A COMPARATIVE STUDY OF GREEN TEA BEVERAGE AND GREEN TEA EXTRACT SUPPLEMENTATION ON CLINICAL VARIABLES AND OXIDATIVE STRESS IN SUBJECTS WITH METABOLIC SYNDROME (MeS)

By

NATALIE SHALYNN BRIGGS

Bachelor of Science in Nutritional Science

Northeastern State University

Tahlequah, OK

2006

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
July, 2008

A COMPARATIVE STUDY OF GREEN TEA BEVERAGE AND GREEN TEA EXTRACT SUPPLEMENTATION ON CLINICAL VARIABLES AND OXIDATIVE STRESS IN SUBJECTS WITH METABOLIC SYNDROME (MeS)

Thesis Approved:

Dr. Arpita Basu

Thesis Adviser

Dr. Edralin Lucas

Dr. Nancy Betts

Dr. A. Gordon Emslie

Dean of the Graduate College

ACKNOWLEDGMENTS

I take this opportunity to acknowledge my advisor, Dr. Basu, for all of her support and guidance during the development of my thesis. I would like to thank Dr. Basu for offering assistance and advice throughout this process. I express my sincere gratitude to Dr. Basu for increasing my knowledge in the research process and training me in proper lab techniques.

I would also like to acknowledge my committee members for their assistance and support. I would like to thank both Dr. Betts and Dr. Lucas for serving on my committee and offering time and encouragement throughout the research process. In addition, I would like to thank Dr. Betts for assisting me in the quest for an advisor.

I would like to convey special thanks to everyone at the General Clinical Research Center (GCRC) at University of Oklahoma Health Sciences Center who aided in the data collection and statistical analysis for this clinical trial. I also would like to extend my thanks to the Human Nutrition Laboratory at OSU for providing the proper equipment to complete the laboratory analyses. I would like to thank all of the subjects who participated in this clinical trial. With them, this project was made possible.

Finally, I would like to express my gratitude to my family and friends, especially my mom and fiancé, who were there to share the stresses of the past year. My family means the world to me and I would like to thank them for all their love and encouragement.

TABLE OF CONTENTS

Chapter Page

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

CHAPTER I

INTRODUCTION

Background

Drinking tea is a cultural pastime that has been around for centuries. Tea is derived from brewing leaves of *Camellia sinensis* in hot water. Along with water, tea (primarily green or black) is the most widely consumed beverage worldwide (Khan and Mukhtar, 2007). For centuries, many Asian cultures have relied on various forms of tea to treat an array of common medical problems including headache, indigestion, and dizziness.

Green tea can be taken in various forms including beverage and extract. However, green tea is primarily utilized in the beverage form. Several studies have suggested that consumption of green and black tea beverages may result in favorable health benefits.

The three primary forms of tea consumed are green, black, and oolong. The difference among these three forms of tea is in the processing. Green tea is made from freshly harvested leaves that have been promptly steamed to prevent fermentation (Khan and Mukhtar, 2007). Black tea, however, is made from leaves that have been fully oxidized prior to desiccation while oolong tea leaves are partially oxidized before drying (Khan and Mukhtar, 2007; Trevisanato and Kim, 2000).

Green tea is a rich source of polyphenolic flavonoids, primarily catechins. Catechins are potent antioxidants (Coimbra et al., 2006). The key polyphenols in green

tea consist of epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin (EC), and epicatechin gallate (ECG) (Chow et al., 2003). Of the catechins found in green tea, EGCG possesses the most anti-oxidative activity and is the polyphenol found most abundantly in green tea encompassing 50%-80% of the total catechin content of green tea (Khan and Mukhtar, 2007; Coimbra et al., 2006). Flavonoids are a class of antioxidants that are known to neutralize free radicals in the body. Free radicals are known to damage cells by oxidative stress (Trevisanato and Kim, 2000). Tea flavonoids are absorbed from the gut. Following absorption, tea flavonoids significantly increase the antioxidant capacity of the blood. This leads to increased antioxidant capacity of the body and the reduction of oxidative damage of important biomolecules (Rietveld and Wiseman, 2003). As a result, green tea may aid in decreasing oxidative stress.

Oxidative stress is an imbalance between the production of free radicals and the antioxidant status of cells in the body (Forrester and Libby, 2007). When an individual is in an oxidative stress situation inflammation may follow. Inflammation is defined as a biological response to harmful stimuli. Internal balance is maintained by actively eliminating damaged cells. Vascular inflammation plays a central role in atherogenesis (Forrester and Libby, 2007). Chronic inflammation, which occurs when the body loses the ability to control inflammatory responses, may lead to an array of problems including atherosclerosis and arthritis. If green tea increases antioxidant capacity then inflammation should, consequently, decrease. Therefore, a healthy lifestyle along with medication, when appropriate, may avert the inflammatory process and thus promote healthy aging (Libby, 2006).

Introduction to Problem

According to Hino et al., 2007 the occurrence of obesity is rapidly escalating in western countries and around the globe. Metabolic syndrome (MeS) is a cluster of risk factors associated with obesity. This cluster includes such factors as high blood pressure, abdominal obesity, hyperglycemia, and dyslipidemia. While the severity of each individual risk factor may be mild, the combination of 3 or more of the various risk factors is associated with increased cardiovascular morbidity and mortality. The development of metabolic syndrome is thought to be drastically influenced by lifestyle. For instance, the frequency of metabolic syndrome is lower in Japan where the beverage of choice is green tea. Therefore, it may be implied that the routine consumption of green tea is inversely associated with metabolic syndrome (Hino et al., 2007). Metabolic syndrome is associated with increased inflammation and oxidative stress (Hino et al., 2007). There exists limited clinical data on green tea supplementation and oxidative stress and inflammation in subjects with metabolic syndrome.

Few studies have looked primarily at the safety of green tea supplementation. The adverse effect that green tea has on iron absorption, due to tannins, has been questioned. However, few studies have reported the adverse effects of green tea supplementation. One concern of supplementation may be hepatoxicity when green tea is supplemented in high doses for an extended period of time (Basu and Lucas, 2007). According to Chow et al., 2003 some unfavorable occurrences that have been reported during various studies include gas, upset stomach, nausea, heartburn, abdominal pain, dizziness, headache, and muscle pain. In the majority of cases, these events were reported as mild and did not occur any more in the supplemented group than in the placebo group. In previous studies,

it has been concluded that green tea supplementation should be considered safe for healthy individuals in doses equivalent to the EGCG content in 8-16 cups of green tea once or in divided doses twice a day for 4 weeks. All side effects seem to be dose dependent. The higher the dose of green tea administered the greater the chance of developing one or more of the minor health maladies, with special regards to nausea, bloating, and vomiting. Caffeinated green tea supplementation may cause agitation, dizziness, insomnia, restlessness and tremors. No observational studies reported toxicity when green tea was consumed as a daily beverage. However, there is a need for further research on the safety of green tea supplementation in human subjects.

Tea consumption in general, has been linked to low incidences of a collection of pathological conditions including CVD, diabetes, obesity and cancer. Drinking tea, especially green tea, daily is an inexpensive and low calorie approach to provide the body with powerful antioxidant protection. Overall, many studies have shown evidence to suggest that the health benefits of green tea supplementation may greatly outweigh the risks (Cooper, Morre, Morre 2005). However, there exists limited data on the safety of green tea supplementation in individuals with MeS and more studies are needed to determine the safety of green tea supplementation in these individuals.

Objective/Hypothesis

The aim of the present study is to look at the correlation between chronic green tea supplementation and its effects on hematological profile and oxidative stress in subjects with MeS. We will also address the safety of chronic green tea supplementation in these subjects. Thus, our study will address the research gap on the safety of green tea supplementation and its effects on oxidative stress and safety in at-risk subjects and the role of green tea in medical nutrition therapy (MNT). The hypotheses to be tested include the following:

- Green tea extract supplementation or green tea beverage will have no effects on safety parameters in individuals with MeS.
- Green tea extract supplementation or green tea beverage will not lower biomarkers of oxidative stress in subjects with MeS.

CHAPTER II

REVIEW OF LITERATURE

The following review of literature focuses on several observational, cell culture, animal model and clinical studies that have analyzed the effects of green tea supplementation on hematology and oxidative stress as well as the safety parameters of green tea supplementation. This review also focuses on the effects of myloperoxidase on oxidative stress.

Green Tea and Health Benefits

Green tea along with black and oolong are the three primary types of tea consumed worldwide (Popkin et al., 2006). These three forms of tea, which originate from the plant *Camellia sinensis,* are grown primarily in Southeast Asia as well as China and are consumed globally. Green tea can be utilized in several forms including beverage, dietary supplementation, and topical preparations. The major differences in the three forms of tea may be due to variations in the fermentation (oxidation) process. Green tea is in the form of fresh/dried unfermented leaves, black tea is fermented and oolong is incompletely fermented (Cooper, Morre and Morre, 2005).

There are several health benefits that may be attributed to tea consumption including an increase in bone density and a decrease in cavities and tooth decay (Popkin et al., 2006). Green tea may also produce antibacterial and antiviral activity. According to Craig, 1999 herbal remedies, such as green tea extract, are used by one out of every three

Americans to treat common ailments such as arthritis, headache, and depression (Craig, 1999).

Tea is a diverse substance that is comprised of flavonoids, antioxidants, micronutrients, and a few amino acids (Popkin et al., 2006). The flavonoids in green tea have anti-inflammatory properties (Craig, 1999). Flavonoids act as antioxidants and prevent tissue damage by the scavenging of free radicals (Trevisanato and Kim, 2000). In a previous study, green tea was shown to have ~4.5-6% greater antioxidant capacity than the antioxidant rich kale and strawberries (Cao, Sofic and Prior, 1996). The potent antioxidant compounds in tea may protect low-density lipoprotein (LDL) cholesterol from oxidation and inhibit lipid peroxidation (Craig, 1999).

Decaffeinated green tea (brewed) contains around 3mg of caffeine compared to caffeinated green tea with approximately 30mg per 8 fl oz. The suggested daily consumption of tea per day is roughly 28 fl oz while the average consumption is 36 fl oz (Popkin et al., 2006). Green tea is inexpensive, provides ~0kcal per serving, has not shown any significant side effects, provides several health benefits, and previous data has indicated that it is safe in recommended amounts for daily use (Trevisanato and Kim, 2000). However, the use of green tea extracts, along with any other herbal remedy, needs to be further investigated to determine their safety (Craig, 1999). The major polyphenols in green tea are EGCG, EC, EGC, and ECG which belong to the family of catechins (Cooper, Morre and Morre, 2005) with EGCG being the most abundant in green tea (90mg/cup) and therefore regarded as the most important green tea catechin in the production of green tea extracts for dietary supplementation (Wang, Provan and Helliwell, 2000).

Observational Studies

Green tea supplementation has been shown to promote weight loss which may in turn have a positive effect on metabolic syndrome (Cooper, Morre and Morre, 2005). Metabolic syndrome is a cluster of risk factors (dyslipidemia, high blood pressure, hyperglycemia, abdominal obesity, etc.) associated with an increase in incidence of certain disease states including cardiovascular disease and type 2 Diabetes Mellitus (DM). Each individual risk factor may be a mild indicator; however, the cluster of risk factors is associated with increased morbidity and mortality. Until recently the frequency of metabolic syndrome has been lower in Japan than in western countries. Green tea is the drink of choice in Japan. Due to westernization there has been a decrease in green tea consumption and an increase in metabolic syndrome Hino et al., 2007. Hino et al., 2007 analyzed the relationship between habitual green tea and coffee drinking and their relationship to metabolic syndrome in the general Japanese population. Food frequency questionnaires were completed by each individual participant to obtain dietary habits. This data was compared to data obtained from a National Nutrition Survey that was completed in 1999. More participants consumed green tea than coffee. In this study, no relationship was found between green tea consumption and metabolic syndrome (Hino et al., 2007).

Green tea consumption has been attributed to a decrease in the risk of cardiovascular disease. The consumption of green tea may lower LDL cholesterol and reduce the risk of myocardial infarction. Furthermore, moderate green tea consumption has been shown to have a hypolipidemic effect, due to the catechin content, and inhibit LDL oxidation in humans (Cooper, Morre and Morre, 2005). Green tea

consumption/supplementation has also been shown to have anti-inflammatory properties which may lead to a decrease in cardiovascular disease since inflammation plays an important role in atherogenesis Forrester and Libby, 2007. Inflammation is the body's natural immune response to external agents in an attempt to maintain internal balance (Forrester and Libby, 2007). The reduction of inflammation can be achieved through antioxidant rich foods, such as green tea. A decrease in inflammation plays a key role in the decrease in the risk of cardiovascular disease.

Cell Culture

Tea polyphenols are potent antioxidants that provide benefits against oxidative stress through the increase of antioxidant defense and scavenging of reactive oxygen species (Raza and John, 2005). Green tea has been reported to have anti-oxidative properties and has been shown to inhibit lipoxygenase and promote LDL oxidative modification in vitro (Yang and Koo, 2000). EGCG, which is a major catechin in green tea, has been shown to aid in the prevention of copper (Cu+) induced LDL oxidation (Miura et al, 1994) and anti-oxidants provide protection to LDL oxidation. It has been shown that oxidized LDL can be an initiator/promoter of atherogenesis. Cholesterol oxidation and lipoprotein oxidation are enhanced through oxygen radicals that are generated by lipid peroxidation and Cu+ mediated LDL oxidation.

In a study by Miura et al, 1994 fresh whole blood samples were taken from normolipidemic males and LDL was separated, concentrated, and then catalyzed by Cu+. The investigators found that antioxidant potency was greatest in EGCG followed by ECG. These catechins had the greatest inhibitory effects on Cu+ catalyzed LDL oxidation and the inhibition of oxidized cholesterol. EGCG has two triphenolic groups in structure

and EGCG and ECG both have galloyl moieties which may account for their enhanced antioxidant activities. Catechins were also shown to inhibit the increase of lipid peroxidation in the Cu+ catalyzed LDL oxidation.

In a study by Ishikawa et al., 1997 catechins were added to plasma of normolipidemic healthy males, incubated, and the LDL fraction was separated. Oxidation of LDL was measured by conjugated dienes and lipid peroxides, thiobarbituric acid-reactive substance (TBARS). The study found that catechins significantly and dose-dependently prolonged the lag time of LDL oxidation. EGCG had the greatest effect in prolonging the lag time and, along with ECG, had the strongest effects on oxidative stress. The decrease in TBARS and lipid peroxide formation was observed in LDL samples in which green tea catechins were added (Ishikawa et al., 1997).

When Raza and John, 2005 investigated the effects of EGCG on PC12 cell compartments, they found a positive alteration in oxidative stress of the cell compartments. The catechins in green tea act as free radical scavengers and have been shown to inhibit the activity of lipoxygenase and inhibit Cu_+ induced LDL oxidation.

Lung Chen tea, which is a non-fermented Chinese green tea, has the highest concentration of catechins and EGCG among the green teas (Yang and Koo, 2000). A study which analyzed the effect of Lung Chen tea on LDL oxidation in endothelial cells found that this tea inhibited LDL oxidation in vitro (Yang and Koo, 2000). In addition, Lung Chen tea dose-dependently lowered lipid peroxidation products (Yang and Koo, 2000). In vitro studies have primarily shown that the effects of green tea on oxidative stress and lipid peroxidation are dose-dependant, with EGCG being the primary contributor (Miura et al., 1994).

Animal Studies

Green tea leaves are high in polyphenols along with many other compounds including, aluminum (Marouani et al., 2007). Polyphenols found in green tea may lead to a decrease in iron status; however, the correlation is unclear. Green tea decoction, which is obtained by cooking dried tea leaves for an extended amount of time, may be a cause of iron deficiency anemia. Green tea decoction may cause an increase in aluminum and /or the formation of insoluble complexes in the gastrointestinal lumen. An increase in aluminum may lead to less availability of iron for absorption. This is due to the fact that iron and aluminum compete at different stages of erythropoiesis for storage.

In an 8 week study by Ishige, Schubert and Sagora, 2001, 42 male Wistar rats (2 months old) were divided into 6 random groups with 7 rats in each group. A control group was given an experimental diet and water, while the remaining groups were given the experimental diet and various amounts of green tea decoction and aluminum. This study, also showed, that a decrease in iron stores in organs which led to a decrease in overall iron status was a directly correlated to aluminum absorption and low iron status along with a decrease in hemoglobin (9%) and hematocrit (7%) (Ishige, Schubert and Sagora, 2001).

Green tea may decrease circulating lipids in body fluids which may reduce oxidative stress in the body. Researchers state that a redox balance exists in organisms and changes in the antioxidant status of the body may disrupt this balance. Oxidative stress may be induced for a variety of reasons including alcohol-abuse, exercise, age, and various disease states such as diabetes mellitus and metabolic syndrome. A reduction in antioxidant status may also lead to oxidative stress. Oxidative stress may lead to cellular

oxidation. This may lead to dyslipidemia and an enhancement in the aging process. In a study analyzing the effect of green tea on oxidative stress in diabetic rats, it was found that lipid peroxide levels decreased significantly as the antioxidant status improved (Babu et al., 2006). It was also found that there was an inverse relationship with food intake and treatment inhibited weight loss (Babu et al., 2006). Tuczaj et al., 2004 analyzed the effect of ethanol-induced oxidative stress in male Wistar rats and found that oxidative stress was lowered when green tea was ingested. Furthermore, the amount of lipid peroxidation products decreased. Green tea prevented oxidative modification by increasing antioxidant status. This was achieved by a synergy within green tea catechins, the most potent being EGCG. In addition, oxidation was decreased in the serum of ethanol-fed Wistar rats. Alessio et al. 2002 investigated exercise induced oxidative stress (in the kidney and liver) in male Sprague-Dawley rats that consumed green tea for 6.5 weeks. Results showed that green tea had no significant effects on measured physiological parameters including glucose and cholesterol but did decrease the accumulation of lipid peroxide products in the kidney.

MPO

Myeloperoxidase (MPO) is used as a marker of inflammation and may be responsible for oxidative stress in several tissues. Liu et al. 2005, investigated the effect of lycopene, and antioxidant, on exercise induced oxidative stress in male Sprague-Dawley rats. MPO in the muscle of the exercise group was significantly higher (30%) than that of the control. In addition, MPO activity was significantly lower in the muscle of the exercise group that was given the highest dose of lycopene. This suggests that antioxidant status may decrease MPO activity during exercise induced oxidative stress.

Therefore, antioxidants may decrease oxidative damage by decreasing MPO activity. MPO is an enzyme that is found in neutrophils, monocytes and macrophages. The level of MPO activity that is present in a suspension of neutrophils is directly proportional to the number neutrophils over a wide range of concentrations. Krawisz et al. 1984, used MPO activity to assess inflammation in two animal models (acetic acid induced colitis in the rat and *Clostridium difficile* induced enteritis in the hamster). MPO was found to have a have a liner relationship with the number of neutrophil cells in purified cell populations. Therefore, MPO may reflect the level of inflammation in intestinal mucosa and MPO activity should provide an estimation of the average level of inflammation. MPO determinations are simple for use in large populations and could be useful as a marker of inflammation in human and animal models. However, the effects of green tea polyphenols on MPO have not been clearly investigated and there is a further need for the investigation of MPO in subjects with MeS.

Clinical Trials

Green tea supplementation has been has been shown to cause a decrease in oxidative stress 9Coimbra et al., 20060. Changes in oxidative stress appear to be related to dietary habits, lifestyle, and to the experimental protocol used in each study. In a study by Coimbra et al., 2006 individual parameters of oxidative stress were evaluated after drinking 1 liter of water daily for 3 weeks followed by drinking 1 liter of green tea daily for 4 weeks. Subjects were advised to drink the assigned beverages throughout the day and maintain their usual food and drink patterns at mealtime. Blood samples were collected at rest and fasting for 12 hrs after 3 weeks of water daily and 4 weeks of green tea daily. In addition, colorimetric assay was used to evaluate total antioxidant status.

This study measured total antioxidant status (TAS), malonyldialdehyde (MDA), and malonyldialdehyde+4-hydroxy-2(E)-nonenal (MDA+4-HNE). There was a significant decrease in membrane bound haemoglobin (MBH) suggesting reduced oxidative stress within the cell (Coimbra et al., 2006).

Tea consumption has been associated with a decrease in the incidence of chronic diseases in which oxidative stress plays a key role. In a study by Erba et al., 2005 involving 24 healthy females, 20-29 yrs old with an average BMI of 19, were evenly/randomly divided into two groups (control and green tea supplementation). The study lasted for 42 days. The supplemented group consumed a cup of green tea with breakfast and a cup of green tea with dinner (160mg green tea extract with 200ml of water). Both groups maintained a controlled diet throughout the study. Blood samples were taken at the beginning and end of the study. This study showed a slight decrease in LDL as compared to the control group and an improved plasma antioxidant status. The mean plasma concentration of green tea catechins was also above the baseline average when green tea is consumed regularly. This increased plasma concentration of EGCGremained for at least 12 hrs after ingestion. This study suggests increased plasma antioxidant status and protection of lymphocytes from DNA oxidative damage following moderate green tea consumption (Erba et al., 2005).

LDL oxidation plays an essential function in atherogenesis. In previous studies green tea polyphenols have been shown to inhibit the oxidation of LDL (Miura et al., 2000). Catechins (primarily EGCG) may play a role in the inhibition of LDL oxidation. When EGCG gets absorbed in the plasma at a maximum concentration, this may lead to increased antioxidant activity in the bloodstream (Nakagawa et al., 1999). Miura et al.,

2000 found that the lag time of LDL oxidation was prolonged approximately 14 minutes following the ingestion of 300mg of green tea polyphenol extract. This same study found that 56% of EGCG in the free form remained in the plasma following a blood draw one hour following ingestion of the green tea extract (Miura et al., 2000). Another study by Nakagawa et al., 1999 reported similar results stating green tea extract (254mg catechins) significantly increased the plasma EGCG concentration in humans 60 minutes following ingestion (Nakagawa et al., 1999). Studies have shown that tea phenolics may produce considerable antioxidant activity and in turn neutralize free radicals. One study which investigated the ingestion of various phenol-rich beverages found that 300ml bolus of green tea (along with red wine) was the most efficient in protection of LDL oxidation (Serafini et al., 2000). However, green tea alone showed maximum decrease in LDL oxidation 30 minutes after ingestion (Serafini et al., 2000).

There is evidence to suggest that tea is a hypoglycemic agent. It is suggested that catechins and theaflavins may enhance insulin activity which may aid in the prevention of hypoglycemia. One study looked at the effects of tea extract supplementation on glucose control in 49 individuals (49-86 yrs old) with recently diagnosed (past 6 months) type 2 diabetes mellitus and without insulin treatment (Mackenzie, Leary and Brooks, 2007). After randomization, subjects received one of the 4 regimens which included; 2 capsules per day of extract, 1 capsule per day of extract, 2 capsules per day of placebo, or 1 capsule per day of placebo for3 months. The extract contained green tea catechins (40%) and black tea theaflavins (20%); equivalent to the catechins of 7 cups of green tea. Adverse events included profuse sweating and a rash. No significant improvements in

HbA_1c were observed suggesting that tea extracts may not have a positive effect on glucose control in individuals with type 2 DM (Mackenzie, Leary and Brooks, 2007).

The safety of green tea supplementation needs to be determined to make the utilization of green tea in interventions a viable option. A study by Chow et al., 2003 investigated the safety/pharmacokinetics of green tea supplementation. Multiple doses of epigallacetechin gallate and polyphenol E were given to 40 healthy individuals (men and women) over 18 years of age. The polyphenon E capsules consisted of EGCG (200mg), EGC (37mg), EC (31mg), and other green tea polyphenols along with minimal structural ingredients. Caffeine was not present in either the EGCG capsules or the polyphenon E capsules. Fasting blood samples were taken from the subjects before and after the 4 week treatment period. The laboratory analysis included glucose, urea nitrogen, creatinine, total protein, albumin, alkaline phosphatase, total billirubin, and iron. One of five treatments (800 mg EGCG once daily, 400 mg EGCG twice daily, 800 mg EGCG polyphenon E once daily, 400 mg EGCG polyphenon E twice daily, of a placebo once daily) were given for 4 weeks after an initial 2 week period free of tea, tea products, dietary and herbal supplements. The study participants were then followed for 4 weeks and any adverse reactions were documented. Reported events included excess gas, upset stomach, nausea, dizziness, headache, etc. However, all reported events were not significantly greater than in the placebo group and all events were reported as mild. Nausea did increase, however, as the dosage of EGCG increased to 800 mg. No significant changes were observed in hematology. Overall, the study concluded that green tea polyphenol supplementation is safe for healthy individuals in doses equivalent to 8-16 cups of green tea per day (Chow et al., 2003).

This review of literature focused on several observational, cell culture, animal, and clinical studies which looked at the safety of green tea beverage and supplement along with the lowering effect that green tea has on oxidative stress and inflammation. However, there seems to be a gap in knowledge with regards to the safety of green tea beverage and supplement in subjects with MeS. There is also a gap in knowledge with regards to green tea and its lowering effects on oxidative stress and inflammation in subjects with MeS. The following study looks at the effect of green tea beverage and supplement on oxidative stress by measuring LDL oxidation and MPO in subjects with MeS. Furthermore, this study looks at the safety of green tea beverage and supplement in these individuals.

CHAPTER III

METHODOLOGY

Subjects

Twenty-eight subjects (n=28) with MeS were enrolled in the study between January 2007 and January 2008. According to the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) criteria, MeS is defined as having any three of the following five features: waist circumference (≥40 inches in men & ≥35 inches in women), triglycerides ≥150 mg/dL, HDL (<40 mg/dL in men; < 50mg/dL in women), blood pressure ≥130/85mm Hg, and fasting glucose (≥100mg/dL). Adult subjects (> 21 years) with normal hemoglobin (Hb), white blood cells (WBC), platelets, liver, renal, and thyroid function tests, and on stable medications were included in the study. Smokers, heavy drinkers (> 1 oz alcohol/day), those taking mega doses of antioxidants or fish oil supplements (> 1g/day), along with individuals who had a history of cancer, coronary heart disease, or type 2 diabetes were excluded from the study. Subjects were asked to abstain from consuming green tea or green tea-related products, other than those provided in the study, and maintain usual diet and lifestyle throughout the study period. All subjects were screened with a complete medical history and signed the informed consent form.

The study examined the effects of green tea (GT- 4 cups/day), green tea supplement (GS- 2 capsules, 500 mg EGCG, 4 cups water/day), and control (C- 4 cups

water/day) on clinical variables (fasting glucose, insulin resistance, lipid profile, complete blood count, liver, renal, and thyroid function tests), and biomarkers of oxidative stress in this eight-week, randomized controlled trial.

Age and sex-matched trios of participants with MeS were randomly assigned to one of three groups. The study was conducted at the University of Oklahoma Health Sciences Center (OUHSC), General Clinical Research Center (GCRC). A total of 5 clinic visits (screening and follow-up visits at 2, 4, 6, & 8 weeks) were conducted for all 3 groups. However, subjects in the green tea group made daily visits (5 days/week) to the GCRC for a fresh supply of tea, especially to ensure compliance and consistency. The green tea group consumed 2 cups of green tea in the morning at GCRC and were provided with another 2 cups in a container and asked to consume later in the day (6-8 hours later). Tea was also provided for the weekends. The Bionutrition unit at GCRC, headed by the research dietitian, prepared the green tea for the subjects and monitored compliance.

Those in the control and supplement groups were provided with containers to measure 4 cups of water to be consumed on a daily basis. Participants in the supplement group received a 2-week supply of capsules during their follow-up visits and compliance was confirmed by a pill count. Participants were instructed to take 2 capsules a day, one capsule with lunch and dinner. Fasting blood draws and anthropometric measurements were performed at screening, four and eight weeks of the study. All participants were compensated at screening and bi-weekly follow-up visits.

Supplements

Decaffeinated green tea supplements were purchased from Solaray® (Park City, UT). The capsules were manufactured from the same lot numbers of raw materials and the label claimed 500 mg of green tea extract providing 250 mg of EGCG. Other ingredients in the capsule and filler included vegetable cellulose, magnesium stearate and silica.

Tea preparation

Green tea bags (from one production lot) were purchased from RC Bigelow Inc.© (Fairfield, CT). Green tea was prepared by trained Bionutrition staff at OUHSC GCRC by steeping 4 decaffeinated green tea bags in 4 cups of boiling water (8 oz/cup) for 10 minutes. No sugar or milk was added to the tea, but according to the preference of the participants, artificial sweetener may be used. The subjects consumed 2 cups at GCRC while being monitored, and the remaining 2 cups were provided in a container. Participants were told not to reheat the tea which they consumed later in the day, but drink it straight from the container. Subjects received 100 mg of EGCG per cup of green tea and 400 mg of EGCG per day. Catechin analyses were performed in the Human Nutrition Laboratory at OSU.

Sample collection and storage

Fasting blood samples taken by a certified phlebotomist were collected at screening, and after 4 weeks and 8 weeks. Blood samples were collected in heparinized tubes, in vials containing EDTA, and serum separator tubes (SST). Serum and plasma samples were separated, flushed with nitrogen, and stored at -80° C for later analysis.

Biochemical analyses

Blood samples were collected immediately after each draw at GCRC and transported to the University of Oklahoma Medical Center (OUMC) Laboratory for analyses of fasting glucose, lipid profile {total cholesterol, triglycerides, low-density lipoproteins (LDL), high density lipoproteins (HDL)} and safety parameters including hemoglobin (Hb), platelets, white blood cells (WBC), liver enzymes, creatinine, and blood urea nitrogen (BUN) were analyzed. ELISA was performed for the analyses of oxidized LDL and myeloperoxidase (MPO).

Oxidized LDL assay

Mercodia Oxidized LDL (Mercodia Uppsala Sweden) competitive ELISA was used to determine LDL oxidation. A fixed amount of oxidized LDL is bound to the microtiter well. This oxidized LDL competes with the oxidized LDL in the sample. The absorbance was then measured at 450 nm and read spectrophotometrically Data was analyzed using a calibration curve for each assay.

MPO ELISA

MPO is an iron containing glycoprotein that is traditionally used as a marker of inflammation. MPO plays a potential role in atherosclerosis and therefore may be a marker of cardiovascular disease (CAD). Plasma levels of MPO were determined by commercially available ELISA (Mercodia Uppsala Sweden) using the manufacturers instruction.

Statistical analyses

The data was first graphed to identify outliers and/or errors. The student's t-test for two independent groups was applied to address any differences between groups at

baseline. A *P* value of <0.05 was considered statistically significant. Initially, the subjects were randomized to trios by age and gender. However, due to imbalance and incompletion within trios due to a small sample size, the data was analyzed by group. Changes from zero to eight weeks were assessed within groups with the paired t-test. Two sample t-tests were applied to measure changes from zero to eight weeks between groups. Due to sample size, the data was not corrected for multiple hypotheses testing. Instead, the data was reviewed for consistencies.

CHAPTER IV

RESULTS

Baseline Characteristics

A total of 28 subjects completed this randomized 8 week study. Three men and twenty-five women were enrolled and completed this study. There were 12 subjects in the control group, 11 subjects in the green tea beverage group, and 5 subjects in the supplement group. The baseline characteristics of these subjects are depicted in Table 1. As depicted in Table 1, the BMI in all groups was greater than 30 which is an indicator of significant obesity. Waist circumference was high in all three groups. Triglycerides were greater than 150 mg/dL in the green tea beverage group alone. However, there was not a significant difference between groups. Control and green tea supplement groups had a triglyceride level less than 150 mg/dL on average. HDL cholesterol was low in all three groups. Blood pressure was not high in any group but the majority of subjects were on blood pressure medication. All subjects were matched well based on age. The mean age in all groups was between 40-50 years. There were no statistically significant differences in baseline characteristics among the three groups.

Body Weight and BMI

The change in body weight (Figure 1) and BMI (Figure 2) were significantly greater in the green tea group ($p<0.05$), when compared to the controls. Weight decreased by an average of 1.9 kg and BMI by 0.7 in the green tea group. The supplement group

experienced a loss of 1.3 kg and BMI decreased by 0.4, which was not significant compared to controls (Figure 1 and Figure 2). The control group showed a slight increase in both body weight (Figure 1) and BMI (Figure 2) at the end of this 8 week study.

Safety Parameters and Hematology

Blood draws were done by a certified phlebotomist at screen, 4 weeks, and 8 weeks. At screening, subjects with abnormal hematology, liver function or renal function tests were excluded from the study. The safety parameters analyzed in this study were liver function tests {alanine transaminase (ALT), aspartate transaminase (AST), and total bilirubin (TBIL} along with renal function {blood urea nitrogen (BUN) and creatinine (Cr)}. Total bilirubin decreased in both the control and green tea beverage group while there was no change seen in the supplemented group. However, this decrease was not statistically significant. There was a non-significant decrease in AST in both the green tea beverage and supplement groups while an increase was shown in the control. Both AST and ALT remained within normal limits from screening to 8 weeks. There was an increasing trend in BUN in all groups while Cr increased in the control group and green tea beverage group with no change seen in the supplement group at 8 weeks when compared to baseline. However, all groups were within normal limits with regard to renal function (BUN=7-18mg/dL and Cr = females-0.7-1.2 mg/dL and males-0.8-1.2mg/dL) which may indicate that green tea beverage or supplement had no harmful effects on renal function. Other hematological parameters addressed in this study were WBC, RBC, Hb, HCT, MCV, and PLT all of which were within normal limits at both baseline and 8weeks. However, there was an increase in platelets within the supplement group versus the control group ($p<0.05$). The lack of change in most of the safety parameter and

hematology, except platelets, at 8 weeks when compared to baseline or control group is a good indication that green tea beverage and supplements are safe for use in subjects with MeS. Furthermore, no adverse events were reported with green tea beverage or supplement during this 8 week clinical trial.

Biomarkers of Oxidative Stress

LDL Oxidation

In this study ox-LDL was used as a measurement of oxidative stress in the subjects. There was a significant decrease in ox-LDL in the green tea group at 8 weeks when compared to baseline ($p<0.05$). This decrease in the green tea group was non-significant in comparison to controls. However, this is still an indication that green tea beverage has a positive effect in lowering oxidative stress in subjects with MeS. A non-significant increase in ox-LDL was seen in the supplement group at 8 weeks when compared to baseline. Therefore, the green tea beverage, not supplement, caused a significant decrease in ox-LDL at baseline compared to 8wks, but not in comparison to controls (Figure 3).

MPO

The green tea group showed a decreasing trend in MPO levels compared to controls ($p<0.1$). Interestingly, the green tea beverage group showed a significant decrease in MPO when compared to the supplement ($p<0.05$). The supplement group showed no significant decrease in MPO levels and the control group showed a non-significant increase in MPO levels. Thus, green tea beverage intake showed a decreasing trend in MPO levels when compared to controls ($p<0.1$) (Figure 4).

Table 1

Baseline Characteristics of all Study Participants by Treatment Group

Variable	Control (n=12)	GT (n=11)	GS (n=5)
Age (years)	44.6± 3.2	45.3±3.0	40.0±4.2
Male (n)	1	1	1
Female (n)	11	10	4
Body weight (kg)	102.7±6.6	100.8±5.8	116.5±8.3
BMI	36.4±2.8	36.9±2.0	41.5±2.1
Waist circum. (in)	42.5±1.9	43.0±1.5	49.7±3.7
TG (mg/dL)	129.2±21.1	168.0±27.1	148.4±38.4
HDL (mg/dL)	41.8±1.9	41.7±2.1	35.6±3.2
SBP (mm/he)	129.8±2.6	133.5±4.8	127.4±6.4
DBP (mm/he)	79.6±2.1	81.9±2.5	80.8±2.7
Medication users (%)	45.4	85.7	60

(Mean±Standard Error)

GT-green tea, GS-green tea supplement, BMI-body mass index, TG-triglycerides, HDL-high density lipoprotein, SBP-systolic blood pressure, DBP-diastolic blood pressure

No statistically significant differences between groups ($p>0.05$)

Table 2

Effect of Green Tea Supplementation on Hematology and Safety Parameters of all Study Participants

Variable	Control (n=12)		GT (n=11)		GS (n=5)	
	0wk	8wk	0wk	8wk	0wk	8wk
WBC (K/mm3)	6.3±0.4	6.0±0.3	6.6±0.5	6.9±0.4	7.8±0.6	9.2±1.3
RBC (K/mm3)	4.5±0.1	4.4±0.1	4.5±0.1	4.5±0.1	4.9±0.2	4.9±0.3
Hb (g/dL)	13.5±0.3	13.3±0.3	13.3±0.2	13.2±0.2	14.5±0.7	14.5±1.0
HCT (%)	39.8±0.9	39.1±0.9	39.0±0.7	38.7±0.5	42.2±2.0	42.5±3.0
MCV (fL)	89.2±1.0	88.8±1.0	86.7±1.0	86.2±0.8	85.6±1.5	86.1±1.6
PLT (K/mm3) **	265.0±17.5	264.8±19.8	310.9±21.1	317.4±19.4	351.4±19.3	389.2±11.6
BUN (mg/dL)	11.3±1.1	12.6±0.8	10.0±0.8	11.1±0.3	10.0±0.9	12.4±1.9
Cr (mg/dL)	0.8±0.0	0.9±0.0	0.7±0.1	0.8±0.0	0.8±0.1	0.8±0.1
AST (Units/L)	26.7±3.2	31.3±5.7	23.4±2.1	23.2±1.4	26.8±6.3	20.8±1.8
ALT (Units/L)	34.8±6.9	34.5±6.9	24.0±2.3	27.9±4.0	33.4±9.9	25.4±4.6
TBIL (mg/dL)	0.7±0.0	0.6±0.0	0.6±0.0	0.5±0.0	0.8±0.3	0.8±0.2

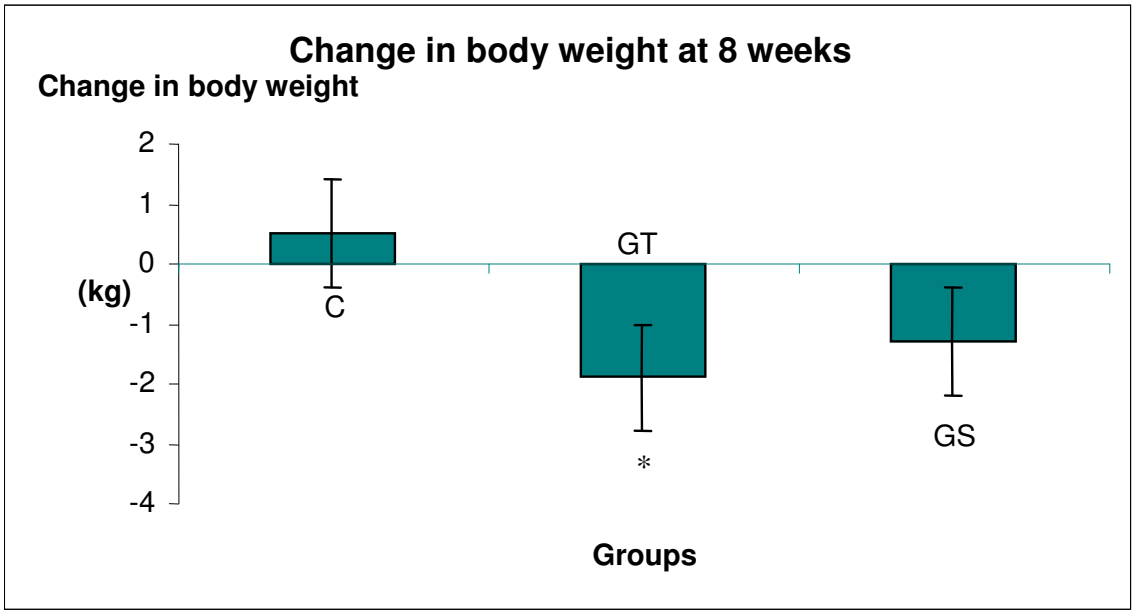
(Mean±Standard Error)

GT-green tea, GS-green tea supplement, WBC-white blood cells, RBC-red blood cells, Hb-hemoglobin HCT-hematocrit, MCV-mean cell volume, PLT-platelet, BUN-blood urea nitrogen, Cr-creatinine, AST-aspartate transaminase, ALT-alanine transaminase, TBIL-total bilirubin

* $p<0.05$, change significantly different from control
** $\triangle$ indicated significance {Control-(-0.2), GT-(6.5), **GS-(37.8)}

Figure 1

Effect of Green Tea Supplementation on Body Weight Change of Participants



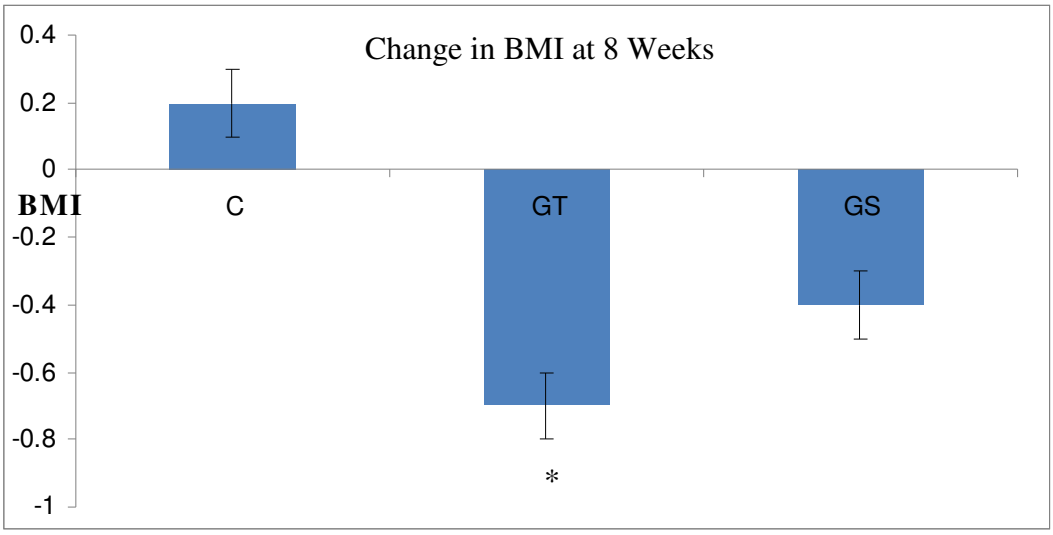
(Mean±Standard Error)

C- Control, GT- Green tea, GS- Green tea supplement

***p<0.05 compared to controls**

Figure 2

Effect of Green Tea Supplementation on BMI of Participants



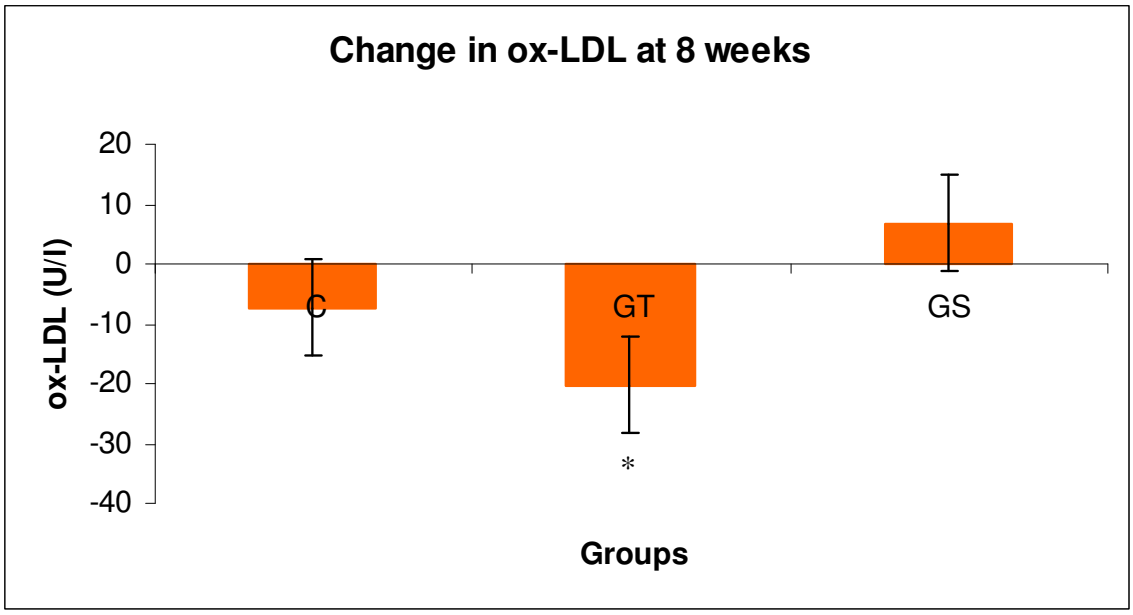
(Mean±Standard Error)

C- Control, GT- Green tea, GS- Green tea supplement

***p<0.05 compared to controls**

Figure 3

Change in oxidized-LDL in Participants by Treatment Group



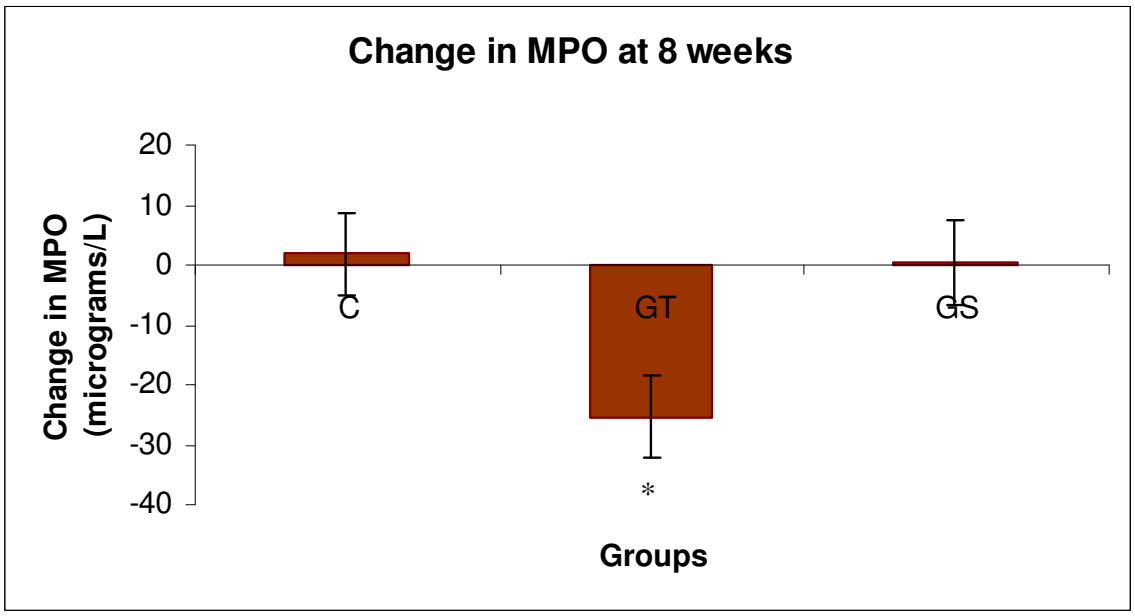
(Mean±Standard Error)

C- Control, GT- Green tea, GS- Green tea supplement

***p<0.05 compared to baseline**

Figure 4

Change in myeloperoxidase (MPO) in Participants by Treatment Group



(Mean±Standard Error)

C- Control, GT- Green tea, GS- Green tea supplement

***p<0.1 compared to control, p<0.05 compared to supplement**

CHAPTER V

CONCLUSION

Discussion

The current study investigated the effects of green tea flavonoid supplementation on hematology, safety parameters, and oxidative stress in obese middle-aged subjects with MeS. Decaffeinated green tea beverage (4 cups/day) and green tea supplement (500mg EGCG/day) was used in the present study. The tea was decaffeinated to avoid any conflicting variations that caffeine may have on the parameters tested. Recorded clinic visits and pill counts helped in the determination of a high participant compliance rate.

There were no statistically significant differences in age and markers of MeS among the three groups at baseline (Table 1). At the end of the study, the changes in weight and BMI were significantly greater in the green tea group ($p<0.05$), when compared to the controls. One of the important findings of our study lies in the fact that weight decreased by and average of 1.9 kg and BMI by 0.7 in the green tea group with was significantly different from controls. The supplement group, however, exhibited a decreasing trend, compared to controls ($p<0.1$). Therefore, it is probable that with a longer study duration green tea beverage and supplement would have caused weight loss decreased BMI. According to Cooper et al., 2005 green tea supplementation has been shown to promote weight loss which may in turn have a positive effect on metabolic

syndrome. However, Hino et al., 2007 found no relationship between green tea consumption and metabolic syndrome when analyzing the relationship between habitual coffee and green tea supplementation in the general Japanese population. Thus, green tea beverage intake may help promote weight loss specifically in obese subjects with metabolic syndrome.

Safety parameters (liver function and renal function tests) were also investigated in this study. There was a non-significant decrease in total bilirubin in both the control and green tea beverage group while there was no change seen in the supplemented group. In addition, there was a non-significant decrease in AST in both the green tea beverage and supplement group while an increase was shown in the control. Both AST and ALT remained within normal limits throughout the duration of the study. This may suggest that green tea beverage and supplement is safe for use in individuals with MeS.

All groups were within normal limits with regard to renal function which may indicate that green tea beverage or supplement had no harmful effects on renal function. There were no significant changes in safety parameters during this 8 week study. The lack of change in most of the safety parameters in this study show that green tea beverage (up to 4 cups/day) and supplement (up to 500mg EGCG per day) may be safe for use in individuals with MeS. Furthermore, no adverse events were reported with green tea beverage or supplement during this 8 week clinical trial which is also an indication of safety. However, the small sample and short study duration may explain the fact that no significant differences were noted in the safety parameters. When Chow et al., 2005 investigated the safety/pharmacokinetics of green tea supplementation for weeks, they found that green tea polyphenol supplementation is safe for healthy individuals in doses

equivalent to 8-16 cups of green tea per day. The reported adverse events noted by Chow et al. included excess gas, upset stomach, nausea, dizziness, and headache. However, these events were mild and not significantly more than the placebo group which was an indication of safety and are in concordance with the findings of our study.

Hematological parameters included in this study were WBC, RBC, Hb, HCT, MCV, and PLT all of which were within normal limits at both baseline and 8 weeks. However, there was an increase in platelets within the supplement group versus the control group. This could be due to a wide variety of factors including medication use, hydration status and the small sample size in this group. It should however be noted that the increase was within the normal range of platelets. The lack of change in most of the hematology, except platelets, at 8 weeks when compared to baseline is a further indication that green tea beverage and supplement are safe for use in subject with MeS. According to Chow et al., 2003 no changes in hematology were observed during a 4 week study period when participants were given either 800 mg once a day or 400 mg twice a day of EGCG or EGCG as polyphenon E.

Biomarkers of oxidative stress measured in this study included ox-LDL and MPO. Green tea catechins (primarily EGCG) may play a role in the inhibition of LDL oxidation. Miura et al., 2000 reported that tea polyphenols inhibited the oxidation of LDL. With regards to ox-LDL there was a significant decrease in the green tea beverage group at 8 weeks when compared to baseline ($p<0.05$). However, this decrease in the green tea group was non-significant in comparison to controls. Furthermore, a non-significant increase in ox-LDL was seen in the supplement group at 8 weeks when compared to baseline. A 42 day study by Erba et al., 2005 that involved 24 healthy

females found a slight decrease in LDL as compared to the control group and an improved plasma antioxidant status. Furthermore, Serafini et al., 2000 found that green tea showed a maximum decrease in LDL oxidation 30 minutes after ingestion.

Green tea group showed a decreasing trend in MPO levels compared to controls. This may be an indication that (with a longer study duration) green tea beverage may show a significant decrease in MPO levels when compared to controls. The supplement group showed no significant decrease in MPO levels while the controls showed a non-significant increase in MPO levels.

When analyzing ox-LDL and MPO levels at baseline, 4 weeks and 8 weeks, green tea beverage seemed to have the greatest reducing effects on the biomarkers of oxidative stress. There was a significant decrease within the green tea beverage group in both ox-LDL (when compared to baseline) and MPO (when compared to supplement). This shows that green tea beverage may have a greater positive effect on biomarkers of oxidative stress compared to the supplement group and controls. Therefore, freshly brewed green tea may have a greater bioavailability than encapsulated green tea extract. However, the supplement did show a non-significant decrease in MPO which, if the study were extended, may have been significant. The use of medication should be taken into account when analyzing the results from any of the clinical parameters measured in this study. Previous clinical trials have not investigated the effects of green tea flavonoid supplement on MPO levels. Thus, to our knowledge this is a unique finding of decreasing trend in MPO levels following green tea flavonoid supplement.

Limitations

The small sample size in the present study in conjunction with the short duration of the study is a limitation of the current study. This may also account for the trends that did not reach significance. A larger sample size and longer study duration in future clinical trials on green tea intervention may improve findings and better show the effects of green tea beverage and supplement on subjects with MeS.

Compliance and lifestyle changes are possible limitations to this study. Even though pill counts were administered and there was monitoring of green tea beverage consumption, there was no monitoring of compliance outside GCRC. Subjects were instructed to maintain their typical physical activity patterns. However, physical activity was not monitored and lifestyle changes could have been made outside the GCRC.

Along with compliance and lifestyle changes, medication use is another possible limitation of this study. The benefits of green tea beverage and/or supplement may have been masked by medication use and this may have affected the parameters tested. Future studies need to address the interactions of green tea beverage and supplement in conjunction with medication use.

Implications for Future Practice and Research

This clinical trial showed that the daily consumption of up to 4 cups/day of freshly brewed green tea is safe for use in individuals with MeS. Furthermore, it was shown that intake of 500 mg EGCG/day is safe for use in individuals with MeS. A longer study duration may have shown more significant changes on hematology and safety parameters. Green tea beverage decreased ox-LDL significantly at 8 weeks when compared to baseline ($p<0.05$) and the green tea group showed a decreasing trend in

MPO levels compared to controls. Additional research is needed to determine the effects of green tea supplementation on biomarkers of oxidative stress in subjects with MeS and determine the differences in bioavailability of green tea beverage and supplementation. Future studies on subjects not on stable medication may show a different result on the clinical parameters measured.

Hypotheses

The present study looked at the correlation between chronic green tea supplementation and its effects on hematological profile, safety parameters and oxidative stress in subjects with MeS. The null hypotheses tested, both of which we reject, included the following:

- Green tea extract supplementation or green tea beverage will have no effects on safety parameters in individuals with MeS. We reject the null hypothesis. There was a significant change in platelets ($p<0.05$) in the supplement group versus control.
- Green tea extract supplementation of green tea beverage will not lower biomarkers of oxidative stress in subjects with MeS. We reject the null hypothesis. Ox-LDL decreased in the green tea group significantly versus baseline ($p<0.05$); decreasing trend in MPO in green tea groups vs controls.

Conclusion

According to the findings of this study, freshly brewed green tea and green tea supplement is safe for use in middle-aged obese adults with MeS and green tea beverage was shown to lower biomarkers of oxidative stress. A decreasing trend was shown with

regards to MPO when analyzing the effects of the supplemented group. Although this group did not reach statistical significance, a longer study duration may have presented different results. In addition, it is possible that the decreasing trend in MPO levels and ox-LDL would have reached a greater statistical significance in both the green tea beverage group and a statistical significance in the supplement group with the use of a larger sample and longer study duration. Thus, it can be concluded that green tea beverage and supplement may provide safe effective means of improving features of MeS and decreasing oxidative stress in these subjects.

REFERENCES

Alessio H M, Hagerman A E, Romanello M, Carando S, Threlkeld M S, Rogers J, Dimitrova Y, Muhammed S, Wiley R L. Consumption of green tea protects rats from exercise-induced oxidative stress in kidney and liver. Nutrition Research. 2002; 22:1177-1188.

Babu P V A, Sabitha K E, Shyamaladevi C S. Therapeutic effect of green tea extract on oxidative stress in aorta and heart of streptozotocin diabetic rats. Chemico-Biological Interactions. 2006; 162:114-120.

Basu A, Lucas E A. Mechanisms and effects of green tea on cardiovascular health. Nutrition Reviews: 2007; 65:361-375.

Cao G, Sofic E, Prior R L. Antioxidant capacity of tea and common vegetables. J. Agric Food Chem. 1996; 44:3426-3431.

Chow S, Iman Y C, Hakim I A, Crowell J A, Shahi F, Brooks C A, Dorr R T, Hara Y, Alberts D S Pharmacokinetics and safety of green tea polyphenols after multiple-dose administration of epigallocatechin gallate and polyphenon E in healthy individuals. Clinical Cancer Research. 2003; 9:3312-3319.

Coimbra S, Castro E, Rocha-Pereira P, Rebelo I, Rocha S, Santos-Silva A. The effect of green tea in oxidative stress. Clinical Nutrition. 2006; 25:790-796.

Cooper R, Morre D J., Morre D M. Medicinal benefits of green tea: part I. review of noncancer health benefits. The Journal of Alternative and Complementary Medicine. 2005; 11:521-528.

Craig W J. Health-promoting properties of common herbs. Am J. Clin Nutr 1999; 70 (suppl):491S-499S.

Crespy V, Williamson G. A review of health effects of green tea catechins in *in vivo* animal models. J. Nutr. 2004; 134:3431s-3440s.

Erba D, Riso P., Bordoni A, Foti P, Biagi P L, Iestolin G. Effectiveness of moderate green tea consumption on antioxidative status and plasma lipid profile in humans. J. Nutr Biochem. 2005; 16:144-149.

Forrester J S, Libby P. The inflammation hypotheses and its potential relevance to statin therapy. Am J. Cardio. 2007; 99:732-738.

Hino A, Adachi H, Enomoto M, Furuki K, Shigetoh Y, Ohtsuka M, Kumagae S-I, Hirai Y, Jalaldin A, Satoh A, Imaizumi T. Habitual coffee but not green tea consumption is inversely associated with metabolic syndrome. an epidemiological study in a general Japanese population. Diabetes Research and Clinical Practice. 2007: 76:383-389.

Ishige K, Schubert D, Sagara Y. Flavonoids protect neuronal cells from oxidative stress by three distinct mechanisms. Free Radical Biology and Medicine. 2001; 30:433-446.

Ishikawa T, Suzukawa M, Ito T, Yoshida H, Ayaori, M, Nishiwaki M, Yonemura A, Hara Y, Nakamura H.

Effect of tea flavonoid supplementation on the susceptibility of low-density lipoprotein to oxidative modification. Am J Clin Nutr. 1997;66:261–266.

Khan N, Mukhtar H. Tea polyphenols for health promotion. Life sciences. 2007; 81:519-533.

Krawisz J E, Sharon P, Senson W F. Quantitative assay for acute intestinal inflammation based on myeloperoxidase activity. Assessment of inflammation in rat and hamster models. Gastroenterology. 1984; 87: 1344-1350.

Libby P. Inflammation and cardiovascular disease mechanisms. Am. J. Clin Nutr. 2006; 83(suppl):456S-460S.

Liu C-C, Huang C-C, Lin W-T, Hsieh C-C, Huang S-Y, Lin S-J, Yang S-C. Lycopene supplementation attenuated xanthine oxidase and myeloperoxidase activities in skeletal muscle tissues of rats after exhaustive exercise. British Journal of Nutrition. 2005; 94:595-601.

MacKenzie T, Leary L, Brooks W B. The effect of an extract of green and black tea on glucose control in adults with type 2 diabetes mellitus: double-blind randomized study. Metabolism Clinical and Experimental. 2007; 56:1340-1344.

Marouani N, Chahed A, Hedhili A, Hamdaoui M H. Both aluminum and polyphenols in green tea decoction (*Camellia sinesis*) affect iron status and hematological parameters in rats. Eur J. Nutr. 2007; 46:453-459.

Miura S, Watanabe J, Tomita T, Sano M, Tomita I. The inhibitory effects of tea polyphenols (flavan-3-ol derivatives) on Cu2 + mediated oxidative modification of low density lipoprotein. Biol Pharm Bull. 1994; 17:1567-1572.

Miura Y, Chiba T, Miura S, Tomita I, Umegaki K, Ikeda M, Takako T. Green tea polyphenols (flavon 3-ols) prevent oxidative modification of low density lipoproteins: an ex vivo study in humans. J. Nutr. Biochem. 2000; 11:216-222.

Ninomiya M, Okubo T, Aoi N, Juneja L R, Kim M, Yamanaka K, Miyazawa T. Tea catechin supplementation increases antioxidant capacity and prevents phospholipid hydroperoxidation in plasma of humans. J. Agric Food Chem. 1999; 47:3967-3973.

Osada K, Takahashi M, Hoshina S, Nakamura M, Nakamura S, Sugano M. Tea catechins inhibit cholesterol oxidation accompanying oxidation of low density lipoprotein in vitro. Comp Biochem Physiol C Toxicol Pharmacol. 2001;128:153–164.

Popkin B M, Armstrong L E, Bray G M, Caballero B, Frei B, Willett W.C.. A new proposed guidance system for beverage consumption in the united states. Am J. Clin Nutr. 2006; 83:529-542.

Raza H, John A. Green tea polyphenol epigallocatechin-3-gallate differentially modulates oxidative stress in PC12 cell compartments. Toxicology and Applied Pharmacology. 2005; 207:212-220.

Rietveld A, Wiseman S. Supplement: proceedings of the third international scientific symposium on tea and human health. antioxidant effects of tea: evidence from human clinical trials. J. Nutr 2003; 133:3285S-3292S.

Serafini M, Laranjinha J A N, Almeida L M, Maiani G. Inhibition of human LDL lipid peroxidation by phenol-rich beverages and their impact on plasma total antioxidant capacity in humans. J. Nutr Biochem. 2000; 11: 585-5990.

Trevisanato S I, Kim Y-I. Tea and health. Nutrition Reviews. 2000; 58: 1-11.

Tuczaj W, Waszkiewicz E, Skrzydlewska E, Roszkowska-Jakimiec W. Green tea protection against age-dependent ethanol-induced oxidative stress. Journal of Toxicology and Environmental Health, part A. 2004; 67:595-606.

Want H, Provan G J, Helliwell K. Flavonoids: their functions, utilization and analysis. Trends in food science and Technology 2000; 11:152-160.

Yang TT, Koo MW. Inhibitory effect of Chinese green tea on endothelial cell-induced LDL oxidation. Atherosclerosis. 2000; 148:67–73.

APPENDIX A

Oklahoma State University Institutional Review Board

Date	Wednesday, November 21, 2007 **Protocol Expires:** 11/20/2008
IRB Application No:	HE06103
Proposal Title:	Effects of Chronic Green Tea Favonoid Supplementation on Biomarkers of Oxidative Stress and Inflammation, and Body Fat Composition Among Subiects with Metabolic Svndrome (MeS)
Reviewed and Processed as:	Expedited **Continuation**

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

Principal Investigator(s) :

Arpita Basu
416 HES
Stillwater, OK 74078

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

Karah Sanchez
14900 N. Penn Ave Apt. 1224
Oklahoma City, OK 73134

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

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

Signature : Sue C Jacobs

Sue C. Jacobs, Chair, Institutional Review Board

Wednesday, November 21, 2007
Date

APPENDIX B

OUHSC IRB # 13154
OSU IRB # HE 06103
Version date: Oct 30, 2007

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

Title: Effects of chronic Green Tea Flavonoid Supplementation on Biomarkers of Oxidative Stress and Inflammation, and Body Fat Composition among Subjects with Metabolic Syndrome (MeS)

Investigator(s): Dr. Timothy Lyons, University of Oklahoma Health Sciences Center
Dr. Arpita Basu, Oklahoma State University

Sponsor: Department of Nutritional Sciences, Oklahoma State University

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 metabolic syndrome. (MeS). Metabolic syndrome is a condition where you have at least three of the five following features: being overweight, having high blood pressure, having increased blood sugar, having increased lipids, and low levels of good lipids. This condition puts you at a high risk of developing diabetes and heart disease.

Why Is This Study Being Done?
The purpose of this study is to find out about the health effects of green tea compared to green tea supplement intake on certain markers in your blood associated with cell damage linked to MeS. The green tea and green tea supplements do not contain significant amounts of caffeine. We will also find out if green tea or green tea supplement will help you reduce body weight and lead to better use of glucose in your body.

What is the Status of the Drugs (Devices or Procedures) involved in this study?
This study involves the use of green tea or green tea supplements which do not contain significant amounts of caffeine. The green tea supplement is not approved by FDA. We will compare the effects of drinking 4 cups of green tea or taking two capsules of green tea extracts on certain markers in blood.

How Many People Will Take Part In The Study?
About 60 people will take part in this study

What Is Involved In The Study?
This is an 8 week study that will be conducted at the General Clinical Research Center (GCRC) at Oklahoma City, OK. If accepted into the study you will visit the GCRC five times during the 8 weeks.

APPROVED
NOV 1 4 2007
OUHSC IRB

Okla. State Univ. IRB
Approved 11/2/07
Expires 11/2/08
IRB # HE-08-103

APPROVAL EXPIRES
AUG 3 1 2008
OUHSC IRB

Screening visit: During this 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, waist, and the amount of fat in your body;
- Drawing about 3-4 tablespoons of fasting blood for measuring your blood sugar, lipids, blood cell counts, and to do some tests to find out how well your cells, liver, kidney, and thyroid are working;
- Providing you with guidelines and forms for 3-day food record.

If you qualify, we will let you know over the telephone and you will be randomized into one of three groups: green tea beverage group, green tea extract group and a group that uses no green tea (to serve as a control). Randomization means that you are put in a group by chance. You have a 1 in 3 chance of being in any of these groups. A computer program at the study sponsor will make this random assignment. Neither you nor your physician will choose which group you will be in. If you take part in this study and qualify, you could be assigned to any of the three following groups:

Control Group: You will follow your usual diet and lifestyle, and drink an additional 4 cups of water per day.

Green Tea Group: You will be drinking 4 cups of green tea per day and will be making daily visits to the clinic (except on weekends) to get a supply of the fresh tea.

Green tea supplement Group: You will be taking two capsules of green tea supplement per day, and drink an additional 4 cups of water daily.

The following visits will be required for all qualified participants:

- 2 weeks- turn in 3-day food records, short talk on how well you are doing on this study.
- 4 weeks- turn in 3-day food records, draw about 3-4 tablespoons of fasting blood for measuring your blood sugar, lipids, and do some tests to find out how well the cells in your body are working. We will also measure your body weight, blood pressure, and the amount of fat in your body, and do some safety tests.
- 6 weeks- turn in 3-day food records, short talk on how well you are doing on this study.
- 8 weeks- This will be your final visit; turn in 3-day food records, draw about 3-4 tablespoons of fasting blood for measuring your blood sugar, lipids, and do some tests to find out how well the cells in your body are working. We will also measure your body weight, blood pressure, and the amount of fat in your body, and do some safety tests.

How Long Will I Be In The Study?

We think that you will be in the study for a period of 8 weeks and 5 total visits. The duration of each visit will be between ½ - 1 hour. If you are in the green tea group, you will be making daily visits (except on weekends) to get freshly made green tea.

The researcher may decide to take you off the study if you develop any serious side effects while drinking green tea or taking green tea supplements.

You can stop participating in this study at anytime. However, if you decide to stop participating in the study, we encourage you to talk to the researcher and your regular doctor first. However, there are no serious consequences of sudden withdrawal from the study.

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.
Green Tea Group:

Likely: the risks involved with drinking 4 cups of green tea per day may include some stomach pain, gas, or headache.

Less likely: you may develop some allergies

Green Tea Supplement Group:

Likely: the risks involved with green tea supplements may be some stomach pain, gas, loose stools, or headache.

Less likely: you may develop some allergies. Some studies have shown liver problems upon taking very high doses of green tea supplements. However, this is less likely to happen at the dose we are using in this study.

Are There Benefits to Taking Part in The Study?
If you agree to take part in this study, there mayor may not be direct medical benefit to you. We hope that the information learned from this study will benefit other patients with this disease in the future.

What Other Options Are There?
You may choose not to participate in the study and please talk to your doctor about other options.

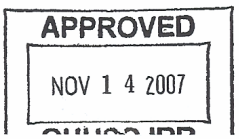
What About Confidentiality?
Efforts will be made to keep your personal information confidential. All participants will be assigned a code and data will be stored using that code. You will not be identifiable by name or description in any reports or publications about this study. We cannot guarantee absolute 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 Oklahoma State University at Stillwater, and the OUHSC Institutional Review Board.

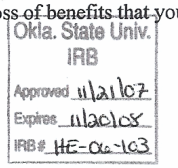
What Are the Costs?
The study sponsor will pay for all costs related to your participation in this study.

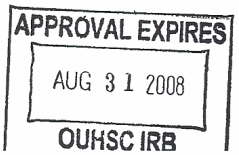
Will I Be Paid For Participating in This Study?
You will not be paid for participating in this study but you will be reimbursed $ 30 per visit to cover travel and expenses; a total of$150. Also, if you participate in the green tea beverage group, you will not be compensated for the expenses involved while you come and pick up your tea each day.

What if I am Injured or Become Ill While Participating in this Study?
It is not anticipated that you will be injured participating in this study. No funds have been set aside by Oklahoma State University or The University of Oklahoma Health Sciences Center (General Clinical Research Center) 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 take part or may leave the study at any time. If you agree to take part and then decide against it, you can withdraw for any reason. Leaving the study will not result in any penalty or loss of benefits that you would otherwise receive.







We will tell you about any new information that may affect your health, welfare or willingness to stay in this study. You may also obtain green tea beverages and supplements outside of the study if you choose not to participate.
You understand that you have the right to access the medical information that has been collected about you as a part of this research study. However, you agree that 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 the study or have a research-related injury, contact Dr. Arpita Basu at 405-744-4437 (9AM -5PM, Monday-Friday)or at 916-607-4143 (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 have questions about your rights as a research participant, you may contact Dr. Sue C. Jacobs, OSU IRB Chair, 219 Cordell North, Stillwater, OK 74078, 405-744-1676 or irb@okstate.edu or the OUHSC Director, Human Research Participant Protection Program 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:

Research Subject: _____
Date:_____

Subject's Printed Name: _____

Person Obtaining Informed Consent:_____
Date:_____

APPROVED
NOV 1 4 2007
OUHSC IRB

Okla. State Univ. IRB
Approved 11/21/07
Expires 11/20/08
IRB # HE-07-103

4

APPROVAL EXPIRES
AUG 3 1 2008
OUHSC IRB

University of Oklahoma Health Sciences Center

Research Privacy-Form 1
PHI Research Authorization

IRB No.:13154

AUTHORIZATION TO USE or DISCLOSE
PROTECTED HEALTH INFORMATION FOR RESEARCH

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

Title of Research Project: **Effects of chronic Green Tea Flavonoid Supplementation on Biomarkers of Oxidative Stress and Inflammation, and body fat composition among subjects with Metabolic Syndrome (MeS)**

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

Address: **Department of Nutritional Sciences, 416 Human Environmental Sciences, Stillwater, OK 74078-6141**

Phone Number: **405-744-4437**

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

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

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

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

Confidentiality. Although the researchers may report their findings in scientific journals or meetings, they will not identify you in their reports. The researchers will try to keep your information confidential, but confidentiality is not guaranteed. Any person or organization receiving the information based on this authorization could re-release the information to others and federal law would no longer protect it.

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

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

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

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

End of Permission. Unless you revoke it, permission for OUHSC researchers to use or share your private information for their research will end on 01/2009. You may revoke your permission at any time by writing to:

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

Giving Permission. By signing this form, you give OUHSC and OUHSC's researchers led by Arpita Basu, PhD, permission to share your private information for the research project called Effects of chronic Green Tea Flavonoid Supplementation on Biomarkers of Oxidative Stress and Inflammation, and body fat composition among subjects with Metabolic Syndrome (MeS).

Patient/Subject Name: _____

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

Or

_____ _____
Signature of Legal Representative** Date

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

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

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

IRB No.: 13154

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

SCREENING QUESTIONNAIRE FOR GREEN TEA STUDY

NAME: _____

ADDRESS: _____

_____

PHONE (WORK): _____

PHONE (HOME): _____

AGE: _____ DATE OF BIRTH: _____ GENDER: _____

SCREENING QUESTIONS:

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

Are you pregnant or lactating? YES NO N/A

Do you smoke? YES NO

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

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

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

Do you exercise ≥ 60 min/day? YES NO

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

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

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

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

Do you have any gastrointestinal problems? YES NO

Do you have anemia? YES NO

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

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

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

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

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

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

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

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

FEATURES OF METABOLIC SYNDROME (Check all that apply):

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

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

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

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

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

CITI Collaborative Institutional Training Initiative

Human Research Curriculum Completion Report
Printed on Wednesday, June 4, 2008

Learner: Natalie Briggs (username: shalynn.briggs)
Institution: Oklahoma State University
Contact Information Department: NSCI
Phone: 9184656512
Email: shalynn11@hotmail.com
Biomedical Research Investigator Faculty /Staff/Student:

Stage 2. Refresher Course Passed on 06/04/08 (Ref # 1854312)

Required Modules	Date Completed
Refresher Course 101 Introduction	06/04/08
101 Refresher Course - History and Ethics	06/04/08
101 Refresher Course - Regulations and Process	06/04/08
101 Refresher Course - Informed Consent	06/04/08
101 Refresher Course - Social and Behavioral Research	06/04/08
101 Refresher Course - An Overview of Research with Vulnerable Subjects	06/04/08
101 Refresher Course - Complete the course	06/04/08
Oklahoma State University module	06/04/08

For this Completion Report to be valid, the learner listed above must be affiliated with a CITI participating institution. Falsified information and unauthorized use of the CITI course site is unethical, and may be considered scientific misconduct by your institution.

Paul Braunschweiger Ph.D.
Professor, University of Miami
Director Office of Research Education
CITI Course Coordinator

Return

VITA

Natalie Shalynn Briggs

Candidate for the Degree of

Master of Science

Thesis: A COMPARATIVE STUDY OF GREEN TEA BEVERAGE AND GREEN TEA EXTRACT SUPPLEMENTATION ON CLINICAL VARIABLES AND OXIDATIVE STRESS IN SUBJECTS WITH METABOLIC SYNDROME (MeS)

Major Field: Nutritional Science

Biographical:

Education:

Completed the requirements for the Master of Science in Nutritional Science at Oklahoma State University, Stillwater, Oklahoma in July, 2008. Completed the requirements for the Bachelor of Science at Northeastern State University, Tahlequah, Oklahoma in May, 2006. Completed the requirements for an Associate of Science at Eastern Oklahoma State College, Wilburton, Oklahoma in May 2004

Professional Memberships: American Dietetic Association and Oklahoma Dietetic Association

Name: Natalie Shalynn Briggs Date of Degree: July, 2008

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: A COMPARATIVE STUDY OF GREEN TEA BEVERAGE AND GREEN TEA EXTRACT SUPPLEMENTATION ON CLINICAL VARIABLES AND OXIDATIVE STRESS IN SUBJECTS WITH METABOLIC SYNDROME (MeS)

Pages in Study: 52 Candidate for the Degree of Master of Science

Major Field: Nutritional Science

Scope and Method of Study: Twenty-eight subjects with MeS were enrolled in this 8 week clinical trial. The study examined the effects of green tea (4 cups/day), green tea supplement (2 capsules, 500 mg EGCG, 4 cups water/day), and control (4 cups water/day) on clinical variables and biomarkers of oxidative stress.

Findings and Conclusions: Freshly brewed green tea and green tea supplement is safe for use in middle-aged obese adults with MeS and green tea beverage was shown to lower biomarkers of oxidative stress. The change in body weight and BMI were significantly greater in the green tea group ($p<0.05$), when compared to the controls. A decreasing trend was shown with regards to MPO when analyzing the effects of the supplemented group. In addition, there was a significant decrease in ox-LDL in the green tea group at 8 weeks when compared to baseline ($p<0.05$).

ADVISER'S APPROVAL: Dr. Arpita Basu

Name: Natalie Shalynn Briggs Date of Degree: July, 2008

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: A COMPARATIVE STUDY OF GREEN TEA BEVERAGE AND GREEN TEA EXTRACT SUPPLEMENTATION ON CLINICAL VARIABLES AND OXIDATIVE STRESS IN SUBJECTS WITH METABOLIC SYNDROME (MeS)

Pages in Study: 52 Candidate for the Degree of Master of Science

Major Field: Nutritional Science

Scope and Method of Study: Twenty-eight subjects with MeS were enrolled in this 8 week clinical trial. The study examined the effects of green tea (4 cups/day), green tea supplement (2 capsules, 500 mg EGCG, 4 cups water/day), and control (4 cups water/day) on clinical variables and biomarkers of oxidative stress.

Findings and Conclusions: Freshly brewed green tea and green tea supplement is safe for use in middle-aged obese adults with MeS and green tea beverage was shown to lower biomarkers of oxidative stress. The change in body weight and BMI were significantly greater in the green tea group ($p<0.05$), when compared to the controls. A decreasing trend was shown with regards to MPO when analyzing the effects of the supplemented group. In addition, there was a significant decrease in ox-LDL in the green tea group at 8 weeks when compared to baseline ($p<0.05$).

ADVISER'S APPROVAL: Dr. Arpita Basu