **Antioxidants**

An antioxidant has been defined as "any substance that can delay or prevent oxidation of a particular substrate"[53], or "any substance that, when present at low concentration compared to those of an oxidizable substrate, significantly delays or inhibits oxidation of that substrate"[54]. Antioxidants can be either enzymatic or non-enzymatic. They are reducing agents, or electron donors, that have the ability to reverse oxidation due to their nature of donating electrons and hydrogen ions[8].

Dietary or non-enzymatic antioxidants include micronutrients like vitamins A, C, and E, selenium, and zinc, as well as polyphenols like flavonoids, tannins, anthocyanins, and ellagic acid[8,40,55-57]. Food sources that contain high amounts of antioxidants include whole grains, vegetables, and fruits, particularly berries, and other yellow- and orange-colored fruits and vegetables. Among popular beverages, green tea and cocoa drinks also have noteworthy amounts of antioxidants[58]. These potent antioxidants have been shown to inhibit lipid oxidation and therefore reduce the likelihood for CVD and its risk factors[59].

Furthermore, antioxidants prevent DNA and protein damage, which could lessen the likelihood of developing chronic diseases like diabetes mellitus, a risk factor for CVD[55]. Enzymatic antioxidants are an integral constituent of the cellular defense mechanisms such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX). Low antioxidant status has been correlated with increased serum total cholesterol concentrations, while high plasma concentrations of antioxidants correlate with higher plasma HDL-cholesterol levels[8].

Vitamins as antioxidants

Among all vitamins, A, C and E deserve special attention as dietary antioxidants. Vitamin A, or retinol, can be synthesized in the body from dietary carotenoids, especially α-carotene and β-carotene. Orange and yellow vegetables, spinach, collards, and turnip greens are significant sources of β- and α-carotene. Retinol is a fat-soluble vitamin and will not be absorbed unless consumed with a fat-containing meal. Carotenoids in plants function as antioxidants and are quenchers of singlet oxygen, with lycopene being one of the most effective quenchers of this element[18].

Vitamin C, also known as ascorbate or ascorbic acid, is a water-soluble vitamin that is found in a variety or fruits and vegetables like papaya, oranges, broccoli, green peppers, grapefruit, and strawberries. This antioxidant is a reducing agent that is protective in aqueous solutions like in the blood and intracellular matrix. Ascorbate has a noteworthy reduction potential in that it will readily donate electrons to regenerate other antioxidants, such as vitamin E and glutathione. While vitamin C is known to be an antioxidant, it can also reduce transition metals like cupric ions (Cu^{2+}) to cuprous (Cu^{3+}) and ferric ions (Fe^{3+}) to ferrous (Fe^{2+}), while the vitamin itself is oxidized to a semidehydroascorbate radical. Ascorbic acid can later be regenerated by two semidehydroascorbate radicals to produce two ascorbate molecules[8].

Vitamin E has eight configurations, of which four are tocopherols and four are tocotrienols, and all contain the same phenolic functional group on a chromane ring and a phytyl side chain[8]. Vitamin E can be found in both plant and animal derived foods, including canola oil, olive oil, whole-grain cereals, and fatty tissues of animals. Vitamin E functions principally in maintaining the integrity of the cell membrane, where it protects cell damage by inhibiting peroxidation of phospholipids that make up the membrane. α-tocopherol is most effective in the ability to donate electrons, which act quickly to neutralize free radicals, like peroxyl radicals, thus reducing damage to the cell membrane[8].

vi. Trace elements: iron, zinc, copper, and selenium

Trace elements (minerals) are vital for biochemical reactions necessary for cell survival and human health, though they constitute only <0.01% of our total body weight. Trace elements like selenium and zinc are essential in reducing oxidative stress, and in performing antioxidant functions, while iron and copper can behave as an oxidant[8].

Iron

Iron in the body is primarily found in hemoglobin, but is also present in myoglobin, enzymes, blood, and in storage in much smaller amounts. Iron in the diet is found in either heme or nonheme form. Heme iron is mainly a component of animal products since it is present in hemoglobin and myoglobin. Nonheme is primarily present in plant foods like nuts, grains, tofu, fruits, and vegetables, but is also present in dairy products in smaller amounts[8]. Once released from the food components, most of the nonheme iron is present as ferric (Fe^{3+}) iron in the stomach, while some of it may be reduced to ferrous (Fe^{2+}) iron. Ferrous iron is absorbed in the small intestine by transporter binding at the brush border on the enterocyte; ferric state is reduced to ferrous state by ferrireductases and vitamin C in the intestine[60,61]. Ferric iron absorption

mechanisms are not well known, but an acidic environment is preferred and facilitation by chelation of iron with chelators or ligands helps solubilize ferric iron. Its absorption across the brush border requires membrane protein integrin, which is part of the paraferritin complex, also including mobilferrin and ferrireductase[61,62].

Chelators or ligands that bind with nonheme iron can either inhibit or enhance its absorption, part of which depends on the nature of the iron-chelate/ligand complex. Components in the diet that may enhance nonheme iron absorption include sugars, acids, meat, poultry, fish, and mucin, for each of these components act as chelators and can increase absorption of nonheme iron. Vitamin C acts as a reducing agent, forming a chelate with nonheme ferric iron in an acidic environment, and the chelate remains soluble in the small intestine which improves its absorption[62]. Factors in the diet that inhibit iron absorption include polyphenols, oxalic acid, phytates, calcium, calcium phosphate salts, and zinc. Polyphenols consumed with an iron-rich meal can reduce absorption of iron by 40-60%[63]. Phytates and oxalates bind to many minerals, and the resulting complexes are insoluble and poorly absorbed[64]. Calcium and phosphorus ingested in large amounts interact with iron absorption by chelate formation at the intestinal mucosa layer and can decrease absorption up to 70%. Zinc and iron may compete for the same transporters and negatively affect each other's absorption[65-67].

During transport in the blood, iron in its oxidized ferric state is bound to protein transferrin which also acts as an antioxidant. If iron is left unbound, harmful free radicals can result. Iron can behave as a pro-oxidant as free ferrous iron can readily react with H_2O_2, producing a free hydroxyl radical that is extremely reactive and damaging to cells. The binding of iron to protein is equally important to prevent free iron that can be used by bacteria to grow and proliferate[68]. However, iron is also important in the antioxidant defense system, such as in CAT which has four heme groups and converts two molecules of H_2O_2 to water and molecular oxygen.. Thus, while

free iron can act as a pro-oxidant, iron as part of antioxidant enzymes plays a crucial role in alleviating oxidative stress[69].

Zinc

Zinc is almost universally found as Zn^{2+}, its divalent ion, and is complexed with amino acids in food sources like red meat, seafood, poultry, pork, and dairy products. Whole grains and leafy or root vegetables represent good plant sources of zinc, but have a lower zinc content and the zinc located in plants isn't as efficiently absorbed as meat products[70]. Like iron, zinc must be hydrolyzed from its bound form with amino acids before absorption can take place. Zinc is absorbed into the intestinal cell by a carrier-mediated process and low zinc intakes are absorbed better than higher intakes. Passive diffusion and paracellular zinc absorption also may occur during periods of high zinc intake[71,72]. Zinc absorption can be enhanced or inhibited by chelators or ligands, depending on the absorbability of the chelate formed like in the case with iron[73]. Citric acid, prostaglandins, certain amino acids, pancreatic secretions, glutathione, tripeptides, and low zinc status tend to enhance zinc absorption. Absorption may be inhibited by phytate, oxalate or oxalic acid, polyphenols, folate, other divalent cations, and sometimes calcium[8,64,74].

Zinc, as a vital trace mineral, is present along with copper as a structural constituent of SOD, which is an endogenous antioxidant enzyme that takes part in antioxidant defense roles by catalyzing superoxide O^{-}_{2} radicals, into water and free molecular oxygen. Zinc is also known to be part of other enzyme systems essential for physiological functions. When zinc binds with protein thionein in the body, the complex is known as metallothionein. Although metallothionein serves as a storage site, it is also increased during states of stress and is known as an acute phase (reactant) protein. This protein has been found to stabilize membranes, regulate zinc and copper metabolism, and detoxify heavy metals. More importantly, this protein functions as a radical scavenging antioxidant of, for example, hydroxyl radicals. Zinc may contribute to the antioxidant

protection of the structure of cell membranes by eliminating free radicals, as a structural component of metallothionein[8,15,74,75].

Copper

Copper is present in either the cuprous state (Cu^{+}) or cupric state (Cu^{2+}) and is found in a wide variety of foods. Organ meats and shellfish are the richest sources, while plant sources high in this mineral include dried fruits, legumes, nuts, and seeds[8]. Most copper in foods is bound to amino acids and other organic components, which requires bound copper to be removed before absorption can take place[76]. Hydrochloric acid and pepsin in the stomach facilitate the release of bound copper. The stomach absorbs some copper, but most absorption takes place at the brush border of the small intestine. While copper's absorption process is not completely understood, it appears to be taken in by active carrier-mediated transporters as well as by passive diffusion. However, before absorption can occur, the copper ions must be reduced by copper reductase, stimulated by vitamin C. Copper status and available dietary copper influence the amount of copper that is absorbed in a given time. There are several enhancers of copper absorption, including amino acids, citric, gluconic, lactic, acetic, and malic acids. Yet, copper absorption can be inhibited by compounds such as phytate, zinc, iron, calcium, phosphorus, and a high pH environment[77].

Free copper, like iron, is typically bound to amino acids and glutathione, as free copper ions may damage cells by oxidizing reactions. More importantly, copper participates as an enzyme cofactor and allosteric component of enzymes, where in many functions copper will serve as an intermediate electron transfer[78,79]. Ceruloplasmin is a multifaceted copper-containing enzyme and antioxidant found in blood and bound to plasma membrane cell receptors. This glycoprotein oxidizes minerals like iron and manganese to bind to transferrin and is then transported to tissues. Ceruloplasmin also functions as a scavenger of oxygen radicals to protect cells and is a modulator

of inflammation[80,81]. SOD functions to catalyze removal of superoxide radicals where copper is reduced with the oxygen radical to generate molecular oxygen and then re-oxidized to hydrogen peroxide (H_2O_2). In absence of adequate SOD, superoxide radicals can damage unsaturated double bonds in cell membranes, fatty acids, and other cell molecules by formation of hydroxyl radicals. Peroxidation of cell membranes is found to be increased in copper deficiency[8].

Selenium

Selenium is a nonmetal that exists in a variety of oxidation states. Concentrations of selenium in plant foods will vary greatly depending on soil selenium concentration in different regions of the world. Seafood is known to represent a better source of selenium except that it may be poorly absorbed due to mercury-selenium complexes. Cereals, grains, organ meats, muscle meats, and dairy products contain selenium, but ranges vary among these sources. Dietary selenium is found primarily as selenomethionine in plants, and selenocysteine in animals. Selenium is well absorbed primarily in the duodenum. Vitamins A, C, and E, and reduced glutathione (GSH) enhance its absorption, while heavy metals and phytates inhibit selenium absorption by chelation and precipitation[8].

Selenium is an essential cofactor for enzyme glutathione peroxidase (GPX) and there are several forms of this endogenous enzyme. GPX catalyzes H_2O_2 and hydroperoxide removal from tissues, therefore assisting in the actions of an antioxidant system[16]. The availability of selenium affects GPX activity. Deficiency of selenium results in decreased GPX activity, GPX concentrations, and GPX mRNA concentrations[82-84]. GPX found in the cytosol is a selenoprotein with protective antioxidant effects on hemoglobin in red blood cells[16].

Selenoprotein P is a glycoprotein functioning as an antioxidant by removing peroxynitrite radicals, which are activated by white blood cells and can cause DNA single-strand breaks and lipid peroxidation[85]. Selenium function can be affected in the body by iron and copper

deficiencies; iron deficiency decreases hepatic GPX synthesis and reduces tissue selenium concentrations, and copper deficiency decreases GPX activities[86-88].

B. Trace element status in individuals with obesity and dyslipidemia

There are a number of studies reporting trace mineral status in obese individuals and those with the metabolic syndrome[89-92]. In a study by Liang et al. in overweight/obese subjects, trace elements, such as iron, zinc, copper, and selenium were significantly increased in the plasma compared to normal weight individuals[93]. In another study, elevated iron stores were correlated with abnormal glucose metabolism, a predictor of type 2 diabetes[94]. Iron has also shown to be a pro-oxidant in subjects with dyslipidemia due to its ability to oxidize LDL cholesterol[95,96]. Iron being a catalyst for hydroxyl radical formation, when present in high concentrations, may promote oxidative stress in pancreatic β cells[97]. Thus, iron may play a role in increasing risks for developing type 2 diabetes or insulin resistance in the obese population, mainly due to the associations with increased oxidative stress, insulin resistance and obesity[98]. Zinc and selenium are typically lower in obese subjects compared to normal weight individuals, while copper levels have been reported to be inconsistent and have been shown to be elevated or lowered in obese subjects vs. normal weight individuals[9-12].

Table 1: Observational studies on trace element status in obesity, dyslipidemia and related CVD risk factors

Trace Element	Sample Population	Study Design and Methods	Study Findings	Reference
Iron	Overweight male adults (n=1,416); BMI 26.66 kg/m^2; 20-49 years	Cross-sectional; NHANES (1988-1994)	Waist-to-hip ratio positively correlated with serum ferritin	Gillum et al., 2001[99]

Iron	Overweight male adults (n=860); BMI 28.29 kg/m^2; 40-74 years	Cross-sectional; NHANES (1988-1994)	Plasma TG levels positive correlated with serum ferritin levels in age subgroups 40-69 years	Gillum et al., 2005[100]
Iron	Overweight male (n=2,880) and female (n=3,069) adults with metabolic syndrome; waist circumference ≥102 cm for men and ≥88 cm for women; ≥20 years	Cross-sectional; NHANES (1988-1994)	Ferritin levels positively correlated with metabolic syndrome and individual components, including plasma TG	Jehn et al., 2004[101]
Iron	Overweight male (n=436) and female (n=379) adults; BMI 25.0 kg/m^2; mean age 26 years	Longitudinal cohort	In women: serum ferritin positively correlated with waist measurement, BMI, and TG. In men: positive correlation with serum ferritin and waist measurement, BMI, HDL, and TG	Williams et al., 2002[102]
Zinc	Obese male (n=11) and female (n=12) children and adolescents; BMI 30.1 kg/m^2; mean age 11 years	Case-control	Lower plasma Zn concentrations in obese individuals	Marreiro et al., 2004[9]
Zinc	Overweight female (n=23) adults who were on hypocaloric, Kawaga healthy diet for 6 months; BMI 25.5 kg/m^2; mean age 54 years	Randomized controlled	↑ Plasma Zn before tx ↓ body weight, BMI and percent body fat	Ishikawa et al., 2005[103]
Zinc	Overweight male (n=51) and female (n=190) adults; BMI 31.56 kg/m^2; mean age 39 years	Case-control	↓ serum Zn and SOD activity in the overweight group compared to control ↑ serum Zn and SOD activity in female overweight group compared to male overweight group	Tungtrongchitr et al., 2003[10]
Zinc	Obese male (n=62) and female (n=81) children and adolescents; BMI	Case-control	↓ serum Zn in obese compared to control and inversely correlated with skinfold thickness	Perrone et al., 1998[11]

	>95th percentile; mean age 11 years			
Copper	Overweight male (n=51) and female (n=190) adults; BMI 31.56 kg/m^2; mean age 39 years	Case-control	↑ serum ceruloplasmin and Cu in overweight subjects compared to control ↑ serum ceruloplasmin and Cu in female overweight group compared to male overweight group	Tungtrongchitr et al., 2003[10]
Copper	Obese male (n=62) and female (n=81) children and adolescents; BMI >95th percentile; mean age 11 years	Case-control	Serum Cu inversely correlated with age	Perrone et al., 1998[11]
Copper	Overweight male (n=167) and female (n=173) adults, BMI 26.8 kg/m^2; mean age 44 years	Cross-sectional	↓ Cu in obese subjects ↑ in sedentary subjects (obese or normal weight)	Sanchez et al., 2010[104]
Selenium	Overweight male (n=2,605) and female (n=2,847) adults; BMI 28.1 kg/m^2; mean age 43 years	Cross-sectional; NHANES (1988-1994)	↑ serum Se correlated with total cholesterol, HDL, LDL, and TG	Bleys et al., 2008[105]
Selenium	Male adults (n=364); BMI not reported; ages 21-59 years	Cross-sectional	Se positively correlated with serum cholesterol after adjustment for age and BMI	Jossa et al., 1991**[106]**
Selenium	Male (n=264) and female (n=309) children; BMI 18.9 kg/m^2 (15% overweight and 14.7% obese); mean age 10 years	Case-control	↓ serum Se in children with excess weight (BMI>P85) compared to normal weight children ↓ Se intake in obese children compared to normal weight Serum Se negatively correlated with all anthropometric variables recorded Serum Se negatively correlated with BMI	Ortega et al., 2012[12]

BMI – body mass index; NHANES – National Health and Nutrition Examination Survey; TG – triglycerides; Zn – zinc, tx – treatment, HDL – high-density lipoprotein, LDL – low-density lipoprotein, SOD – superoxide dismutase, Cu – copper, Se – selenium, CHD – coronary heart disease

Excess stored iron and increased risk for CVD was first identified in 1981 in a study assessing iron status and sex differences[107]. Ferritin, an iron storage protein, is found in cells of the liver, spleen, heart and kidneys. Serum ferritin concentrations correlate well with iron stored in the body. In a study assessing iron status in Mexican-American men aged 20-49 years, the researchers measured waist-to-hip ratio, waist-to-thigh ratio, ratio of subscapular to triceps skinfold thickness, and central-peripheral skinfold ratio, and revealed positive relationships with serum ferritin levels in all age groups of the study sample population[99]. Another study in adults 20 years or older reported increased serum ferritin with both increased abdominal adiposity and the prevalence of elevated triglycerides in men and postmenopausal women[101]. A third study in 26 year old males also found correlations between iron and waist circumference, BMI, and triglycerides, while men showed positive correlations between iron and BMI and waist circumference, but negative correlations with HDL-cholesterol and triglycerides. Serum ferritin in women was also strongly associated with CRP, thus increasing risks of CVD[102]. Abundant iron stores could promote lipid peroxidation and accelerate atherogenesis and CVD[108-111]. Catalytic iron converts ROS into highly reactive radicals, leading to elevated oxidative stress[108].

Serum zinc has been found to be lower in obesity than in healthy weight individuals in several studies[9,10,44,112]. Normal weight individuals have a higher zinc status and lower risks for developing diabetes, while lower zinc status was observed for obese subjects[113]. A study examining plasma levels of biomarkers after weight-loss in obese women showed higher zinc concentrations after weight loss and decreased BMI compared to baseline levels[103]. Similar results were seen in a study conducted in adolescents and children[114].

Higher serum ceruloplasmin and copper have been observed in some obese populations than in non-obese subjects[10,115]. These elevated levels could be a result of the body's demand for copper as an antioxidant to reduce LDL oxidation (in obese subjects with dyslipidemia). Yet, since

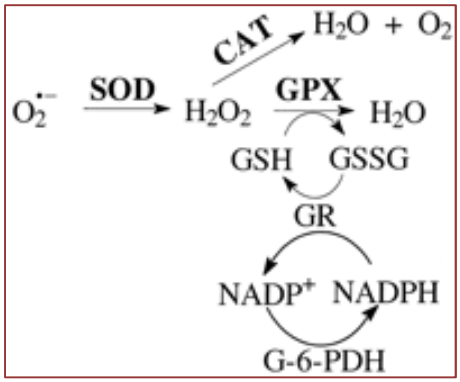
copper can act as a pro-oxidant, high serum copper can cause peroxidation of LDL cholesterol[115]. Serum copper has been found to be inversely related to HDL cholesterol, and the zinc/copper ratio showed a positive correlation with HDL cholesterol as well[116]. However, some studies show low copper levels in obese individuals versus non-obese individuals[11].

Selenium levels in normal weight, overweight, and obese subjects showed a positive association with total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides[105]. Other studies have also reported similar correlations[106,117-119]. Since selenium is a cofactor for the GPX enzyme, it has been studied in association with CVD. While high levels of selenium in plasma haven't been shown to be beneficial, low amounts have been correlated with CVD risk factors[119-122]. Low amounts of plasma selenium have been associated with vascular dysfunction, and this could also be linked with dyslipidemia[123].

C. Endogenous antioxidants

Cells protect themselves against oxidative stress by endogenous, enzymatic antioxidants, including SOD, CAT, and GPX, in a highly organized endogenous defense system[124]. These enzymes are important in reducing oxidative stress; SOD converts a superoxide radical into a hydroxyl radical and then to H_2O_2, followed by the action of CAT and GPX converting the H_2O_2 molecule into free oxygen and water molecules[125]. They are also important regulators of redox-sensitive signaling pathways[14].

Figure 1: The antioxidant defense system including SOD, CAT, and GPX



Li et al., 2000[126]

SOD converts superoxide radical into a hydroxyl radical and then to H_2O_2, as evidenced by increased levels of H_2O_2 with increased cellular SOD activity[127]. There are three different isoforms of SOD found in mammals, all of which catalyze the same reaction: CuZnSOD (SOD1), MnSOD (SOD2), and ecSOD (SOD3). SOD1 is found in the cell cytoplasm, mitochondrial inter membrane space, nucleus, lysosome, and peroxisome. SOD2 is found in mitochondrial matrix, while SOD3 is located in extracellular matrix, cell membrane, and extracellular fluids. SOD location within the cell is important for redox signaling. SOD conversion of O_2^- to H_2O_2 includes reduction and reoxidation of a transition metal at the enzyme's active site, such as copper or manganese. Thus, SOD requires the presence of a catalytic metal to carry out its role in the antioxidant defense system[127].

Catalase (CAT), found in the cytosol and cell peroxisomes, contains four heme iron groups which are helpful to recognize and react to H_2O_2 as its substrate[128,129]. CAT functions at a very low affinity. Thus, catalase may mainly function when H_2O_2 levels are above the physiological level, such as during an oxidative burst in response to stress. Peroxisomes produce H_2O_2 under physiologic conditions, but not O_2^-. Thus, catalase is found in this organelle to decompose H_2O_2 and prevent an accumulation of this damaging compound. If a peroxisome is badly damaged and

catalase is down-regulated, H_2O_2 will be released into the cytoplasm and contribute to significant oxidative stress[128].

GPX activity is said to represent the initial defense response to optimize H_2O_2 concentration under both normal and stressful conditions[38]. A high selenium intake is thought to increase GPX activity, thus decreasing the amount of H_2O_2 in the cell[130]. Glutathione, the co-factor for GPX is synthesized in the cytosol and is found in large concentrations in the cytosol, nucleus, and mitochondria[124,131]. Glutathione is protective against oxidative stress with its many roles, including as a cofactor of detoxifying enzymes that prevent oxidative stress (GPX, glutathione transferase, etc.), participation in singlet oxygen scavenging abilities, H_2O_2 and lipid peroxide detoxification, and its ability to regenerate vitamins C and E back to their reduced forms[124].

Glutathione may be found in two forms: oxidized glutathione disulphide (GSSG) and reduced glutathione (GSH). GPX is the enzyme that calalyzes the compound H_2O_2 to H_2O and free oxygen, while GSH is oxidized to GSSG simultaneously. GSH's role in the nucleus is to maintain the redox balance for necessary DNA repair and expression. An accurate measurement of oxidative stress in an organism is to determine the GSH/GSSG ratio. Excessive GSSG concentration may impair GPX activity, as GSH is GPX's substrate and is oxidized to GSSG when GPX acts to catalyze H_2O_2[132].

Table 2: Plasma antioxidant status in individuals with obesity and/or dyslipidemia

Sample Population	Study Design and Methods	Significant Findings about Plasma Antioxidants	Other Significant Study Findings	Reference
Obese male (n=100) and female (n=160)	Observational	BMI positively correlated with	Severe obesity positively	Olusi et al., 2002[41]

adults; BMI 19->50 kg/m^2; mean age 41 years		plasma MDA BMI negatively correlated with SOD and GPX activity	correlated with lipid peroxidation	
Obese female adults (n=36); mean BMI 38.5 kg/m^2; mean age 35.4 years	Randomized control	Before weight loss, obese women had significantly ↓ GPX compared to healthy weight controls	After weight loss, ↑ GPX compared to before weight loss After weight loss, ↓ GPX compared to control	Bougoulia et al., 2006[133]
Obese male (n=23) and female (n=31) children; BMI 25.4 kg/m^2; mean age 9 years	Case-control	↑ SOD in obese children compared to control	↑ cholesterol in obese children compared to control	Sfar et al., 2013[134]
Obese (n=27) and non-obese (n=57) female adults with and without PCOS; BMI of obese group 33.6 kg/m^2; mean age 31 years	Case-control	↑ CAT activity in obese women with and without PCOS compared to non-obese control	↑ serum GPX activity in obese group without PCOS compared to non-obese group without PCOS	Bausenwein et al., 2010[135]
Obese male (n=30) and female (n=103) adults; BMI 31.4 kg/m^2; mean age 43 years	Case-control	↑ serum GPX-3 in obese group compared to control	↑ TAG and ↓ HDL in obese compared to control	Baez-Duarte et al., 2012[136]

MDA – malondialdehyde (reactive species); BMI – body mass index; GPX – glutathione peroxidase; SOD – superoxide dismutase; CAT – catalase; GPX-3 – Nonselenocysteine-containing phospholipid hydroperoxide glutathione peroxidase; PCOS – polycystic ovary syndrome; WHR – waist-to-hip ratio; TAG – triacylglycerides

Antioxidant enzymes are found in the vascular wall in order to reduce ROS and defend cells from oxidative damage and prevent development of diseases like atherosclerosis[129]. There is no current evidence expressing a correlation between dyslipidemia presence and plasma antioxidant status, but the effect of obesity on antioxidant status varies. In a study examining levels of GPX and CuZn-SOD enzymes in humans, the authors reported that BMI negatively correlated with levels of these endogenous antioxidant enzymes, while BMI positively correlated with plasma MDA[41].

Similar results of decreased plasma antioxidants were found in a number of other studies in obese adults[137], obese children[138], obese male children[139], and obese female children[140]. Yet, Olusi et al. proposed that initiation of obesity is associated with a stimulation of antioxidant enzyme activity. However, once obesity becomes persistent, the antioxidant enzyme activity and reserves become depleted, as indicated by the findings in the study[41]. Over time, low activity of cytoprotective enzymes like GPX and catalase may lead to progressive tissue damage, atherosclerosis, cancer, and other chronic conditions[41]. Many other findings shown in Table 2 indicate that activity of SOD, catalase, and GPX may be elevated in the presence of obesity[134-136], and that weight loss may potentially improve these values compared with baseline values[133].

D. Polyphenols

Polyphenols are commonly consumed plant-derived compounds in the diet and are present mostly in fruits, vegetables, fruit juices, wine, black tea, green tea, coffee, as well as in cereals, chocolates, and dry legumes. These compounds contribute to the food's color, odor, flavor, and oxidative stability[17]. Since the mid-1990s, these compounds have been recognized for their health benefits and disease prevention characteristics[19,141-143]. Strong supporting evidence shows that polyphenols may confer protection against CVD, cancer, osteoporosis, neurodegenerative diseases, and diabetes mellitus[18-20]. Polyphenols have been shown to reduce CVD risk factors, such as lowering total TGs, total cholesterol, LDL-cholesterol, BMI, and waist circumference[22,144]. The polyphenol content of selected foods is shown below in Table 3:

Table 3: Commonly consumed polyphenol-containing foods in the US

Food source	Polyphenol content per serving (mg/serving)	Type of polyphenol	Calories per 100 g (kcal)
Strawberry	27.01	Anthocyanins	32

	4.6 1.65 0.26	Flavan-3-ols Flavonols Flavanones	
Blackberry	100.61 42.4 4.52	Anthocyanins Flavan-3-ols Flavonols	43
Blueberry	163.3 6.69 10.63 0.2	Anthocyanins Flavan-3-ols Flavonols Flavones	57
Gala Apple	1.22 8.21 3.8	Anthocyanins Flavan-3-ols Flavonols	52
Soybeans	37.41	Flavan-3-ols	70
Red wine	19.27 11.08 2.4 1.57	Anthocyanins Flavan-3-ols Flavones Flavonols	85
Candies, milk chocolate	10.88	Flavan-3-ols	535
Potato	1.5	Flavonols	97

Manach, et al., 2004; United States Department of Agriculture, 2009[145-147]

Many factors affect polyphenol content in fruits , including exposure to light, ripeness of the fruit, soil type in which the fruit was grown, fruit yield per tree, processing, and storage[148]. Food processing can significantly alter polyphenol content, especially those involving vacuum and convection methods of drying[149]. Wojdylo et al. reported that freeze-drying versus vacuum microwave drying methods did not significantly alter polyphenol compounds in strawberries[149].

Polyphenols in cocoa and berries have been shown to lower blood pressure, increase HDL-cholesterol[150,151], decrease inflammatory markers, and overall decrease risks for cardiovascular mortality[151,152]. Polyphenols such as those found in green tea may decrease lipid digestion and absorption by decreasing digestive enzyme activity and lipid emulsification[153]. Tea catechins neutralize reactive oxygen and nitrogen radicals, as well as act upon metal ion chelators that are active in the redox system[154]. Green tea, resveratrol, and curcumin have been shown to reduce adipocyte fat accumulation by activation, inhibition, and down-regulation of lipogenic enzymes and genes[155-158]. Furthermore, phenolic compounds in various foods have been shown to affect

trace mineral and endogenous antioxidant levels, as well as LDL cholesterol oxidation, thus modulating disease risks in humans[8,57,159-165].

In cell culture studies, polyphenols have been shown to alter trace minerals, such as iron and zinc absorption in the human enterocyte[166-169]. Reported studies include polyphenol-rich supplements such as green tea, green tea extract, or grape seed extract. Ma et al. reported a significant decrease in iron absorption when enterocytes were treated with polyphenols and iron, when compared to the iron only treated control group[166]. Iron transfer across the basolateral membrane was significantly lower in the enterocytes treated with iron and polyphenols versus iron only, leading to the observation that polyphenols may form complexes with iron in the cell and prevent iron from leaving the basolateral membrane. However, zinc uptake has been shown to be increased or unchanged in the presence of EGCG and green tea compared to the control group[170,171]. Specific polyphenols may bind with zinc and form a complex which improves mineral absorption, but the transport and cellular bioavailability of the complex thereafter remains unknown. Thus, polyphenols can significantly alter trace element status in vivo, especially those that are known to modulate oxidative stress and antioxidant status.

Table 4: Effects of polyphenols on trace element uptake in cell models (human Caco-2)

Duration	Polyphenol-Rich Source	Significant Study Findings	Reference
3 hour incubation	Phytate (66 mg/L); EGCG (46 mg/L); GT (46 mg/L); or GSE (46 mg/L)	↓ Fe uptake with EGCG, GT, and GSE tx ↓ apical Zn uptake with GSE tx ↓ Zn uptake with phytate tx	Kim et al., 2000[170]
7 hour incubation	EGCG (0.46, 4.6, or 46 mg/L); GT (0.46, 4.6, or 46 mg/L); or GSE (0.46, 4.6, or 46 mg/L)	↓ Fe uptake by EGCG, GSE and GT compared to control	Ma et al., 2012[172]
7 hour incubation	EGCG (46 mg/L) or GSE (46 mg/L)	↓ Fe uptake by EGCG and GSE tx	Ma et al., 2010[167]
3 hour total	2 mL RW, RGJ, or GT; or 200 μmol/L tannic	↑ Zn uptake with RW, RGJ, and GT tx compared to control	Sreeniva-sulu et al.,

incubation	acid, quercitin, catechin, gallic acid, or caffeic acid	↓ Zn uptake with RW fraction excluding polyphenols ↑ Zn uptake with tannic acid and quercitin tx compared to control	2010[171]
24 hour incubation	0.73 mL apple, pear, white grape, RGJ, prune, grapefruit, or orange juice	↓ Caco-2 cell ferritin formation (from iron) with RGJ and prune juice tx compared to control	Boato et al., 2002[173]

Fe – iron, Zn – zinc, treatment – tx, DMEM – Dulbecco's Modified Essential Medium, EGCG – epigallocatechin-3-gallate, GT – green tea, GSE – grape seed extract, RW – red wine, RGJ – red grape juice

Altered levels of trace elements have been observed in healthy animal models following dietary supplementation of polyphenols[174-176]. Polyphenols have also been shown to alter endogenous antioxidant activity in animals models of diet-induced obesity, which may alter absorption and activity of the trace minerals that are structural constituents of endogenous antioxidant systems[177,178].

Table 5: Effects of polyphenols on general antioxidant status in diet-induced animal models of obesity

Animal Model	Duration	Polyphenol-Rich Source	Significant Study Findings	Reference
Male Sprague Dawley rats (n=40), fed normal or HF diet with or without red grape skins	Four weeks	Red grape skins, 20 mg/kg/day	↓ CAT activity in HF diet compared to normal diet control ↑ CAT and SOD in HF and normal diet with red grape skin tx ↑ GSH/GSSG ratio in normal diet and red grape skin tx + normal diet	Lee et al., 2009[174]
Sprague Dawley female rats (n=36), fed normal, HF, or HF diet + GTP	Eight months (four months with GTP)	GTP, 0.5% (wt/vol) of distilled water	↑ liver GPX activity in HF + GTP compared to HF diet control	Shen et al., 2012[175]
Obese Zucker fatty rats (n=24), fed normal diet or diet + RWP	Eight weeks	RWP, 20 mg/kg/day	↓ O_2^- from aorta, carotid arteries, and SMA with RWP tx compared to control	Agouni et al., 2009[176]

HF – high-fat, CAT – catalase, SOD – superoxide dismutase, GSH – reduced glutathione, GSSG – oxidized glutathione, GTP – green tea polyphenols, RWP – red wine polyphenols, SMA - small mesenteric artery

i. Strawberries: bioactive compounds

Strawberries, among many other berries, are rich in vitamins and polyphenols beneficial in disease prevention and overall health promotion. Phytochemicals of particular interest found in strawberries include glycosides anthocyanins and 2,5-dimethyl-4-hydroxy-3-[2H] furanone (DMHF), catechins, ellagic acid and hydroxycinnamic acid derivatives, and flavanols, such as quercetin and kaempferol[149,179-181]. Strawberries contain both free and bound phenolics. Bound polyphenols may remain undigested in the stomach and intestine and then be acted upon by bacteria in the colon. Strawberries contain 92.3% of their polyphenols in the free form, allowing the small intestine to absorb them and then these may be utilized in the body[182]. Polyphenol content, ferric reducing ability of plasma (FRAP), and Trolox equivalent antioxidant capacity (TEAC) in strawberries are significantly decreased during processing methods, including mashing, pressing juice, filtering juice, pasteurizing juice, and pureeing. However, anthocyanin composition is significantly increased during processing methods like mashing and pureeing[183].

Table 6: Nutritive value and composition of fresh strawberries

Nutrient	Unit	Value per 100 g
Water	g	90.95
Energy	Kcal	32
Total lipid	g	0.3
Protein	g	0.67
Fiber, total dietary	g	2.0
Total carbohydrate	g	7.68
Sugars	g	4.89
Iron	mg	0.41
Zinc	mg	0.14
Vitamin A	IU	12
Vitamin C	mg	58.8
Vitamin E	mg	0.29

Agricultural Research Service USDoA, 2014

Strawberries are high in vitamin C and polyphenols, as well as fiber and thus have been known to reduce serum cholesterol levels and CVD risks in some studies[180,184,185]. Strawberry consumption has been shown to decrease serum glucose, total cholesterol and LDL cholesterol in obese adults[186,187]. Studies involving anthocyanin and ellagitannin consumption have shown that these phytochemicals may prevent incidence of cancer, CVD, and age-related decreased neurodegenerative function[180,188,189]. These preventative effects are thought to be associated with phenolic acids, mainly due to their antioxidant properties and ability to neutralize ROS in prevention of chronic diseases[181].

Furthermore, consumption of strawberries has been shown to improve plasma antioxidant enzyme activity and reduce oxidative stress in healthy human subjects[181,190]. Experimental studies have also shown compounds in strawberries to possess ROS-scavenging activity[182,191-193]. Compounds in strawberry juice have shown to scavenge free radicals such as, O_2^-, H_2O_2, and $^{\bullet}OH$. Lin et al. found that among different berries, strawberries and blackberries have the highest ROS scavenging capacity when exposed to the individual reactive compounds, thus providing evidence that strawberries contain bioactive antioxidants[192].

E. Effects of polyphenols on trace minerals/general antioxidants in human subjects with obesity and/or dyslipidemia

While polyphenols have been found to be beneficial in healthy human populations, they have also been shown to reduce risks of chronic diseases in populations with obesity and/or dyslipidemia. Mechanisms by which polyphenols may prevent weight gain include their ability to increase lipolysis, decrease lipogenesis, stimulate β-oxidation, inhibit adipocyte growth and differentiation, and debilitate inflammatory response and oxidative stress[194]. Another important property that polyphenols, especially flavonoids and tannins, possess is the tendency for phenolic

hydroxyl groups to chelate metal ions, further contributing to their antioxidant activity[164]. The capacity to bind metals may be relevant to the phenolic antioxidant activity, considering free transition metal ions are pro-oxidants which produce free radicals in the presence of H_2O_2. Due to the presence of phenolic hydroxyl groups, electron delocalization principles allow phenolic groups to be readily ionized and thus act as weak acids which can influence chemical reactivity of phenolics[159].

Many studies have shown improved levels of selected trace elements[195,196], increased total antioxidant capacity[21,190,195,197,198], and decreased LDL cholesterol oxidation[190,197,199] in study participants supplemented with a polyphenol-rich source (Table 7).

Table 7: Effects of polyphenols on trace elements and general antioxidant status/endogenous antioxidants in overweight or obese participants with/without dyslipidemia

Sample Population	Study Design and Methods	Duration	Polyphenol-Rich Source	Significant Study Findings	Reference
Obese male (n=23) and female (n=23) adults; BMI 32.76 kg/m^2; mean age 50 years	Randomized, double-blind, placebo-controlled	Three months	379 mg GTE	↓ BMI, waist circumference, serum TC, LDL-cholesterol, and TG levels compared to control ↑ TAC after tx compared to control ↓ serum Fe and Zn after tx compared to baseline	Sulibur-ska et al., 2012 [21]
Male (n=5) and female (n=20) adults with mild dyslipidemia;	Randomized control	12 weeks	Three tablets of 280 mg mulberry leaf powder, three times daily	↓ TC/HDL ratio and serum TG and LDL levels compared to control	Aramwit et al., 2013[200]

BMI 23.18 kg/m^2, mean age 36 years					
Overweight female adults (n=8); BMI 26.1 kg/m^2; mean age 67 years	Clinical	Four hours	240 g fresh strawberries	↑ serum antioxidant capacity during initial four hours after consumption compared to control	Cao et al., 1998[190]
Overweight male adults (n=21); BMI 26.9 kg/m^2; mean age 38 years	Clinical	14 days	7 mL/kg cranberry juice	↓ plasma oxLDL levels compared to baseline ↑ plasma antioxidant capacity compared to baseline	Ruel et al., 2005[197]
Obese male (n=13) and female (n=15) adults with HTN; BMI 32.5 kg/m^2; mean age 49 years	Randomized double-blind, placebo-controlled	Three months	379 mg GTE	↑ TAC compared to control	Bogdan-ski et al., 2012 [198]
Obese male (n=22) and female (n=25) adults; BMI 30.59 kg/m^2; mean age 57 years	Randomized, parallel and double-blind case-control	Four weeks	1.4 g cocoa extract (645.3 mg of polyphenols)	↓ plasma oxLDL and MPO in tx group compared to control	Ibero-Baraibar et al., 2013[199]
Obese male (n=8) and female (n=27) adults with metabolic syndrome; BMI 36.33 kg/m^2; mean age 42 years	Single-blinded randomized controlled	Eight weeks	4 cups/day green tea, or 2 capsules (870 mg catechins) GTE	↑ whole blood GSH and plasma antioxidant capacity in GTE tx group and green tea tx group compared to control ↓ plasma Fe in GTE tx group compared to baseline ↑ plasma Cu in green tea tx group compared to GTE tx group after tx	Basu et al., 2013[195]

Overweight male (n=10) and female (n=14) adults with hyperlipidemia; BMI 29.2 kg/m^2; mean age 51 years	Randomized, single-blind, placebo-controlled, crossover	12 weeks (6-week crossover point)	10 g FDS beverage (338 mg total phenolic compounds) with high-fat meal	↓ plasma oxLDL after FDS beverage supplementation compared to placebo	Burton-Freeman et al., 2010[201]

GTE – green tea extract, BMI – body mass index, TC – total cholesterol, LDL – low-density lipoprotein, TG – triglycerides, TAC – total antioxidant capacity, Zn – zinc, Fe – iron, tx – treatment, GPX – glutathione peroxidase, HDL- high-density lipoprotein, HTN – hypertension, oxLDL – oxidized low-density lipoprotein, MPO – myeloperoxidase, GSH – glutathione, Cu – copper, FDS – freeze-dried strawberry

Adults with obesity and dyslipidemia have increased oxidative stress and an increased risk for CVD. Trace elements have been shown to be altered in subjects with obesity and dyslipidemia, as iron is typically elevated and has a pro-oxidant effect, while zinc and selenium can act as antioxidants and are typically decreased in this population. Dietary and antioxidant defense enzymes are typically lower in obese and dyslipidemic populations compared to the healthy population, Plasma trace element and antioxidant levels are significant indicators of oxidative stress, which may contribute to the pathophysiology of chronic diseases, especially CVD. Strawberries and other polyphenol-rich foods have been shown to improve trace element and antioxidant status in subjects with obesity.

While research provides some evidence on the role of polyphenols in improving antioxidant status and altering trace element levels, there is a lack of clinical data on the role of strawberries in modulating these biomarkers, especially in subjects with obesity and dyslipidemia. With the dramatic increase in the prevalence of obesity, dyslipidemia, and related chronic health conditions like CVD associated with elevated oxidative stress, there is an urgent need for research in this area. Thus, we intend to address this research gap and examine the effects of strawberries on plasma trace elements, especially iron, copper, zinc, and selenium, and antioxidants such as catalase and GSH in subjects with obesity and dyslipidemia. We hypothesize that the

consumption of freeze-dried strawberries will affect the levels of trace elements, especially lower plasma iron, in obese individuals with above optimal serum lipids when compared to the control group. We also hypothesize that the consumption of freeze-dried strawberries will affect the levels of endogenous antioxidants, especially plasma catalase and GSH in obese individuals with above optimal serum lipids when compared to the control group in a 12-week intervention trial.

CHAPTER III

METHODOLOGY

3.1 Participants and study design

Male and female adults (≥21 years) with abdominal adiposity (waist circumference in men >40 inches, women >35 inches) and dyslipidemia (two of four criteria: fasting total cholesterol >200mg/dL, triglycerides >150mg/dL, LDL-cholesterol >100 mg/dL, or HDL-cholesterol (men<40mg/dL, women <50 mg/dL), as defined by National Cholesterol Education Program (NCEP), Adult Treatment Panel (ATP) III were included in the study. Subjects with normal liver, kidney, and thyroid function tests were included in the study. Subjects on stable multivitamin/mineral supplements or prescription medications (except hypolipidemic, hypoglycemic, and steroid agents) were included in the study. Subjects with any form of pre-existing disease, e.g. cancer, heart disease, diabetes (fasting blood glucose ≥126mg/dL), liver, or renal disorders, anemia, pregnancy and lactation, taking mega doses of antioxidants/fish oil supplements (> 1g/day), taking hypolipidemic, hypoglycemic, and steroid medications, abnormal hemoglobin (normal range: 12.0-18.0 g/dL), white blood cell (normal range: 4.0-11.0 thousands per cubic milliliter (K/mm^3)), or platelets (140-440 K/mm^3), hypo/hyperthyroidism (normal range for thyroid stimulating hormone: 0.35- 4.940 µIU/mL), abnormal liver enzymes (normal range for aspartate aminotransferase (AST): 7-40 units/L; alanine aminotransferase(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), smoking, and drinking alcohol (>1oz/day) were excluded from the study. Both males and females, as well as individuals from any ethnic group, who qualify, were included in the study. People who have ever been allergic to strawberries were excluded from the study. The study was approved by the ethics committees at the Oklahoma University Health Sciences Center (OUHSC) and at Oklahoma State University (OSU), and all participants provided written informed consent prior to any study procedures.

Subjects were recruited at the General Clinical Research Center (GCRC) at Oklahoma University (OU) and at the Department of Nutritional Sciences (NSCI) at OSU via flyers and campus e-mail advertisements (30 subjects/site). Following an initial telephone screen, subjects were scheduled for a screening visit and qualification was confirmed based on the two qualifying measurements of abdominal adiposity and dyslipidemia, as mentioned earlier. Upon qualification, subjects were then randomized using a block randomization design to account for the effects of age and gender on the variables of interests. Participants were recruited into quartets matched for age (± 5 years) and gender. The age and gender for a quartet was determined by the first participant assigned to that quartet. The next consecutive participant that met the matching criteria of that quartet was assigned as the second participant of that quartet, and so on. Each quartet had one participant in each of the four intervention groups: low fiber/calorie control, low freeze-dried strawberries (FDS) beverage, high fiber/calorie control, or high FDS beverage. While quartets had been filled consecutively within the matching parameters, the intervention to which the first, second, third, and fourth participants in the quartet was assigned was pre-determined by random permutation.

All participants consumed two cups of FDS or control beverage. Each cup contained 12.5 g FDS blended in 1 cup water with added Splenda (optional) in the low dose group, or 25 g FDS blended in 1 cup water with added Splenda (optional) in the high dose group. The matched control beverage contained 2 g fiber (vegetable fibers and natural gums, containing both insoluble and

soluble fiber (1:2) per serving (Fiberstir)) and 2 tsp sugar blended in 1 cup water in the low fiber/calorie control group, or 4 g fiber (vegetable fibers and natural gums, containing both insoluble and soluble fiber (1:2) per serving (Fiberstir)) and 4 tsp sugar blended in 1 cup water in the high fiber/calorie control group. The control beverage also had artificial strawberry flavor and food color added to the drinks. The low dose FDS and matched control beverage contained equal amounts of fiber (4 g) and calories (75 kcal) per day, while the high dose FDS and matched control contained 8 g fiber and 150 kcal per day for 12 weeks (Table 8).

All subjects came for three days/week of mandatory visits to the research facilities to ensure compliance and the remaining quantity of the beverages had been provided in containers for later consumption. All subjects were asked to follow usual diet and lifestyle, and refrain from other sources of berries and related products while on the study. Subjects also maintained 3-day food records at screen, 6 and 12 weeks of the study. Height, weight, blood pressure, and waist circumference was measured by trained personnel at GCRC and at NSCI at screen and at 12 weeks of the study. Blood draws were performed by trained nurses at GCRC and by trained phlebotomists at Stillwater Medical Center at OSU.

Fasting blood samples were immediately sent to OU Medical Center laboratory (Oklahoma City, Oklahoma) or the Stillwater Medical Center laboratory (Stillwater, Oklahoma) for comprehensive metabolic panel (CMP) including glucose, insulin, glycated hemoglobin, lipid panel, electrolytes, liver, kidney, thyroid tests, and complete blood count. Remaining plasma and serum samples were stored at -80ºC for subsequent analyses.

3.2 Plasma trace elements

Plasma levels of iron, zinc, copper, and selenium were measured using inductively coupled plasma mass spectroscopy (Elan 9000; Perkin Elmer, Norwalk, Connecticut) based on previously published procedures[202]. Plasma samples were diluted 1:50 in 0.05% Triton X-100 (Sigma-

Aldrich, St. Louis, Missouri) and analyzed by inductively coupled plasma mass spectroscopy (ICP-MS, Elan 9000, Perkin Elmer, Norwalk, Connecticut) with gallium as internal standard. Plasma samples were diluted 1:20 in 0.1% nitric acid (GFS Chemicals, Powell, Ohio) and analyzed by ICP-MS using gallium as internal standard. The OSU Nutritional Sciences laboratory participates in the CDC Laboratory and Multielement Proficiency (LAMP) program. Tri-level blood lead control samples and plasma control samples were utilized (Utak Laboratories, Inc., Valencia, California) and were within expected ranges. A standard cocktail solution containing iron, zinc, copper, and selenium at a concentration of 100 µg/L was prepared (from commercial standard 1-g/L solution) and stored in pre-cleaned polyethylene volumetric flasks. A simulated blank solution was used to correct for interferences from polyatomic ions [0.14 mol/L nitric acid, internal standard (100 µg/L gallium), sodium chloride, sodium nitrate, cysteine, and calcium nitrate.] Quantitative analyses were performed using the scanning mode data acquisition. For each analyte (iron, zinc, copper, and selenium), peak area (signal) was divided (normalized) by the signal of the internal standard. For each element, the average normalized signal of the blank solution was subtracted from the average normalized signal of the diluted plasma solution. The inter-assay CV was within 5% for each element.

3.3 Catalase and reduced glutathione

Serum catalase enzyme activity was measured using Cayman Chemical Company (Ann Arbor, Michigan) spectrophotometric Catalase Assay Kit based on the manufacturer's protocol. Samples were pipetted to wells, where 100 µL diluted Assay Buffer, 30 µL methanol, and 20 µL of sample were added in duplicates. The reaction was initiated by adding 20 µL of diluted hydrogen peroxide to all wells and were incubated on a plate shaker for 20 minutes. Next, 30 µL of diluted potassium hydroxide were added to each well to terminate the reaction and the addition of 30 µL of catalase purpald (chromogen) followed. Catalase purpald forms bicyclic heterocycles with aldehydes, which change from colorless to a purple color upon oxidation. The plate was

incubated on the plate shaker for ten minutes, followed by the addition of 10 µL of catalase potassium periodate to each well. The wells were incubated on a plate shaker a final time for five minutes at room temperature and then immediately analyzed by the microplate reader (Bio-Tek Synergy HT Multi-Detection, Winooski, Vermont) at 540 nm.

Reduced glutathione content in heparinized whole blood sample was measured using the method described by Beutler et al[203]. Briefly, 100 mL of hemolyzed blood sample and 200 mL of 2.5 Mm 5,5′-dithiobis-2-nitrobenzoic acid (Sigma, St. Louis, Missouri) were mixed in tubes containing 1.9 mL Tris-HCL buffer, Ph 8.0. The absorbance of the yellow thiolate anion was measured at 412 nm. GSH (Sigma, St. Louis, Missouri, USA) was used as a standard. Calibration curve was used to calculate concentration and was expressed as µg/g hemoglobin. The average inter-assay CV was 5.2%.

3.4 Statistical analyses

Descriptive statistics were calculated for all parameters, and graphs were drawn to look for outliers. The primary objective was to identify differences in means of antioxidant and trace element parameters at screening (week 0) and end of study (week 12) between low FDS beverage and control, and high FDS and control treatment. Within-group differences were analyzed using paired *t* test. Means at screen and 12 weeks were compared using the multivariate analysis of variance (MANOVA). All data were expressed as means ± standard deviation for the variables of interests, with significance level set at 0.05. SPSS for Windows (version 15.0, 2006; SPSS Inc, Chicago, Illinois) was used for the statistical calculations.

CHAPTER IV

FINDINGS

Baseline characteristics

Among the 85 participants screened for the study, 66 met the inclusion and exclusion criteria, and were enrolled in the study. Among the 66 enrolled, six participants dropped out of the study due to their time constraints and inability to make the mandatory three visits per week to the study centers. Thus, 60 participants completed the 12-week study in strawberry and control arms. Among these participants, compliance was 100% for the strawberry groups and 97% for the control groups, as assessed by mandatory weekly visits and any unconsumed beverages. No adverse events were reported in the study. At baseline, no significant differences in clinical and demographic characteristics were noted in the low dose FDS (25g/day) vs. low dose control group and in the high dose FDS (50g/day) vs. HDC (Table 9).

Trace elements

i. Iron and copper

As shown in Table 10, at baseline ^{54}Fe was not significantly different between high dose FDS and low dose FDS or between high dose FDS and HDC. However, at baseline, ^{54}Fe was significantly

lower in the low dose FDS versus LDC ($p<0.05$). At baseline, ^{57}Fe was not significantly different among any groups. There were no significant differences the two iron isotopes measured at 12 weeks. There were no significant differences in copper at baseline or at 12 weeks among any groups. Within-group comparisons revealed no significant differences in plasma iron and copper.

ii. Zinc and selenium

No significant differences in zinc or selenium were noted at baseline or at 12 weeks between high dose FDS and low dose FDS as well as their respective matched controls. Within-group comparisons revealed no significant differences in plasma zinc and selenium (Table 10).

Catalase

At baseline, catalase activity was not significantly different between high dose FDS and HDC or between low dose FDS and LDC. Yet, at baseline, catalase activity was significantly higher in the high dose FDS versus low dose FDS (Table 11.) However, no significant differences were noted at 12 weeks among any of the groups. In the high dose FDS group, within-group comparison revealed catalase activity to be significantly lower at 12 weeks compared to baseline (Table 11).

Reduced glutathione (GSH)

As shown in Figure 3, GSH was not significantly different between high dose FDS and HDC, but was significantly higher in the high dose FDS versus low dose FDS and was significantly lower in the low dose FDS versus LDC at baseline. At 12 weeks, GSH was significantly higher in high dose FDS versus low dose FDS, as well as when compared to its respective control group. Within-group comparisons revealed that GSH was significantly higher at 12 weeks compared to baseline, in both low dose FDS and high dose FDS groups (Table 12).

Table 8: Composition of strawberry and control beverages administered in the 12-week study*

Composition	Low dose FDS	Low dose control	High dose FDS	High dose control
FDS (g)[1]	25	-	50	-
Fiber (g)[2]	4.0	4.0	8.0	8.0
Calories (kcal)	75	80	150	144
Protein (g)	1.8	-	3.5	-
Fat (g)	0.3	-	0.5	-
Carbohydrates (g)	16	20	32	36
Ash (g)	1.5	-	3.2	-
Vitamin C (mg)	55	-	109	-
Total phenolics (mg gallic acid equivalents)	1001	-	2005	-
Total anthocyanins (mg cyaniding-3-glucoside equivalents)	78	-	155	-
Ellagic acid (mg)	106	-	220	-
Phytosterols (mg)	23	-	50	-

* Strawberry composition information provided by the California Strawberry Commission

[1]FDS, freeze-dried strawberries (California Strawberry Commission, CA)

[2]Fiber, insoluble and soluble fiber (FiberStir, LLC, Minneapolis, MN) for low and high dose control beverages

Table 9: Baseline characteristics of the study participants[1]

Variable	Low dose FDS	Low dose control	High dose FDS	High dose control
n	15	15	15	15
Age (years)	50±10	48±10	49±11	48±10
M/F (n/n)	1/14	1/14	2/13	1/14
Waist (inches)	41±3	43±3.2	45±5	42±2.6
Height (cm)	165±5.8	165±6.7	164±6.5	168.5±7.6
Weight (kg)	94.6±13.5	100±12	101±18	99±15
BMI (kg/m^2)	34.5±4.4	37±4.4	38±7	35±5
BUN (mg/dL)	14±2.3	15.7±4.5	16±4.2	17±5
Creatinine (mg/dL)	0.8±0.2	0.8±0.2	0.8±0.2	0.9±0.3
AST (U/L)	29±10	26±7.1	25±4.3	25±7.2
ALT (U/L)	34±12	34±12	30±11	31±12
WBC (K/mm^3)	6.6±1.2	6.7±1.4	6.7±1.7	7.1±1.5
RBC (M/mm^3)	4.6±0.5	4.5±0.3	4.6±0.6	4.8±0.4
Hb (g/dL)	14±1.4	13.6±1.3	14±1.4	14±1.6
Multivitamin users (%)	20.0	20.0	20.0	10.0
Fruit servings, cups[2]	1.2	1.0	1.2	1.0
Vegetable servings, cups[2]	1.0	1.1	1.1	1.2

[1]Values are mean ± SD

[2]Servings consumed based on diet recall at baseline

FDS – freeze-dried strawberries; M/F – male/female; BMI – body mass index; BUN – blood urea nitrogen; AST – aspartate aminotransferase; ALT – alanine aminotransferase; WBC – white blood cell; K/mm^3 – thousands per cubic milliliter; RBC – red blood cell; M/mm^3 – millions per cubic milliliter; Hb - hemoglobin

Table 10: Plasma trace element levels at baseline and 12 weeks after FDS supplementation

Group	Time Point	^{54}Fe (mg/L)	^{57}Fe (mg/L)	^{63}Cu (mg/L)	^{65}Cu (mg/L)	^{66}Zn (mg/L)	^{82}Se (mg/L)
Low dose FDS	Baseline	1.0±0.4*	1.4±1.0	1.3±0.4	1.1±0.3	1.0±0.7	0.1±0.1
	Week 12	0.9±0.4	1.8±0.9	1.5±0.4	1.3±0.4	0.7±0.4	0.1±0.1
Low dose control	Baseline	1.7±1.8	2.5±2.4	1.5±0.5	1.3±0.4	1.4±0.9	0.2±0.1
	Week 12	0.9±0.4	1.5±0.9	1.6±0.5	1.4±0.5	0.8±0.6	0.1±0.8
High dose FDS	Baseline	1.0±0.4	1.3±1.2	1.9±1.1	1.6±1.1	1.0±0.4	0.1±0.1
	Week 12	1.1±0.5	1.8±1.0	1.6±0.7	1.5±0.6	0.8±0.5	0.1±0.1
High dose control	Baseline	0.9±0.3	1.1±0.7	1.3±0.4	1.2±0.4	0.9±0.6	0.1±0.1
	Week 12	1.2±0.8	2.0±1.3	1.6±0.4	1.4±0.4	0.9±0.7	0.1±0.1

Footnote: Fe – iron; Cu – copper; Zn – zinc; Se – selenium; FDS - freeze-dried strawberries

*$P < 0.05$ versus low dose control at baseline

Table 11: Plasma catalase activity levels at baseline and 12 weeks after FDS supplementation

Intervention Group	Baseline (nmol/min/mL)	Week 12 (nmol/min/mL)
Low dose FDS	38.5±39.3	13.2±4
Low dose control	77.7±73.2	32.3±28.4
High dose FDS	114.6±82.9*	14.6±3.3**
High dose control	63.7±74	84.7±88.7

Footnote: FDS – freeze-dried strawberries

* $P < 0.05$ versus low dose FDS at baseline

** $P < 0.05$ versus high dose FDS at baseline

Table 12: Whole blood GSH levels at baseline and 12 weeks after FDS supplementation

Intervention Group	Baseline (µg/g Hb)	Week 12 (µg/g Hb)
Low dose FDS	1657.1±180**	2294.9±500.4 †
Low dose control	1898.8±164.1	1775.3±153
High dose FDS	1824.2±119.5*	2860.2±381.6*** †
High dose control	1789.1±199.5	1862.2±195.1

Footnote: GSH - glutathione; FDS – freeze-dried strawberries

* $P < 0.05$ versus low dose FDS at baseline

** $P < 0.05$ versus low dose control at baseline

*** $P < 0.05$ versus low dose FDS and high dose control at 12 weeks

† $P < 0.05$ compared to baseline

CHAPTER V

CONCLUSION

Overall findings

To our understanding, this is the first clinical study to examine the effects of freeze-dried strawberries (FDS) in a dose-response manner, on trace elements and endogenous antioxidant markers in obese subjects with dyslipidemia. No significant effects were observed in plasma iron, copper, zinc, and selenium, except ^{54}Fe which was significantly lower in low dose FDS versus LDC at baseline only. In the high dose FDS supplemented group, catalase activity at 12 weeks was significantly lower when compared with baseline values. At baseline, GSH values were significantly higher in high dose FDS and low dose FDS, while significantly lower in low dose FDS versus LDC. When compared with the fiber- and calorie-matched controls, significant increases in GSH were detected in both high dose FDS and low dose FDS groups after 12 weeks of FDS supplementation Furthermore, GSH was significantly higher in high dose FDS versus low dose FDS at 12-weeks.

Trace element:

Trace elements are critical to human health and cellular function via participation in biochemical reactions, as components of enzymatic structure, and also act as enzymatic cofactors[8]. Iron and copper are known to participate in oxidizing reactions which can be damaging to cells[68,69,78],

while zinc and selenium tend to partake in antioxidant activities within cells[16,74,75]. Certain levels of iron, copper, zinc, and selenium are vital to normal bodily functions, but elevated iron and copper as well as decreased levels of zinc and selenium, often seen in the obese population, can result in an oxidant/antioxidant imbalance and lead to oxidative stress[9-12,95,96].

Populations with obesity and/or dyslipidemia are more likely to have high levels of iron and copper, and are also likely to have lower serum zinc and selenium levels[9-12]. When consumed with polyphenol-rich foods, iron uptake is reduced while zinc uptake may be improved as reported in cell culture studies[167,170-172]. Endogenous antioxidants and total antioxidant capacity are also increased in obese participants fed a polyphenol-rich food source, along with some studies finding alterations in trace element levels as well[21,190,195,197,198]. Thus, we hypothesized that in participants consuming high- and low-dose polyphenol-rich freeze-dried strawberries would demonstrate decreased serum iron and copper levels, while increased serum zinc and selenium levels. However, no significance was found among iron, zinc, copper, and selenium levels in our study subjects after 12 weeks of strawberry supplementation. Thus, we reject our hypothesis of predicted modulation of trace elements after low and high dose FDS supplementation.

The forms of trace elements examined in studies must be considered in assessing outcomes. While we measured only free isotopes of selected elements, previous research have reported bound iron as well as transport proteins that play an important role in levels of these trace elements.[99-102]. Sulibursk a et al. found that daily consumption of green tea extract (379 mg) for three months reduced serum iron and zinc compared to baseline in males and females with obesity[21]. Basu et al. reported eight weeks daily consumption of green tea extract capsules significantly reduced plasma iron compared to baseline in men and women with obesity and metabolic syndrome. This study also found copper to be increased after fresh-brewed green tea consumption when compared to green tea extract consumption[195]. These findings compared with ours may indicate that the polyphenols found in strawberries may not significantly affect free

iron, zinc, copper, and selenium levels when compared to the reported effects of green tea and green tea extract. However, these observations need further clarifications in larger trials on the effects of dietary polyphenols in circulating levels of trace elements.

Catalase:

Catalase is an enzyme found in the cytosol and peroxisomes of most cells and functions as an endogenous antioxidant by converting toxic H_2O_2 into water and free oxygen.

Our study found that high dose FDS supplementation significantly lowered catalase activity at 12 weeks compared to baseline. Therefore, we fail to reject the hypothesis that high dose FDS would increase catalase activity at 12 weeks compared to baseline. Since glutathione peroxidase (GPX) is thought to initially respond to both normal and stressful conditions where H_2O_2 may be elevated, catalase activity may have been modified in the participants from our study as a result of possible modified GPX activity[38,128]. However, we did not report GPX activity in our participants. Catalase activity may have also decreased as a result of increased GSH found in our study; GSH being the reduced form of glutathione and is readily able to donate an electron to reduce and stabilize ROS[204]. Although catalase activity was found to be decreased post-intervention compared to baseline, this may possibly indicate improved antioxidant status as a result of increased GSH, which works closely with GPX as a co-enzyme and as a "first-line of defense" in combating increased H_2O_2 levels[124,131].

Our study findings are consistent with previous clinical studies which reported insignificant changes in catalase activity after polyphenol supplementation[174,195,205-208]. On the other hand, animal studies show different results. In rats fed a high-fat or normal diet supplemented with polyphenol-rich red grape skins (20 mg/kg/day) daily for four weeks, catalase activity was increased in both diets when compared with rats fed a high-fat or normal diet alone. Furthermore, catalase activity was decreased in rats fed a high-fat diet compared with a normal diet[174]. In

contrast to our findings, diabetic rats fed polyphenol-rich green tea extract for four weeks revealed a significant increase in catalase activity in the aorta and heart compared to the diabetic rat controls[209]. Thus, polyphenols may differ in their effects in modulating antioxidant enzyme systems in vivo.

In a study by Hokayem et al. two grams per day of grape polyphenols for nine weeks showed no significant changes in activities of catalase, GPX, SOD, or blood GSH/GSSG ratio in overweight and obese adults at increased risk for type 2 diabetes[205]. Another study by Basu et al. showed that supplementation of green tea or green tea extract for eight weeks in obese subjects with metabolic syndrome found no significant changes in catalase activity or reduced glutathione concentrations[195]. In our study, participants with obesity and dyslipidemia supplemented in high doses of strawberries had decreased catalase activity post-intervention. Thus, strawberries may have different effects on catalase activity compared to other polyphenol-rich foods.

Reduced glutathione (GSH):

Reduced glutathione (GSH) is a vital cofactor for the action of GPX and decomposition of H_2O_2. In addition to decreasing H_2O_2, GSH also quenches singlet oxygen and regenerates vitamins C and E into their stable, reduced forms. In the nucleus, GSH maintains the redox balance in order to protect DNA from oxidative stress[124,131,132].

A dose-dependent effect was observed with the significantly higher GSH levels at 12 weeks in the high dose FDS compared with low dose FDS. The significantly increased levels of GSH in high dose compared to low dose, both low dose FDS compared with their respective controls, and both low and high dose FDS at 12 weeks compared to baseline may imply improved antioxidant status as a result of strawberry supplementation. Furthermore, we fail to reject our hypothesis that low and high dose FDS supplementation would increase GSH when comparing the high to low dose FDS, their respective dose-dependent controls, and their respective baseline levels.

Similar results have been reported in animal and clinical studies on the effects of fruit polyphenols on GSH levels. A study in mice fed a berry juice mixture of blueberry, crowberry, and elderberry (1:1:1) for 3-4 weeks had increased total glutathione as a result of altered γ-glutamylcysteine synthetase (γ-GCS) following the supplementation of the polyphenol-rich berry juice[208]. A similar study conducted by Babu et al. in diabetic rats supplemented with green tea extract for four weeks revealed significantly increased serum GSH content in diabetic rats supplemented with the polyphenol-rich extract compared with the control animals[209]. The researchers suggested that green tea directly scavenging ROS in the experimental group may have reduced GSH utilization, leading to an increase in GSH in rats treated with green tea extract[209]. This may also help explain our findings of significantly increased GSH in FDS supplemented participants. An ex vivo study examining the concentration-dependent effects of epicatechin on biomarkers of oxidative stress in blood samples from overweight patients with hypertension, found significantly increased GSH levels after supplementation of epicatechin compared to baseline[210].

Clinical trials have reported similar effects on GSH after polyphenol-rich supplementation. Basu et al. previously reported significantly increased whole blood GSH and plasma antioxidant capacity after eight weeks of daily green tea (4 cups) or green tea extract (two capsules, 870 mg catechins) supplementation compared to the controls in adults with obesity and metabolic syndrome. Many other studies have also reported increased total antioxidant capacity in participants with obesity after consuming polyphenol-rich supplements compared with their respective controls[21,190,197,198]..

Our findings reveal that the supplemented dosage of polyphenols (25-50 g FDS) in strawberries for the study duration of 12 weeks may decrease catalase activity and increase serum GSH in participants with obesity and dyslipidemia. No effects were noted in trace elements. Increased

GSH may indicate improved response to oxidative stress, though these findings warrant further investigation.

Limitations

Our study has some limitations which must be considered in the interpretation of our findings. Our study participants are primarily females, with five out of sixty subjects being males. Our study sample of sixty participants is also small. The fact that our study was confined to those with enlarged waist circumference and dyslipidemia, included participants who were or were not taking vitamin supplements, excluded participants with other chronic conditions and were recruited locally at the OSU and OUHSC campuses limits the generalizability of our study findings.

Our study involved supplementation of freeze-dried strawberries equivalent to 1.5 – 3 cups of fresh strawberries per day which is much higher than the average fruit consumption in the US. CDC state-specific trends show only about 18% of Oklahoma residents consume two or more servings of fruit per day[211]. Thus, while our intervention may have therapeutic implications, the doses used may not be suitable for a preventative strategy. Also, the differences in sensory qualities between the strawberry and the control beverages, the latter containing vegetable fiber and artificial flavor and color, could have led to inadequate blinding of the participants. No diet analysis was performed to analyze trace element consumption of participants at baseline and at 12-weeks. Furthermore, no comprehensive analysis was obtained from subjects to include other antioxidants or enzyme systems (i.e. vitamin E, GPX, SOD, etc.)

Conclusion and recommendations

Strawberry supplementation in our study had no significant effects on trace elements, while catalase activity and GSH concentrations were significantly modulated following strawberry supplementation. Since obesity and dyslipidemia present a condition of elevated oxidative stress

and compromised antioxidant function, our findings may have some implications in the nutritional management of these conditions. Thus including strawberries within the recommended servings of fruits and vegetables, as per the Dietary Guidelines of Americans (2010)[212], may be a prudent approach in improving the dietary sources of antioxidants in participants with CVD risk factors.

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APPENDICES

IRB approval forms:

Oklahoma State University Institutional Review Board

Date:	Thursday, May 20, 2010	Protocol Expires:	2/28/2011
IRB Application No:	HE1015		
Proposal Title:	Chronic Effects of Freeze-Dried Strawberry Beverage on Cardiovascular Risk Factors in Subjects with Abdominal Adiposity and Dyslipidemia		
Reviewed and Processed as:	Expedited **Modification**		
Status Recommended by Reviewer(s)	**Approved**		

Principal Investigator(s):

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The requested modification to this IRB protocol has been approved. Please note that the original expiration date of the protocol has not changed. The IRB office MUST be notified in writing when a project is complete. All approved projects are subject to monitoring by the IRB.

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

The reviewer(s) had these comments:

The modification request to decrease the dosing level of the strawberries is approved.

Signature :

Shelia M. Kennison

Shelia Kennison, Chair, Institutional Review Board

Thursday, May 20, 2010
Date

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.

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.

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

VITA

Stacy Jean Morris

Candidate for the Degree of

Master of Science

Thesis: THE EFFECTS OF DIETARY FREEZE-DRIED STRAWBERRIES ON PLASMA TRACE ELEMENTS AND ANTIOXIDANTS IN ADULTS WITH OBESITY AND ABOVE OPTIMAL SERUM LIPIDS

Major Field: Nutritional Sciences

Biographical:

Education:

Completed the requirements for the Master of Science in Nutritional Sciences at Oklahoma State University, Stillwater, Oklahoma in December 2014.

Completed the requirements for the Bachelor of Science in Nutrition: Emphasis in Dietetics at Texas Woman's University, Denton, Texas in 2013.

Experience:

Graduate Teaching Assistant – NSCI 2114 – January – December 2014

Graduate Research Assistant – Dr. Deanna Hildebrand – June – December 2014

Graduate Research Assistant – Dr. Arpita Basu – August 2013 – June 2014

Professional Memberships:

Student Member – Academy of Nutrition and Dietetics

Student Member – AND DPG – Hunger and Environmental Nutrition

Student Member – American Society for Nutrition