EFFECTS OF CHRONIC GREEN TEA FLAVONOID SUPPLEMENTATION ON FEATURES OF METABOLIC SYNDROME, BIOMARKERS OF OXIDATIVE STRESS AND INFLAMMATION AND PLASMA CATECHIN LEVELS IN SUBJECTS WITH METABOLIC SYNDROME

By

KAVITHA PENUGONDA

Master of Science in Food and Nutritional Sciences
Sri Venkateswara University
Tirupati, India
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Dissertation Approved:

Dr.Arpita Basu

Dissertation Adviser

Dr. Nancy Betts

Dr.Edralin Lucas

Dr. A. Gordon Emslie

Dean of the Graduate College

DEDICATION

I dedicate my research to my great grandmother Koteswaramma. She is an encyclopedia of life and knows how to deal with every life situation. I also want to dedicate this research to my maternal grandfather Nagi Reddy and my paternal grandfather Venkata Reddy. Although they are not with us their blessings are always with me and my family. These persons played a vital role in my achievements. I also dedicate my research to all the people who are suffering mentally and physically in the world, to people who believe in love like Michael Jackson.

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CHAPTER I

INTRODUCTION

Metabolic syndrome (MeS), a constellation of several risk factors including dyslipidemia, hypertension, central adiposity, and impaired fasting glucose, is also a condition associated with increased inflammation and hypercoagulation. Subjects with MeS also possess an increased risk of developing type 2 diabetes and coronary heart disease (CHD) (1). Emerging research shows that obesity, hypertension, diabetes mellitus, dyslipidemia, smoking, aging, diets rich in saturated fats and reduced physical activity are the established risk factors for atherosclerosis and cardio vascular diseases (CVD) (2,3,4,5,6). All these metabolic and degenerative disorders are also characterized by inflammation and oxidant burden (7,8). Oxidative stress and inflammation play a pivotal role at all stages of atherosclerosis and the subsequent development of CHD (9,10). Hence, MeS subjects are at an increased risk for CVD like atherosclerosis.

The incidence of MeS is high among the US population (11). In the year 2000, approximately 64 million adults had MeS in US (12). Oklahoma is the 6th most obese state in US with 30.3% obesity prevalence (13). Also, according to the findings of the Strong Heart Study, the prevalence of MeS is high among the American Indians (43%) residing in Arizona, Oklahoma, North Dakota, and South Dakota (14). MeS, a condition characterized by increased oxidative stress and inflammation has a parallel association with overweight and obesity (15). Ever increasing prevalence of overweight and obesity predict further increase in MeS incidence in US.

Phytochemicals are a group of dietary bioactive compounds that were shown to have health promoting properties. Dietary bioactive compounds and phytochemicals have been shown to play a crucial role in attenuating biomarkers of oxidative stress and inflammation. According to NHANES 1999–2002 data on 24 hour dietary recalls, the mean flavonoid intake among US adults is 189.7 mg/day, with tea (157mg flavonoid/day) as the major flavonoid contributor. Almost 84 % of the total dietary flavonoid was contributed by tea with 21.3% US adults reported drinking tea on a daily basis. Daily total flavonoid intake of tea consumers was over 20 times than tea non consumers (697.9 mg/day vs 32.6 mg/day) (16), indicating that tea is the major flavonoid source in US diets.

Several epidemiological studies found an inverse association between dietary flavonoid intake and mortality. Large cohort and meta-analysis studies found a significant reduction in incidence of myocardial infarction (MI) and relative risk of death due to CVD with increase in green tea consumption (17,18,19). In- vitro studies have suggested the role of flavonoids in cocoa, berries, tea, apples, onions, and red wine, in reducing the biomarkers of oxidative stress and inflammation (20). Epigallocatechin-3-gallate (EGCG), a major bioactive polyphenol present in green tea is a potent antioxidant and also an anti-inflammatory agent (21). Both green tea beverage and green tea extracts showed cardiovascular benefits by decreasing biomarkers of oxidative stress like Ox-LDL (22) and inflammation like C-reactive protein (CRP) (23). The health benefits of green tea are mainly attributed to its flavonoid content that has anti-oxidant and anti-inflammatory characteristics.

Even though green tea was studied extensively for its antioxidant and anti-inflamamtory properties, most of the studies were done in animals and in cancer cell lines. Limited numbers of studies were done on cardiovascular benefits in individuals at risk like MeS subjects. Since there is a limited research data on antioxidant and anti-inflammatory health benefits of green tea flavonoids in MeS subjects, this study was conducted to assess the antioxidant and anti-inflammatory effects of chronic green tea flavonoid intake among subjects with MeS, a group more vulnerable to degenerative diseases. This study will also address the high burden of MeS in the state of Oklahoma. Green tea flavonoid supplementation may lower oxidative stress, thereby reducing the risks of atherosclerosis and other CVD in the subsequent years, in subjects with MeS. Since very little information is available in this field, our findings will add to the existing body of knowledge in the field of dietary bioactive compounds, including green tea flavonoids, and MeS.

Purpose

There is limited information on the health benefits of green tea in MeS. The major purpose of this study is to assess the effects of chronic green tea flavonoid supplementation as a beverage or extract on features of MeS, biomarkers of oxidative stress and inflammation and plasma free catechin concentrations in MeS subjects. As there were not many comparison studies we want to compare the freshly brewed green tea beverage with green tea extract capsule supplementation in improving the components of MeS.

Null hypotheses:

The following null hypotheses are being tested in our study.

1. Green tea beverage or green tea extract supplementation has no effect on anthropometric measures and features of metabolic syndrome in subjects with MeS.
2. Green tea beverage or green tea extract supplementation has no effect on biomarkers of oxidative stress and inflammation in subjects with MeS.
3. Green tea beverage or green tea extract supplementation has no effect on plasma catechin levels in subjects with MeS.

CHAPTER II

REVIEW OF LITERATURE

Metabolic syndrome: prevalence, causes & consequences

Metabolic syndrome is one of the major public health problems and is found to be common in most countries of the world. In western countries like Americas, Europe and Asian countries like India, at least one-fourth of the adults are affected by this syndrome (24). Nearly 50 million US adults aged ≥ 20 yrs had MeS in 1990 and it increased to approximately 64 million by the year 2000. NHANES III 1988-1999 and NHANES 1999-2000 surveys indicate a significant increase in MeS prevalence from 23.1% to 26.7% (12). MeS prevalence appears to be increasing because of the parallel increase in the obesity prevalence from 22% in 1988-1999 to 30.5% in 1999-2000. Another contributing factor for higher prevalence of MeS is aging of the population (24).

Metabolic syndrome is a disorder that predisposes to the development of type 2 diabetes and atherosclerotic diseases. This is mainly due to increased oxidative stress and inflammation that are associated with MeS (25). Hyperglycemia, a common feature of MeS, leads to the production of reactive oxygen species (ROS) like superoxide anion ($O2^-$) and hydrogen peroxide ($H2O2$), through the activation of different pathways (26).

Free radicals are molecules that contain one or more unpaired electrons and are highly reactive. Free radicals are produced as part of the normal metabolic reactions and are cleared by natural and dietary antioxidants present in the body (27). ROS react with nitric oxide and form more potent reactive nitrogen species (RNS) like peroxynitrite. Both ROS and RNS are toxic and affect many physiological processes like endothelial function and lipid peroxidation. (28). ROS also cause oxidative damage to the vital cell

structures like cell proteins and DNA that can lead to cancer, atherosclerosis and heart diseases. Free radicals are not harmful all the time. They are also essential for microbial killing and immune function. Anti-oxidants are the substances that scavenge these radicals. However, excessive levels beyond the endogenous anti-oxidant capacity to neutralize them are harmful (27). Green tea was shown to trap both reactive oxygen and nitrogen species and reduce oxidative stress (29).

Role of oxidative stress and inflammation in CVD

Oxidative stress, an imbalance between free radical formation and antioxidant status, is the major contributor to CVD, and inflammation is a manifestation of oxidative stress. Oxidative stress induces inflammation by acting on the pathways that generate inflammatory mediators like adhesion molecules and pro-inflammatory cytokines (27). Recent human studies have shown significant positive associations between oxidative stress and inflammation and indicators of vascular damage, like impaired endothelial function (30). Oxidative stress and inflammation also induce vascular smooth muscle cell (VSMC) activation and proliferation, angiogenesis, lipid peroxidation and platelet activation (31,32,33,28). All these initiate and promote atherosclerosis and increase CVD risk. Thus, a food rich in anti-oxidants like green tea may help in promoting cardiovascular health by reducing both inflammation and oxidant burden.

Body has different mechanisms to get rid of the oxidant burden and to maintain the redox balance. One of such mechanisms is balance between prooxidant and anti-oxidant enzymes. NAD(P)H oxidase, xanthine oxidase and myeloperoxidase are some of the ROS-generating oxidant enzymes and enhanced activity of these enzymes leads to oxidative stress. Endogenous antioxidant enzymes and vitamins like superoxide

dismutase, glutathione peroxidase, catalase, vitamin E and C scavenge or neutralize the excess free radicals and help in mitigating the oxidative stress and maintaining homeostasis (28).

Biomarkers of oxidative stress and inflammation

C-reactive protein (CRP) is the best validated inflammatory biomarker and a good predictor of future CVD events (33,34). In addition to CRP, adiponectin, vascular cell adhesion molecule-1 (VCAM-1), tumor necrosis factor- α (TNF-α), interleukin-1 (IL-1), interleukin-18 (IL-18), soluble CD40 ligand (sCD40L), monocyte matrix metalloproteinase 9 (MMP-9) are other major biomarkers of inflammation (35,34) Whereas, lipid peroxidation, oxidized-low density lipoprotein(Ox-LDL), urinary 8-isoprostane levels are the oxidative biomarkers of CVD risk factors (36).

CRP is an acute phase protein and its levels may increase up to 1000 fold during acute inflammation. CRP is an independent marker of systemic inflammation. Under laboratory conditions CRP is measured as high sensitivity CRP (hsCRP). According to Li and Fang (2003), CRP is not only an indicator of inflammation, it also participates in the process of atherosclerosis by up regulating the expression of adhesion molecules, promoting the Ox-LDL uptake by macrophages, stimulating macrophages to release pro inflammatory cytokines like interleukin- 6 (IL-6) and TNF-α, activating vascular smooth muscle cells (VSMC) (37,38,32). Hence, CRP measurement in addition to the lipid profile will help in more accurate assessment and early detection of future CVD risk.

It is well recognized that high levels of LDL is associated with CVD risk. Oxidative modification of LDL produces Ox-LDL in the arterial wall. Ox-LDL evokes

inflammatory response and participates in the process of atherosclerosis. Circulatory Ox-LDL levels is an indicator of oxidative stress and predictor of CVD risk (35,38).

Nitric oxide (NOx) is a gaseous molecule with a very short half life of few seconds. NOx is produced from amino acid L-arginine by the action of nitric oxide synthase (NOS) (39). Three isoforms, endothelial NOS (eNOS), neuronal NOS (nNOS) and inducible NOS (iNOS) catalyze this reaction and are expressed in endothelial, neuronal and inflammatory cells respectively. Both eNOS and nNOS are constitutively expressed and produce NOx on demand at low physiological concentrations. This constitutive NOx is responsible for the regulation of many physiological processes like vasodilatation, cell communication and promoting endothelial integrity by preventing adhesion of inflammatory cells and adhesion molecules to endothelial cells.

Where as, iNOS is expressed in immune cells after cell activation by proinflammatory cytokines like tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-2 and interferon gamma (IFN-γ) and results in over production of nitric oxide for a longer period of time (40). Abundance of NOx leads to the production of peroxy nitrite ($ONOO^-$) by reacting with superoxide anion (O_2^-). $ONOO^-$ is a more potent cytotoxic reactive species Even though there is a greater production of NOx by iNOS under inflammatory conditions, its bioavailability and bioactivity will be reduced by formation of $ONOO^-$ (39,41).

Green tea: processing, storage, and stability

Tea is one of the most widely used beverages worldwide. It is one of the most ancient Asian liquid foods. *Camellia sinensis* is the botanical name of tea plant and it belongs to *Theaceae* family. Tea leaves contain polyphenols and an enzyme polyphenol

oxidase. When tea leaves are chopped, this enzyme gets activated and oxidizes polyphenols. Different tea products like green tea, black tea and oolong tea are manufactured by subjecting fresh tea leaves to a series of treatments. (42).

Green tea, most popular and more widely used tea in China, is made by exposing the cut leaves to hot steam or by applying high temperatures. This heat treatment prevents polyphenol oxidation by inactivating polyphenol oxidase (43,44). The basic steps involved in green tea manufacture are plucking, heat treatment either by steaming or pan firing, rolling and high temperature air drying. The major aim of all these processes is to preserve the catechins in the final product. Variation in these processes results in compositional and aromatic differences among different green tea brands (42). Where as, black tea is produced by promoting enzymatic oxidation or fermentation and oolong tea by semi oxidation. Fermentation converts catechins to theaflavins and thearubigins and reduces catechin content in black tea (45).

Tea composition varies with variety, age, position of the leaf, climate and season. Young leaves like leaf bud and first leaf are richest in catechins. However the leaves used for green tea manufacture are relatively low in catechin content compared to the ones used for black tea (42). This may be due to the great commercial value for black tea than other teas.

Green tea chemistry

The principal flavonoid phytochemicals present in green tea are called catechins. Catechins are polyphenolic plant metabolites that belong to flavonoid group, particularly to flavan-3-ols. Catechins are colorless water soluble compounds with astringent taste. Epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), epicatechin-3-gallate (ECG)

and epicatechin (EC) are the major catechins found in green tea. Catechins are phenols composed of three benzene rings with hydroxyl groups. The number of hydroxyl groups indicates the antioxidant capacity. Catechins constitute about 30% of green tea by dry weight (42). EGCG is the major green tea catechin and it accounts for 50-80% of the total catechin content (46).

Digestion, absorption, and metabolism of catechins

Flavonoids in nature are always glycosides with a sugar moiety, but catechins occur as aglycones. Intact flavonoid aglycones reach the small intestine following ingestion. They don't need any transporters and diffuse into enterocyte. In the enterocyte they undergo conjugation reactions and reach the liver for further metabolism. All these metabolites are released into systemic circulation, reach different parts of the body and excreted through urine and feces (47).

However some of the conjugated catechins from liver are transported back to small intestine through bile via enterohepatic circulation. Catechin conjugates cannot diffuse through the intestinal epithelium hence they reach colon, where colonic microflora deconjugate them and releases aglycones. Aglycones may be reabsorbed or undergo further metabolism (48). Microflora convert catechins to phenyl valerolactones and then to phenolic acids. Catechin metabolites may be absorbed in the colon or excreted through feces (46).

Three types of metabolic pathways methylation, glucuronidation and sulfation have been identified for all catechins. Liver, small intestine and colon are the major organs where catechins are extensively metabolized (46). Small intestine has lower specific activity than liver in the methylation. Methylation occurs in cytosol and is

catalyzed by catechol-O-methyltransferase (COMT). The products of methylation are mono or dimethylated catechins. Glucuronidation occurs in the microsomes of liver and small intestine. Glucuronosyl transferases (UGT) catalyze the conjugation and form glucuronides. Sulfation is carried out by sulfotransferases (SULT) in liver and intestinal cytosol (49). All these catechin and catechin metabolites reach liver through portal vein and then reach systemic circulation. They undergo phase I and phase II metabolism at various sites in the body and finally excreted through urine and feces (48).

Both EGC and EC were absorbed much faster than EGCG. Delayed absorption may be due to the extra gallate moiety in EGCG. EGCG was better absorbed when given through oral route than through intra gastric administration in rats, and also the absorption is 3.6 fold higher when it given as green tea beverage rather than a pure EGCG supplement. Another important finding is that EGCG was less readily eliminated from body when given in the form of green tea beverage compared to supplement. This may be due to the competition for elimination enzymes like sulfotransferase and glucuronosyltransferase that result in inhibition of EGCG elimination. Not all the catechins are excreted in a similar way. EGCG is excreted mainly through bile whereas EGC and EC are excreted through both bile and urine (50).

Green tea catechin stability

Several factors like pH, temperature and the presence of other substances influence the stability of green tea catechins (GTC) in either freshly prepared green tea or processed tea drinks (51). A study conducted on eleven brands of dry tea leaves and fourteen brands of canned or bottled tea drinks showed that GTC are more stable in aqueous solutions at room temperature and at low pH. More than 90% of GTC was lost

when pH was increased to 5. Approximately 23% of the catechin was lost when tea was autoclaved at 120 °C for 20 min, whereas the loss was only 15 % in traditional tea drink prepared with freshly boiled water. Sucrose, citric acid and ascorbic acid, the common ingredients of bottled tea drinks, showed different effects at different times on catechin stability (52).

High temperatures employed during the processing of tea drinks promote epimerization of catechins. Epimerization affects catechin bioactivity and heat epimerized catechins, gallo catechin (GC), gallo catechin gallate (GCG) are slightly less efficient anti-oxidants than their precursors (53). These studies indicate the superiority of freshly brewed tea drinks over processed tea drinks in terms of catechin content, bioactivity and stability.

Pharmacokinetics of green tea catechins

Lee et al (2002) administered a single oral dose of green tea (20 mg solids/ kg body wt) or EGCG (2 mg/kg body wt) to human subjects. The maximum plasma concentrations of EGCG, EGC, and EC with green tea were 77.9, 223.4, and 124.03 ng/ml, respectively. The elimination half lives were 3.4, 1.7 and 2 hr respectively. All the catechins reached peak concentration between 1.3 and 1.6 hr. These pharmacokinetic parameters indicate that green tea catechins are rapidly absorbed and eliminated. EGCG stays in the system for a relatively longer period of time compared to other catechins. This may be due to the additional gallate moiety or due to the competition for elimination enzymes. In plasma, most of the EGCG was present in free form where as EGC and EC were found in conjugated form (54).

Bioavailability

Green tea polyphenols (GTP) are less bioavailable. Molecular size, molecular weight, processing and mode of transport are some of the factors that influence the GTP bioavailability (55). Information on bioavailability and biomarkers of green tea consumption is not yet clear. Wang etal (2008), found a dose-dependent increase in green tea plasma catechin levels, particularly EGCG and ECG. They conducted a phase II intervention trial with 500 or 1000 mg of green tea polyphenols among 124 healthy adults for 3 months. In plasma, EGCG existed in free form unlike other catechins. They found a predominance of free polyphenols like EGCG than sulfated and glucuronidated conjugates. Both ECG and EGCG are the reliable biomarkers of green tea consumption in blood (56).

EGCG is the most abundant and most potent catechin of green tea. However, its bioavailability is limited due to the poor absorption from small intestine. In addition to poor intestinal absorption, active efflux by microsomes (57), pahse II bio-transformation and microbial degradation in the colon are some of the factors that contribute to enhanced elimination of catechins from the system and reduced bioavailability (58). Chow et al (2003) conducted a pharmacokinetic study on green tea polyphenols and suggested that up to 800 mg /day of green tea polyphenols in the form of supplements is well tolerated and safe for healthy individuals (59).

Bioavailability can be increased by taking the green tea supplements on an empty stomach (60). In an effort to improve the bioavailability of EGCG, Lambert and colleagues (2006) prepared a paracetylated EGCG derivative (AcEGCG). Acetylation is the process of replacing the hydrogen atoms with acetyl groups thus making the hydroxyl

groups unavailable for phase II transformation (61). The authors found improved cell uptake and increased intracellular AcEGCG concentration. The plasma area under curve was significantly higher for AcEGCG (465.0 μg/ml) compared with EGCG (194.6 μg/ml), and the t1/2 and bioactivity were also increased for AcEGCG (62).

Though acetylation improved the bioavailability of EGCG, we may not predict the same for other catechins. Different green tea catechins and metabolites have different pharmacokinetic behaviors due to their structural differences and presence or absence of gallate moiety. More research is needed on the pharmacokinetics of catechins and catechin conjugates.

Anti-oxidant and anti-inflammatory effects of green tea

Green tea has both anti-oxidant and anti-inflammatory properties. Green tea was more effective against lipid oxidation in cell membranes compared to roasted, oolong and black tea (63). A green tea catechin supplementation study conducted among 40 healthy Japanese adults found significant decrease in Ox-LDL levels from 9.56 ± 9.2 to 7.76 ± 7.7 U/mL. Subjects in catechin group received green tea extract (Polyphenon 70S) capsules containing 500 mg of catechin (equivalent to the dosage found in 6 to 7 cups of green tea) for 4 weeks. This study showed a significant (11.7%) reduction in Ox-LDL levels with green tea consumption (64).

Regular consumption of tea containing 690 mg of catechin per bottle of oolong tea promoted weight loss and fat loss. It also reduced oxidative stress by inhibiting the formation of malondialdehyde modified LDL (MDA-LDL). MDA-LDL is a marker of unstable atherosclerotic CVD. These results indicate the possible role of catechins in

obesity prevention and improvement in obesity related disorders like MeS and Type 2 diabetes (65).

Another study with green tea supplementation for 2 weeks (8g/day) in young smokers significantly improved endothelial function, as measured by flow-mediated vasodilatation, thereby preventing future cardiovascular events in these subjects (66). Where as green tea consumption for 4 weeks reduced intracellular adhesion molecule-1 (ICAM-1), and oxidized LDL levels in adult smokers (67). Smoking is associated with increased oxidative stress and increased risk for CVD. Green tea may improved the CVD risk factors through its anti-oxidant and anti-inflammatory properties.

Anti-Obesity and hypolipidemic effects of green tea

Nagao and colleagues (2007) conducted a clinical trial to study the body fat and CVD risk reducing effects of green tea extract high in catechins. Japanese men and women (n=240) with visceral fat-type obesity received either catechin rich (583 mg/can) green tea or catechin poor (96 mg/can) green tea for 12 weeks. There was a non-significant, greater decrease in all the anthropometric indices of body fat like body weight, body mass index, body fat mass, visceral and subcutaneous fat areas in the catechin group than in the control group. They also found a decrease in systolic blood pressure (SBP) and low-density lipoprotein (LDL) cholesterol among catechin rich group (68).

The possible mechanisms by which green tea catechins reduce body fat are related to increased energy expenditure and fat oxidation and sympathetic nerve induced thermogenesis (69,70). Catechins induce hypolipidemic effects by inhibiting the rate limiting enzyme in the cholesterol synthesis and suppression of fatty acid synthase gene

expression (71). The insignificant results in the Nagao et al (2007) study may be due to the catechin dosage that is not enough to show any marked changes in the tested parameters.

Green tea effects on nitric oxide

Inflammation induces excessive NOx production, which in turn increases oxidative stress by forming RNS. Several clinical studies found an association between high levels of serum/plasma nitric oxide and metabolic syndrome features (72,40,73). A large population based study conducted among 3505 subjects with MeS showed a significantly higher serum NOx values (31.9 Vs 29.8 μmol/L) compared to controls. It also showed a direct association between number of metabolic risk factors and serum NOx levels (72).

Maejima et al (2001) showed higher plasma NOx levels in type II diabetic subjects. Plasma NOx levels were also correlated with the presence of hypertension and microvascular complications (73). Another clinical study also found significantly higher serum NOx concentrations among overweight and obese women. They also found positive correlations of serum insulin with both NOx and TNF-α concentrations.

Summarizing the findings, all these clinical studies indicate that metabolic and degenerative diseases are associated with increased levels of iNOS induced NOx, resulting in impaired endothelial function, insulin secretion and increased risk for CVD.

Green tea, an antioxidant rich beverage, showed a direct scavenging of NOx and O_2^- in-vitro. Catechins with gallate moiety and with tri-hydroxyl groups in their structure like EGCG and ECG showed greater free radical scavenging capacity (29). In a cell culture study, green tea extract inhibited iNOS mRNA expression, iNOS protein and

NOx production in human alveolar and colon epithelial cells in a concentration dependent manner (74).

Another cell culture study on human osteoarthritis chondrocytes found that EGCG (100 μM) inhibits NOx production. Cells were stimulated with IL-1β alone (2ng/ml) and IL-1β+ EGCG. IL-1β is a pro-inflammatory cytokine that increases NOx production by enhancing the iNOS expression. Treatment with EGCG significantly reduced the IL-1β induced NOx production (75). Hence EGCG may help in improving inflammatory diseases like CVD. It seems that green tea is effective in reducing iNOS induced NOx levels both by acting as an antioxidant and anti-inflammatory agent. Previous studies also indicate that green tea works at transcriptional level to inhibit iNOS production.

Green tea is a catechin rich beverage. Though it improves health through its anti-oxidant properties, it also prevents diseases and promotes cardiovascular health through different other mechanisms like mitigating inflammation, inhibiting transcription of inflammatory cytokines, inhibiting activation of inflammatory mediators, promoting endothelial function and through anti-obesity and hypolipidemic effects. Most of the green tea studies were conducted in animal models and cancer cell lines. Existing green tea clinical studies were conducted in healthy subjects in Asian countries like China and Japan. Due to the differences in dietary pattern and relatively longer green tea exposure in Asian subjects, we may not apply the previous study findings to Western subjects. Most often, anti-oxidative and anti-inflammatory effects of green tea were investigated in healthy subjects. There is a gap in knowledge with regards to anti-oxidative and anti-inflammatory effects of green tea in MeS subjects in western countries like US. As the prevalence of obesity and MeS were constantly increasing in US there is an immediate

need to address the problem. Hence, we are investigating the effects of green tea on features of metabolic syndrome and biomarkers of oxidative stress and inflammation in MeS subjects.

CHAPTER III

METHODOLOGY

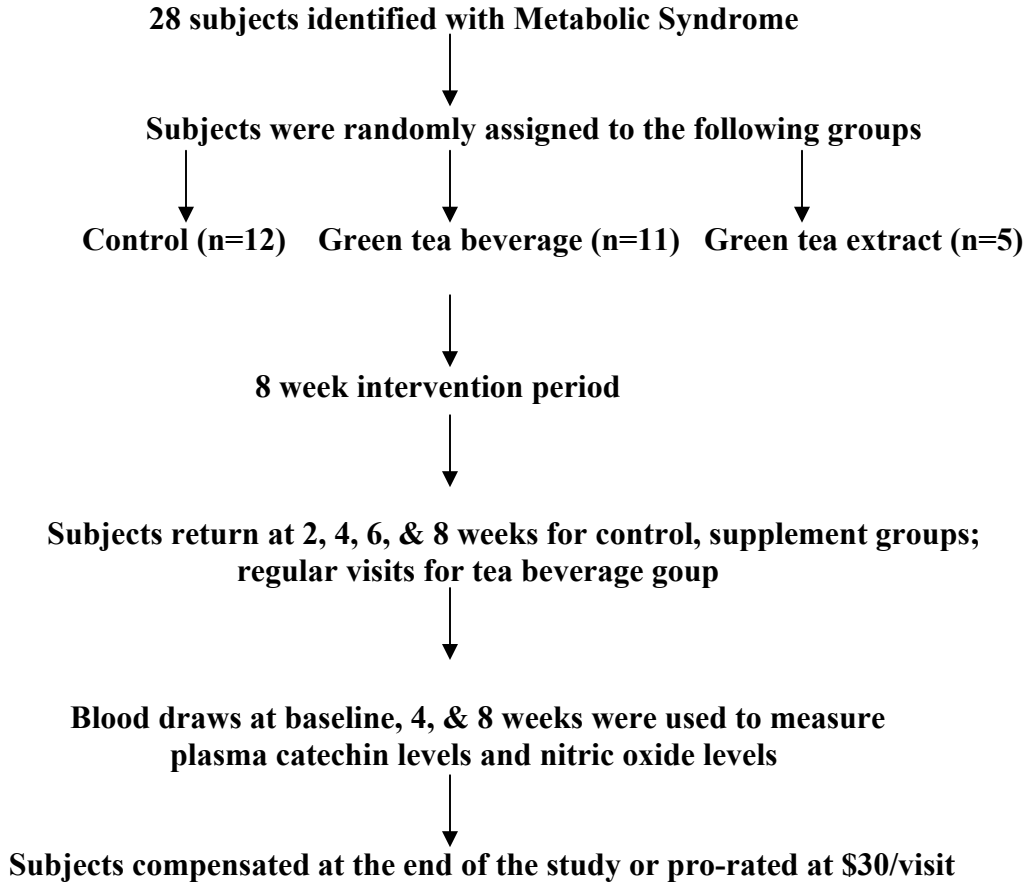
Subjects

28 subjects with metabolic syndrome were recruited through the General Clinical Research Centre (GCRC) at Oklahoma Health Sciences Center at Oklahoma City. Subjects were randomly assigned to the following 3 groups.

- Control group (usual diet + 4 cups of water)
- Green tea beverage group (4 cups green tea/day and usual diet)
- Green tea extract group (amount of EGCG ~green tea beverage and usual diet + 4 cups of water)

Flyers were posted at the GCRC Oklahoma Health Sciences Center, Oklahoma City and interested subjects were screened to examine if they fit the study criteria. Upon qualification, they were enrolled into the study. A telephone questionnaire was used for initial screening. Subjects who qualified were randomized to any one of the three groups using a GCRC randomization sheet. The informed consent was administered prior to the screening blood draw.

Research plan



All subjects were consented and entered into the study following the approval of the institutional review board (IRB) at Oklahoma State University and the corresponding human ethics committees at University of Oklahoma Health Sciences Center.

Inclusion criteria

According to the NCEP guidelines, subject should possess any three of the following features to fall in the category of metabolic syndrome: Waist circumference (≥ 102 cm in men & ≥ 88 cm in women), triglycerides ≥ 150 mg/dL, HDL (< 40 mg/dL in men; < 50mg/dL in women), blood pressure ≥ 130/85mm Hg, fasting glucose (≥ 100mg/dL). Adult subjects ≥ 21 years of age, but with normal Hb, WBC, platelets, liver, renal, and thyroid function tests and met at least three features of metabolic syndrome were included in the study. Subjects on stable medications were included.

Exclusion criteria

Any form of pre-existing disease, e.g. cancer, heart disease, diabetes, liver or renal disorders, anemia, pregnancy and lactation, women on hormone replacement therapy, taking mega doses of antioxidants/fish oil supplements, abnormal Hb (normal range: 12.0-18.0 g/dL), WBC (normal range: 4.0-11.0 K/mm^3), or platelets (140-440 K/mm^3), hypo/hyperthyroidism (normal range for thyroid stimulating hormone: 0.35-4.940 units/mL, abnormal liver enzymes (normal range for AST: 7-40 units/L; 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 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. Any subject with deviations from the normal range of Hb, WBC, platelets, liver enzymes, BUN, creatinine or TSH, during the study period were discontinued from the study. Both males and females, as well as individuals from any ethnic group, who qualify were included in the study. For women, the blood draws and anthropometric measurements were conducted between day 6 & 15 of their menstrual cycle to control for the effect of

hormone on study variables. Children were not included in the study.

Upon qualification, the subjects were randomized to the control and intervention groups as mentioned in Research Plan. Fasting blood draws were done by a certified phlebotomist, at screening, 4, and 8 weeks of the study subjects in the green tea beverage group were supplemented on a daily basis since the study participants in this group were recruited on- campus at OUHSC. Subjects were compensated $ 30 per visit and these visits include screening, 2, 4, 6 and 8 weeks of the study.

Anthropometric and blood pressure

Anthropometric measurements were obtained by trained staff members at the GCRC. Height, weight, blood pressure, WC, and BF% were measured at screening, four, and eight-week visits. Systolic and diastolic blood pressure was collected in mmHg with Spot Vital Signs Device (Welch Allyn, Skaneateles Falls, NY). Waist circumference was taken from subjects at the superior iliac crest with the Gulick II Tape Measure (Vital Signs, Gay Mills, WI). Body fat percentage was determined through bio-electrical impedance assay (BIA) with the Bodystat 1500 (Bodystat Ltd, Isle of Man, Great Britain).

Sample collection

Blood samples were collected in heparinized tubes, and also in vials containing EDTA, and serum separator tubes. Serum and plasma samples were separated by centrifugation, flushed with nitrogen and stored at -80^0C for subsequent analyses of plasma Ox-LDL, CRP, NOx and catechins.

Reagents

Green tea catechin standards epicatechin (EC), epicatechingallate (ECG), epigallo catechin (EGC) were purchased from Sigma-Aldrich (St Louis, MO), and epigallocatechingallate (EGCG) from Calbiochem (La Jolla, CA). Green tea from Bigelow (Fairfield, CT) and green tea supplements from Solaray (Park City, UT) were purchased. All the chemicals were HPLC grade. Acetonitrile and methanol were obtained from Pharmco-Aaper (Brookfield, CT). Phosphoric acid from EMD Chemicals (Gibbstown, NJ), perchloric acid from Sigma-Aldrich (St Louis, MO) and potassium carbonate from J.T.Baker (Phillipsburg, NJ) were purchased. For serum total nitric oxide Nitrate/nitrite colorimetric assay kit from Cayman Chemical (Ann Arbor, MI), for plasma Ox-LDL mercodia oxidized LDL competitive ELISA kits from Mercodia, Uppasala, Sweden, quantikine human C-reactive protein from R and D systems (Minneapolis, MN) were used.

Catechin standards

All four major green tea catechins: EC, EGC, ECG, and EGCG 1 mg each, were individually dissolved in 1 mL of methanol: water (1:1, v/v) and were sonicated for 20 min in amber coloured micro centrifuge tubes. Catechin standard stock solutions were further diluted to obtain 200, 100 and 50 µg/mL concentrations. Standard calibration curves were constructed for all the four catechin standards. Catechin concentrations were calculated from the peak area by using the equation for linear regression obtained from the calibration curve.

Green tea samples

Both green tea leaves and green tea extract capsules were analyzed for their catechin content. 100 mg of dried tea leaves from Bigelow green tea bags were weighed and dissolved in methanol:water (1:1, v/v) in 100 ml volumetric flask and sonicated for 20 min. the same procedure was followed for the green tea supplement. Catechin content of each sonicated sample was analyzed by running through HPLC.

HPLC conditions

The HPLC system consisted of a waters 600 pump, 717 autosampler, and 2447 dual λ absorbance detector with Empower software (Waters Corporation, Milford MA). The mobile phase consists of two solvents; solvent A (freshly prepared 0.2% phosphoric acid) and solvent B (100 % acetonitrile). These two solvents were used under binary linear gradient conditions as follows: 0-5 min, 100% A; 5-15 min, 90-85% A & 10-15% B; 15-25 min, 85-80% A & 15-20% B; 25-30 min, 100% A with a flow rate of 0.5 mL/min. All samples 50 µl injection volumes were filtered (0.22 µm) and analyzed on Waters symmetry C18 (100 mm - 4.6 mm, 3.5 µm) column. The sample run time was 30 min. The column temperature was maintained at 21°C.The absorbance at 200 nm was used for detection and quantification of catechins from standards, green tea samples and plasma samples.

Plasma free catechins

Plasma free catechins were analyzed by the LC-UV modified method developed by Masukawa etal (2006). To 250 µl of the plasma thawed just prior to use, 25 µl 6M perchloric acid and 125 µl acetonitrile were added. The mixture was vigorously mixed for 2 min with a vortex mixer in a polypropylene tube. The mixture was kept at 5 °C for

30 min, and was then centrifuged at 20,000×g for 5 min or 13,000 x g for 10 min at 5 °C in a eppendorf Centrifuge 5417R (Hamburg, Germany). The supernatant and 100 µl 0.75M potassium carbonate solution were mixed moderately for 30 s. Insoluble potassium perchlorate in the tube was precipitated by centrifugation at 20,000×g for 5 min or 13,000 x g for 10 min At 5 °C. The resulting supernatants were analyzed by HPLC analysis (90).

Plasma Ox-LDL

Mercodia Oxidized LDL (Mercodia Uppsala Sweden) competitive ELISA was used to determine LDL oxidation. This assay is based on the monoclonal antibody 4E6. 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.

Human C-reactive protein

CRP was measured by quantitative sandwich immuno assay. Microplates were pre-treated with CRP specific monoclonal antibody. When samples and standards are pipetted into the wells any CRP present in the sample binds to the immobilized antibody. Adding substrate solution produces colour which is proportional to CRP in the sample. Intensity of the colour was measured at 450 nm.

Serum total nitric oxide (NOx)

Determination of NOx radical is difficult as it has a very short half-life of 10-16 seconds. NOx rapidly converts to nitrate (NO_3^-) and nitrite (NO_2^-). Recent studies indicate that these inorganic anions can be converted to bioactive NOx and can be

considered as storage pool of NOx (76). Hence plasma nitrite and nitrate concentrations are used as indicators for NOx radical formation. Serum total nitrate and nitrite levels were measured by using the Nitrate/Nitrite calorimetric kit (Cayman chemical, MI, USA) based on Griess reaction. This method is based on two steps. In the first step nitrate is converted to nitrite by nitrate reductase and in the the second step nitrite is converted to deep-purple azo compound with the addition of Griess reagent I and II. Serum total nitric oxide concentration was determined by photometric quantification of azo compound. Serum samples were ultrafiltered by using 30 kDa molecular weight cut-off (MWCO) filters (Amicon, Ultracel YM 30 membrane), pre-rinsed with Millipore water. Ultrafiltration reduces background absorbance and improve color formation with Greiss reagent. Approximately 300 µl of the serum sample was taken into the filters and was centrifuged at 14,000 g for 20 minutes at room temperature. 40µl of the filtrate was added to duplicate wells and were diluted with equal amount of assay buffer. Enzyme cofactor mixture and nitrate reductase mixture 10µl each were added to all the standard and sample wells. The plate was covered, gently shaked and incubated at room temperature for 3 hours. After incubation 50 µl each of Greiss reagent R1 and R2 were added to standard and sample wells. It was allowed to develop color for 10 minutes at room temperature. Blank wells contained 200µl of assay buffer. After 10 min of color development at room temperature, the absorbance was measured on a multi-detection microplate reader (Synergy HT, Vermont, USA) at 540 nm. Each sample was assayed in duplicate wells. Standard curves were prepared with known concentration of nitrate stock standard (200µM). Care was taken while pipetting to avoid the air bubbles that interfere with the absorbance. The detection limit for total nitric oxide assay is > 2.5µM.

Statistical methods:

All measures had descriptive statistics calculated and graphs drawn to look for outliers. Outliers due to data errors were corrected where possible or removed. Analyses were completed with and without outliers and their influence on the results discussed. Pair wise differences (green tea versus control and green tea extracts versus control) between the groups at baseline were assessed using student t-tests.

Changes in measurements over the eight week study period were assessed by calculating the difference between the pre and post-intervention measurements. The differences calculated for the green tea and green tea extract groups were then conditioned on their respective control; the difference seen in the control participant was subtracted from the difference seen in each corresponding green tea extract participant within age and gender matched trio. These conditional differences for the green tea and green tea extract groups were assesses as being different from zero using student t-tests. All statistical tests were two-tailed with significance level set at 0.05. Significance levels were not adjusted for multiple hypotheses testing, rather, the results were reviewed for consistencies, SPSS for windows (version 15.0, SPSS Inc., 2006) was used for the statistical calculations.

CHAPTER IV

RESULTS

Baseline characteristics and metabolic syndrome features

A total of 28 subjects with at least 3 metabolic syndrome features were included in the analysis. Baseline characteristics and features of metabolic syndrome of the subjects were shown in Table 2. The mean age of the subjects ranged from 40 - 45 yrs. Subjects were randomly assigned to control (n=12), green tea beverage (n=11) or green tea extract (n=5) groups. Waist circumference, fasting blood glucose, triglycerides (TG), high density lipoprotein cholesterol (HDL), systolic and diastolic blood pressure were the metabolic syndrome features used for the selecting criteria. Subjects in all the three groups were obese with mean BMI ranging from 37.4 to 41.5. Many of the subjects were on anti hypertensive drugs. Hence, their systolic (SBP) and diastolic (DBP) blood pressures were lower than the MeS cut-off points of > 130 mm Hg and > 85 mm Hg respectively. In all three groups, almost half of the subjects were on medications with the highest percentage in green tea beverage group (85.7%). Medication use was significantly higher ($p<0.05$) in green tea beverage and green tea extract groups compared to controls. Angiotensin converting enzyme (ACE)-inhibitors, calcium-channel blockers and beta-blockers were the most common forms of blood pressure medications used by the subjects. Apart from medication use, there were no significant differences in baseline parameters among the three groups.

Anthropometric measures and blood pressure

Eight weeks of green tea supplementation showed a significant decrease in body weight and BMI in the green tea beverage group when compared with controls ($P<0.05$). Anthropometrics and blood pressure measurements at baseline and at eight weeks are shown in Table 3. Body weight reduced by 2.3 kg in green tea beverage group. Green tea extract group also showed a non-significant decrease in body weight by 1.9 kg. A greater decrease in SBP (from 135.6 to 126.3 mmHg) and a slight reduction in DBP (from 83.7 to 79.0 mm Hg) were observed in green tea beverage group only versus controls, though not statistically significant. In contrast, both green tea extract and control groups showed an increase in DBP.

Biomarkers of oxidative stress and inflammation

Ox-LDL, nitric oxide and CRP were measured as biomarkers of oxidative stress and inflammation (Table 4). There were no significant differences in any of these three measures between the study groups at baseline and at 8 weeks. However, we observed a decreasing trend in both Ox-LDL ($P<0.1$) and NOx ($P<0.1$) in green tea beverage group compared to baseline. Green tea supplementation decreased Ox-LDL levels from 109 to 87.5 U/L in green tea beverage group. A non-significant decrease in NOx concentrations was observed in both green tea beverage and green tea extract groups. It decreased by 31.2 % in beverage group and 11.5 % in extract group compared to baseline while NOx levels increased by 17 % in control group. CRP levels also showed a non-significant decrease from 6.2 to 5.8 mg/L and 6.3 to 5.8 mg/L in green tea beverage and green tea extract groups, respectively, whereas, CRP levels increased from 5.6 to 5.7 mg/L in the control group.

Plasma free catechins

The four major green tea catechins EGC, ECG, EGCG and EC were measured in the plasma by HPLC and as shown in Table 5. We were not able to detect EC in many plasma samples. In addition to this, standard errors were greater than mean values. Because of these inconsistent results in EC levels, we excluded this data from analysis. There were no significant differences in any of the catechins among the three groups at baseline and at 8 weeks. The plasma free catechin concentrations were lower than those found in actual green tea beverage samples and green tea extract capsules. Table 1. summarizes the amounts of four major catechins in both green tea beverage and green tea extract. Highest plasma concentrations were observed for EGC and lowest were found for EGCG.

TABLES

Table 1. Catechin and caffeine composition of green tea beverage and green tea extracts

Catechins (mg)	Green tea beverage[1] (1 cup ≈ 8 fl oz)	Green tea extract[1] (1 capsule)
Total Catechins (mg)	232.0 (100)	435.0 (100)
EGCG	110.0 (47.4)	230.0 (52.8)
EGC	55.0(23.7)	120.0 (27.6)
ECG	45.0 (19.4)	60.0 (13.8)
EC	22.0 (9.5)	25.0 (5.8)
Caffeine	2.24	1.8

[1] Percentage of total catechin in parentheses.
Total catechin concentration was defined as the sum of EGCG, EGC, ECG and EC values. EGCG- epigallocatechin gallate, EGC- epigallocatechin, ECG- epicatechin gallate, EC- epicatechin

Table 2. Baseline characteristics and metabolic syndrome features of the subjects in all the treatment groups

Variables	C (n=12)	GT (n=11)	GTE (n=5)
Age (yrs)	44.6± 3.2	45.3±3.0	40.0±4.2
Male	1	1	1
Female	11	10	4
Body weight (kg)	104.6±6.9	108.6±4.4	116.5±8.3
BMI (kg/m^2)	37.4±2.8	38.6±1.5	41.5±2.1
Waist circumference (in)	43.0±2.0	44.2.0±1.0	49.7±3.7
Glucose (mg/dL)	88.8±4.4	89.3±3.4	84.8±2.8
TG (mg/dL)	129.2±21.1	168.0±27.1	148.4±38.4
HDL (mg/dL)	40.8±1.8	40.3±2.2	37.7±5.0
SBP (mm Hg)	129.5±2.8	135.6±5.9	127.4±6.4
DBP (mm Hg)	78.7±2.1	83.7±3.5	80.8±2.7
Medication users (%)	45.4	85.7*	60*

Values are Mean ± SE
* $p<0.05$, significantly different from the control
C- Control group, GT- Green tea beverage group, GTE- Green tea extract group
BMI- Body mass index, TG- Triglycerides, HDL- High density lipoprotein, SBP- Systolic blood pressure, DBP- Diastolic blood pressure

Table 3. Anthropometric measures and features of metabolic syndrome at baseline and at 8 weeks of the study subjects

Variables	Week	Contrlol (n=12)	GT (n=11)	GS (n=5)
Body weight (Kg)	0	104.6 ± 6.9	108.6 ± 4.4	116.5 ± 8.3
	8	105.2 ± 6.9	106.1 ± 4.0 *	41.5 ± 2.1
Body weight change (Kg)	GT vs C	-	- 2.3 ± 0.7*	-
	GTE vs C	-	-	- 1.9 ± 0.6
BMI (kg/m^2)	0	37.4 ± 2.8	38.6 ± 1.5	49.7 ± 3.7
	8	37.6 ± 2.8	37.7 ± 1.4 *	41.0 ± 2.0
Body fat (%)	0	45.7 ± 3.1	46.5 ± 3.1	47.7 ± 2.2
	8	45.7 ± 3.4	46.2 ± 2.6	47.5 ± 2.0
Waist circumference (in)	0	43.0 ± 2.0	44.2.0 ± 1.0	49.7 ± 3.7
	8	42.7 ± 1.8	45.2 ± 1.5	48.3 ± 3.0
HDL (mg/dL)	0	40.8 ± 1.8	40.3 ± 2.2	37.7 ± 5.0
	8	39.7 ± 1.7	41.5 ± 2.4	36.2 ± 4.2
Glucose (mg/dL)	0	88.8 ± 4.4	89.3 ± 3.4	84.8 ± 2.8
	8	85.9 ± 4.7	88.8 ± 2.5	83.3 ± 6.4
SBP (mm Hg)	0	129.5 ± 2.8	135.6 ± 5.9	127.4 ± 6.4
	8	128.0 ± 2.8	126.3 ± 5.3	128.0 ± 3.8
DBP (mm Hg)	0	78.7 ± 2.1	83.7 ± 3.5	80.8 ± 2.7
	8	80.2 ± 2.8	79.0 ± 3.7	81.6 ± 3.9

Values are Mean ± SE
* Changes at 8 weeks significantly different from control ($p<0.05$)
** Decrease in trend at 8 weeks compared to baseline ($p<0.1$)
GT- Green tea beverage group, GTE- Green tea extract group
BMI- Body mass index; calculates as kg/m2, TG- Triglycerides, HDL- High density lipoprotein, SBP- Systolic blood pressure, DBP- Diastolic blood pressure

Table 4. Biomarkers of oxidative stress and inflammation at Baseline (0) and at 8 weeks of green tea supplementation

Biomarker	Week	C (n = 12)	GT (n = 11)	GTE (n = 5)
Ox-LDL (U/L)	0	102 ± 11.5	109 ± 5.4	91 ± 9.1
	8	94.6 ± 8.1	87.5 ± 5.2*	93 ± 9.2
Nitric oxide (μM)	0	18.6 ± 4.6	22.7 ± 3.9	25.8 ± 2.6
	8	21.8 ± 6.5	15.6 ± 7.9*	22.8 ± 3.7
CRP (mg/L)	0	5.6 ± 1.5	6.2 ± 2.7	6.3 ± 3.8
	8	5.7 ± 1.7	5.8 ± 3.1	5.8 ± 2.9

Values are Mean ± SE
* ($P<0.1$) compared to baseline
C- Control group, GT- Green tea beverage group, GTE- Green tea extract group, Ox-LDL- Oxidized low density lipoprotein, CRP- C-reactive protein.

Table 5. Plasma free catechin concentrations of the metabolic syndrome subjects at baseline (0) and after 8 weeks of green tea supplementation

Green tea catechin	Week	C (n=12)	GT (n=11)	GTE (n=5)
EGC (μg/ml)	0	65.87 ± 15.87	70.78 ± 19.09	77.89 ± 16.98
	8	69.87 ± 17.65	73.76 ± 17.98	75.98 ± 22.98
ECG (μg/ml)	0	18.98 ± 9.75	26.97 ± 11.76	28.96 ± 15.98
	8	26.86 ± 8.95	31.96 ± 13.67	33.65 ± 14.76
EGCG (μg/ml)	0	10.4 ± 8.64	15.87 ± 9.56	12.76 ± 8.98
	8	10.76 ± 5.66	20.45 ± 8.98	18.95 ± 7.98

Values are Mean ± SE
No significant differences between groups ($p>0.05$)
C- Control group, GT- Green tea beverage group, GTE- Green tea extract group
EGC-Epigallocatechin, ECG- Epicatechin gallate, EGCG- Epigallocatechin gallate

CHAPTER V

DISCUSSION AND CONCLUSION

This is an eight week randomized, single blind controlled study investigating the effects of green tea polyphenols as green tea beverage or green tea extract in MeS subjects. We investigated the effects of green tea supplementation on anthropometrics, MeS features and biomarkers of oxidative stress and inflammation. We found a significant reduction in body weight with green tea beverage. This study also found non-significant improvements in metabolic syndrome features and biomarkers of oxidative stress and inflammation with green tea beverage supplementation.

Anthropometrics and features of metabolic syndrome

Green tea has been shown to exert anti-obesity effects in several clinical studies. The major mechanisms by which green tea reduces body weight are related to increased energy expenditure and fat oxidation (77,70,78). Auvichayapat et al (2008) showed a significant decrease in body weight following 12 weeks of green tea extract supplementation. Pure EGCG supplementation per se increased fat oxidation in obese men (70). Where as, Hsu et al (2008) did not find any difference in body weight with green tea extract supplementation in obese women. Exact reasons for this null effect were not known. However, they were not able to detect any of the green tea catechins in plasma samples collected after 12 hrs. This may partially explain the absence of any effect on body weight (23). A large cross section study conducted in Japan found that increased green tea consumption was associated with decreased serum cholesterol, LDL cholesterol and triglycerides and increased HDL cholesterol concentrations (79). This indicates that green tea can improve components of MeS. In our study, subjects were

obese (grade-II and grade-III) with BMI values ranging from 37.4 to 41.5 kg/m^2. Green tea supplementation reduced body weight significantly in green tea beverage group only. We also observed a non-significant increase in HDL cholesterol with green tea beverage compared to baseline.

Biomarkers of oxidative stress and inflammation

Ox-LDL, NOx and CRP are significantly elevated in subjects with MeS compared to controls in several studies. Hence, we selected these three markers for our study. Oxidative stress is characterized by increased levels of ROS (27). As it is difficult to measure free radicals because of their unstability, Ox-LDL is measured as an indicator of oxidative stress (80). Recent clinical studies have reported higher concentrations of Ox-LDL in subjects with metabolic syndrome (81) and with components of metabolic syndrome like hyperlipidemia and abdominal obesity (3). In contrast, Sjogren et al (2005) found no increase in Ox-LDL concentrations in MeS subjects. However, the percentage of subjects classified as having MeS ($\geq$ 3 MeS features) was only 8%. This relatively low percentage reduces the power to interpret the data (82). In-vitro studies demonstrated that green tea polyphenols can inhibit Cu(2+) induced LDL oxidation (83). Sung et al (2005) found a significant reduction of 21.1% in plasma Ox-LDL levels versus baseline in young men (28-42 yrs) supplemented with 4 cups of green tea/day for 4 weeks (22). Inami et al (2007) found a significant reduction in Ox-LDL (11.7%) levels in Japanese adults with 4 weeks of green tea extract supplementation. In our study, we found a non-significant (19.7%, $p<0.1$) decrease in Ox-LDL in green tea beverage group only compared to baseline. However, we did not find any decrease in Ox-LDL with green tea extract. In contrast, we observed a 2.1% increase in Ox-LDL in green tea extract group. The amount

of the green tea beverage (22) and green tea extracts (64) used in the previous studies is equal to the amount that we used in our study. There might be differences in total catechin content and composition of the green tea beverage used in different studies. In addition the subjects in the previous study (Sung et al, 2005) are healthy and relatively young (28-42 yrs) compared to the subjects in our study. Also, the baseline Ox-LDL concentrations (69.5 U/L) of these subjects are less than the concentrations (100.6 U/L) that we found in our subjects. Healthy young subjects versus metabolic syndrome subjects might have contributed to the differences in the significance of our study results.

Nitric oxide is a gaseous molecule produced by NOS family enzymes. Under inflammatory conditions iNOS is stimulated and results in excessive production of NOx (40). Asl et al (2008) found significantly higher serum NOx levels in MeS subjects (with at least 3 MeS features) compared to controls (72). Obese and overweight women also showed higher levels of both NOx and TNF-α. TNF-α is a pro-inflammatory cytokine that activates iNOS. A positive correlation between these two biomarkers in subjects with metabolic disorders indicates that high NOx concentrations were contributed by TNF-α induced iNOS (40,84). Singh et al (2002) found a significant reduction in IL-1β induced NOx production in human osteoarthritis chondrocytes treated with EGCG (75). Basing on the existing literature it may be concluded that subjects with MeS in our study have high levels of inflammation induced NOx. As green tea showed anti-inflammatory properties in addition to anti-oxidant abilities, we wanted to examine effects on NOx levels in MeS subjects. Reported clinical studies have not investigated the effects of green tea flavonoid supplementation on NOx concentrations in MeS. Thus, to our knowledge this is the first study to show the decreasing trend in NOx levels with

green tea beverage supplementation. In our study, serum NOx levels were decreased in both the green tea supplementation groups with greater decreases in beverage group (31.2%). Our study participants had slightly lower NOx levels ranging from 18.6 μmol/L - 25.8 μmol/L compared to the previous study (26.1 μmol/L - 27.4 μmol/L) by Asl et al (2008) in Iran in MeS subjects (72). In our study most of the subjects were women, in addition to this, differences in age, diet and physical activity and environment might have influenced the NOx levels (85).

CRP is an acute phase protein that increases under inflammatory conditions. CRP is an independent predictor of CVD risk. Younger subjects (25.86 yrs) with MeS showed significantly higher levels of CRP compare to controls. CRP levels increased with increase in number of MeS features (86). Nah and Lee (2006) found a positive correlation between hsCRP and fasting glucose, SBP; and negative correlation with HDL cholesterol in MeS subjects (87). Dietary anti-oxidants have an effect on CRP. In a seven year Swedish cohort study, hsCRP levels were negatively associated with dietary intakes of ascorbic acid and α-tocopherol (88). It indicates that oxidative stress and inflammation are positively associated. Chronic green tea extract (decaffeinated) supplementation significantly reduced CRP levels in hemodialysis patients compared to controls (23). This study clearly demonstrates the anti-inflammatory effects of green tea, as CRP levels increased and reached control levels in the same subjects during washout period. However, both acute and chronic green tea consumption did not show any effect on CRP levels among healthy smokers (67,89). The confounding factors like smoking and high caffeine content of green tea in the later studies limit the interpretation of results. In our study, the mean CRP levels of the subjects in all the three groups at baseline ranged from

5.6- 6.3 mg/L. These high CRP concentrations (> 3 mg/L) confirm that our subjects are with elevated levels of inflammation and at high risk for future CVD. A non-significant decrease in CRP levels in green tea beverage and green tea extract may be due to small sample size and short duration (2 months) of supplementation. Four cups of green tea in our study and in a previous study by Sung et al (2005) may not be enough to cause a significant decrease in CRP levels.

As most of the previous studies were conducted in young healthy subjects and smokers comparing the study results with our findings is difficult. Metabolic syndrome is a condition associated with increased oxidative stress and inflammation. Hence they may require higher doses of green tea and supplementation for longer periods that may cause the same significant improvements in biomarkers of oxidative stress and inflammation as observed in hemodialysis patients (23).

Plasma free catechins

Plasma free catechin concentrations were not significantly different between groups at baseline and at 8 weeks. However, we observed a pattern that is similar to most green tea pharmacokinetic studies (90,58). Catechins were eluted in the order of EGC, EC, EGCG and ECG. Gallated catechins like EGCG and ECG eluted at the end. The relatively longer elution time is due to the presence of gallate moiety that increases their size compared to non-gallated catechins. Green tea catechins are rapidly absorbed and eliminated. Over 90% of the catechins were cleared from the body with in 8 hrs of consumption (91). Hsu et al (2008) were not able to detect EGCG in obese subjects after 12 hrs over-night fast (23). However, Wang et al (2007) detected all four major green tea catechins in 12 hr overnight-fast blood samples (92). We were also able to detect the

green tea catechins in plasma even after 12 hrs of green tea consumption. This indicates the longer retention times in the body in certain subjects. When compared among the four green tea catechins, EGC was found in higher concentration and EGCG in lower concentrations in plasma. Similar findings were observed in pharmacokinetic studies conducted by Yang et al (1998) in healthy humans (91). Gallate moiety and tri-hydroxyl groups in EGCG structure reduce the intestinal absorption and might have contributed to the low plasma levels.

Limitations:

Complete monitoring of the supplement intake was not possible. Subjects in the green tea beverage group consumed only 2 cups of green tea every day in GCRC under the supervision of a study dietitian. Consumption could not be monitored for the remaining 2 cups taken at home. In green tea extract group, pill count was considered for measuring compliance. However, it is difficult to measure the compliance in human subjects.

Lack of information on diet and physical activity is another major limitation. Physical activity was not monitored during the study period. Seasonal changes may cause changes in dietary intakes and physical activity levels. Access to dietary data will allow us to correlate the dietary components like lipids, anti-oxidants, vitamins and sodium to the tested variables like body weight, Ox-LDL, CRP, NOx and blood pressure. Even though subjects were instructed to maintain their usual diet and physical activity, there is a possibility of alteration in these parameters.

Like many green tea studies, information was not complete for nutrient and phytochemical composition of the green tea samples used in the study. This might be

another possible limitation. Some of the minerals and vitamins like zinc and vitamin C have anti-oxidant properties. Data on mineral and vitamin content of green tea samples in addition to the catechin composition might have helped us in understanding the differential effects between green tea beverage and green tea extract.

Medication use among the subjects is another limitation. A significantly higher percentage of subjects were on medication in green tea beverage and green tea extract groups. This might have masked the effects of green tea flavonoid supplementation.

The relatively small sample size and short period of supplementation might have contributed to the insignificant results.

Implications

Green tea beverage may help in reducing obesity prevalence as it showed significant weight loss in subjects with MeS. The study finding implies that, weight reducing effects were contributed by green tea catechins as both green tea beverage and extract used were decaffeinated. Green tea beverage may help in reducing oxidative stress and inflammation as it showed a decreasing trend in Ox-LDL and NOx concentrations. Hence, green tea may be beneficial not only for MeS, but also for other diseases like cancer. Our study indicates that green tea catechins can be detected in the body even after 12 hrs. Green tea is the most widely consumed beverage in the world. Any health promoting effects found with green tea consumption may benefit a wide range of population globally.

Future studies

As of now, only plasma free catechin levels are used as biomarkers of green tea polyphenol bioavailability. This may not be an accurate marker, as green tea polyphenols

undergo phase II biotransformation in the body and produce catechin conjugates. Future studies are needed to explore more accurate green tea polyphenol biomarkers taking into consideration the level of conjugation of catechins. Future studies should be conducted with larger sample and for longer duration in order to see significant effects in biomarkers of oxidative stress and features of MeS. The present study found a difference between green tea beverage and green tea extract in improving components of MeS, oxidative stress and inflammatory indicators. Also, future studies should focus on the compositional changes between different green tea supplements.

Hypotheses

The present study investigated the effects of green tea supplementation on anthropometrics, features of metabolic syndrome, biomarkers of oxidative stress and inflammation and plasma free catechin concentrations.

- We reject the null hypothesis: chronic green tea beverage or green tea extract supplementation has no effect on anthropometric measures and features of metabolic syndrome. There was a significant decrease in body weight and BMI in green tea beverage group versus controls ($p<0.05$).
- We fail to reject the null hypothesis: chronic green tea beverage or green tea extract supplementation has no effect on biomarkers of oxidative stress and inflammation in subjects with MeS.
- We fail to reject the null hypothesis: chronic green tea beverage or green tea extract supplementation has no effect on plasma free catechin levels in subjects with MeS.

Conclusion

Eight weeks of decaffeinated green tea beverage supplementation significantly reduced body weight compared to controls. No significant changes in metabolic syndrome features and biomarkers of oxidative stress and inflammation were observed in any of the groups with green tea supplementation. However, we found a decreasing trend for Ox-LDL and NOx concentrations and increasing trend for HDL levels in the green tea beverage group only. This indicates that green tea beverage may help in reducing oxidative stress and inflammation in subjects with MeS. This study confirms the anti-obesity effects of green tea, as the effects were observed in subjects without any change in diet and life style. Future studies with larger sample size and longer duration are necessary to explain the findings of our study.

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APPENDICES

Oklahoma State University Institutional Review Board

Date	Thursday, September 25, 2008	**Protocol Expires:**	9/24/2009
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
301 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 : 

Shelia Kennison, Chair, Institutional Review Board

Thursday, September 25, 2008
Date

OUHSC IRB # 13154
OSU IRB # HE 06103
Version date: July 22nd, 2008

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 135 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.

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 (like a roll of the dice). 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.

Reproductive Risks For Women:

Risks for females:
If you are a female, you must not be and should not become pregnant nor breast-feed an infant while on this study. Taking the green tea or supplement, undergoing a particular procedure or treatment involved in this study while pregnant or breastfeeding may involve risks to an embryo, fetus or infant, including birth defects which are currently unforeseeable. In order to reduce your risk of pregnancy, you or your partner should use one or more of the acceptable methods of birth control listed below, regularly and consistently while you are in this study.

Risks for both females and males:
Acceptable methods of birth control (continuing throughout the study and for one month after the study) include:

- An approved oral contraceptive (birth control pill)
- Intra-uterine device (IUD)
- Hormone implants
- Contraceptive injection (Depo-Provera)
- Barrier methods (diaphragm with spermicidal gel or condoms)
- Transdermal contraceptives (birth control patch)
- Vaginal contraception ring (birth control ring)
- Sterilization (tubal ligation, hysterectomy or vasectomy)

If you are already using a method of birth control, you should check with the study doctor to make sure it is considered acceptable for this study. Certain drugs may interact with contraceptive agents and reduce their effectiveness, therefore, you should inform the study doctor of all medications (prescription and over-the-counter) that you are currently taking or begin taking during the study.

If you become pregnant or suspect that you are pregnant, or (for males) if you make someone pregnant, during this study, you should immediately inform the study personnel. If you become pregnant or suspect that you are pregnant while on this study, a pregnancy

test will be done. The study agent (green tea or supplement) may be discontinued until the result of the pregnancy test is known. If pregnancy is confirmed, you may be withdrawn from the study. The study physician will assist you in getting obstetrical care and the study doctor and the Sponsor will follow the progress of your pregnancy, and will require access to your and/or your infant's medical records for up to at least eight weeks after delivery. Payment for all aspects of obstetrical, child, or related care will be your responsibility.

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 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. 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. Refusal to participate or 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 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 at 405-744-4437 (9AM -5PM, Monday-Friday)or at 916-607-4143 (any time) or Timothy Lyons, MD at 405-271-5896 (8AM-5PM), or 405-255-3340 (any time), 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. Shelia Kennison, 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
JUL 2 3 2008
OUHSC IRB

Okla. State Univ.
IRB
Approved 9/25/08
Expires 9/24/09
IRB # HE-08-103

5

APPROVAL EXPIRES
JUN 3 0 2009
OUHSC IRB

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? (Cardiovascular disease, rheumatoid arthritis, etc.)	YES	NO
Do you have high blood pressure? If controlled, what medications does the patient take? _____	YES	NO
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) (Female ≥ 35 inches)**	(Value:_____)
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) (Female ≤ 50 mg/dL)**	(Value:_____)
4. _____	**Triglycerides**	**(≥ 150 mg/dL)**	(Value:_____)
5. _____	**Fasting Blood Glucose (≥ 100 mg/dL and < 126 mg/dL)**		(Value:_____)

VITA

Kavitha Penugonda

Candidate for the Degree of

Master of Science

Dissertation: EFFECTS OF CHRONIC GREEN TEA FLAVONOID SUPPLEMENTATION ON FEATURES OF METABOLIC SYNDROME, BIOMARKERS OF OXIDATIVE STRESS AND INFLAMMATION AND PLASMA CATECHIN LEVELS IN SUBJECTS WITH METABOLIC SYNDROME

Major Field: Nutritional Sciences

Biographical:

Education:
Master of Science in Food Science and Nutrition from Sri Venkateswara University, Tirupathi, Andhra Pradesh, India, 2001.
Completed the requirements for the Master of Science in Nutritional Sciences at Oklahoma State University, Stillwater, Oklahoma in July, 2009.

Experience:
Worked as a clinical and community nutritionist in India.
Currently working as a graduate assistant in the department of Nutritional Sciences

Professional Memberships:
American Society for Nutrition (ASN)
Golden Key International Honour Society
Indian Dietetic Association (IDA)

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Name: Kavitha Penugonda Date of Degree: July, 2009

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study:
EFFECTS OF CHRONIC GREEN TEA FLAVONOID SUPPLEMENTATION ON FEATURES OF METABOLIC SYNDROME, BIOMARKERS OF OXIDATIVE STRESS AND INFLAMMATION AND PLASMA CATECHIN LEVELS IN SUBJECTS WITH METABOLIC SYNDROME

Pages in Study: 63 Candidate for the Degree of Master of Science

Major Field: Nutritional Sciences

Scope and Method of Study: Green tea, rich in anti-oxidants, particularly epigallocatechin gallate (EGCG), is inversely associated with cardio vascular disease (CVD). Metabolic syndrome (MeS) is linked with increased oxidative stress & inflammation and subsequent risk for CVD. The main objectives of the study are to examine the effects of green tea beverage and green tea extract supplementation on features of MeS, biomarkers of oxidative stress and inflammation and plasma catechin levels. Obesity has parallel association with metabolic syndrome and Oklahoma is the sixth most obese state with 30.3 % prevalence of obesity. Hence, green tea supplementation study will address this problem. 28 subjects with MeS were randomly assigned to control, green tea beverage (4 cups/day, 440 mg EGCG) or green tea extract (2 capsules/day, 460 mg EGCG) groups for 2 months.

Findings and Conclusions: Chronic green tea supplementation for 8 weeks showed significant weight loss in green tea beverage group (-2.3 kg, $p<0.05$) vs control. This study found no significant difference in markers of oxidative stress and inflammation. However, there is a decreasing trend in Ox-LDL (- 19.7%, $p<0.1$), nitric oxide (- 31.2 %, $p<0.1$) levels and increasing trend in HDL cholesterol in beverage group vs control after 8 weeks of supplementation. Green tea catechins were found in plasma samples collected after over-night fast. This indicates that catechins may present in the system for longer periods of time. Green tea supplementation with higher doses for longer periods is necessary to explore the findings of our study.

ADVISER'S APPROVAL: Dr.Arpita Basu