Influence of *COMT* Genotype Polymorphism on Plasma and Urine Green Tea Catechin Levels in Postmenopausal Women

A THESIS

SUBMITTED TO THE FACULTY OF THE

UNIVERSITY OF MINNESOTA

BY

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

MASTER OF SCIENCE

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September 2014

Abstract

Catechins are the major polyphenolic compound in green tea that have been investigated extensively over the past few decades in relation to the treatment of various chronic diseases including diabetes, cardiovascular disease, and cancer. *O*-methylation is a major Phase II metabolic pathway of green tea catechins (GTCs) via the enzyme catechol-*O*-methyltransferase (COMT). A single nucleotide polymorphism in the gene coding for COMT leads to individuals with a high-, intermediate-, or low-activity COMT enzyme. An epidemiological case-control study suggests that green tea consumption is associated with reduced risk of breast cancer in women with an intermediate- or low-activity *COMT* genotype. A cross-sectional analysis discovered that men homozygous for the low-activity *COMT* genotype showed a reduction in total tea polyphenols in spot urine samples compared to the intermediate- and high-activity genotypes. Several human intervention trials have assessed green tea intake, metabolism, and *COMT* genotype with conflicting results. The aim of the present study was to determine if the *COMT* polymorphism would modify the excretion and plasma levels of GTCs in 180 postmenopausal women at high risk for breast cancer consuming a green tea extract supplement containing 1222 mg total catechins daily for 12 months. All participants in the study were a sub-set from the larger parent study "Green Tea and Reduction of Breast Cancer Risk." Thirty subjects with the high-activity *COMT* genotype, thirty with intermediate-activity *COMT* genotype, and thirty with low-activity *COMT* genotype from each of the placebo and treatment groups were analyzed. No statistically significant differences were found in any urinary or plasma catechin metabolite measurements between the homozygous high-activity and homozygous low-activity *COMT* genotype in

the treatment group. Additionally, no differences were found when high-, intermediate-, and low-activity *COMT* genotypes were all compared in the treatment group. This suggests that the *COMT* genotype does not play a major role in GTC metabolism. Dosing of GTC and timing of biological samples are important factors that may need further research in future trials evaluating the effect of *COMT* genotype and GTC metabolism.

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Introduction

Tea is one of the most commonly consumed beverages in the world with over two-thirds of the world's population drinking it. It is enjoyed for its good taste, attractive aroma, and health-promoting effects. Tea is a water infusion made from the dried leaves of *Camellia sinensis* originating in southern China. The plant is now produced in several countries including China, India, Indonesia, Japan, Taiwan, Sri Lanka, and in countries of central Africa [1]. Over 300 different kinds of teas are presently manufactured from the dried plant leaf, but there are only three general categories of tea. They include the unfermented green tea, the partially fermented oolong or pouchong tea, and the fermented black tea [2]. Black tea is 78% of the total world tea produced and consumed mostly in the US and Europe. Only 2% of world production consists of oolong tea, while green tea makes up 20% of production and is prevalent in Japan and China [3].

Green tea has been studied extensively for its health benefits. Many of the beneficial properties surrounding green tea are associated with a compound called catechins in the tea leaves. Green tea and its catechins have been investigated for its reduction of body weight, protection against diabetes, and prevention of cardiovascular disease and cancer [4]. The biotransformation of green tea catechins (GTC) are extensive causing the understanding of the role green tea plays in the prevention of disease to be complex [5]. Different genetic variations in the enzymes that metabolize GTC may play a crucial role in the function green tea has in disease inhibition between individuals. Some research studies have looked at this genetic variation [6], but more research is needed to give a clear picture of how green tea metabolism may affect disease prevention.

Major Tea Components

Fresh tea leaves have a high content of flavonoids. Flavonoids are a diverse group of polyphenolic compounds that can be divided into several classes. Two of these classes, flavanols and flavonols, are the predominate types of flavonoids found in tea. Catechins, a flavan-3-ol, are the most abundant class of flavonoids in tea accounting for 30-42% of the dry weight of tea. Flavonols and flavonol glycosides make up only 3% of the dry weight of tea. These include quercetin, myricitin, kaempferol, and their glycosides. Roughly 2.5 to 4.0% of the dry weight of tea leaves contains caffeine and very small quantities of theobromine. Phenolic acids and depsides make up 5% of the dry weight of tea. The ash portion of the water-soluble extract solids of tea contains potassium, calcium, magnesium, and aluminum. The tea beverage also contains about 1 mg of fluoride per serving. Approximately 15 to 20% of the dry weight of tea is nitrogenous material. Enzymes compose a large fraction of this, roughly 6% of the weight is amino acids, and 1% of the weight is nucleic acids. There are 19 amino acids found in tea, and theanine is an amino acid unique to the beverage. Carbohydrates make up about 5 to 7% of the dry weight consisting of cellulose, pectin, glucose, fructose, and sucrose [7,8].

Fresh tea leaves are steamed or dried to prevent the enzymatic oxidation of catechins in the manufacturing of green tea. When tea leaves are fermented to manufacture oolong or black tea, the fresh leaves are allowed to wither and are crushed [9]. This process of fermentation is initiated by the oxidation of catechins to reactive quinones. This oxidative polymerization of the catechins is dependent on the enzyme polyphenol oxidase and will form compounds such as bisflavanols, theaflavins, thearubigins and other oligomers.

These products of fermentation give oolong and black tea its orange-yellow to red-brown color and different flavors [2,7]. Catechins are reduced by roughly 85% throughout the manufacturing of black tea [7]. Figure 1 shows the tea extraction processes.

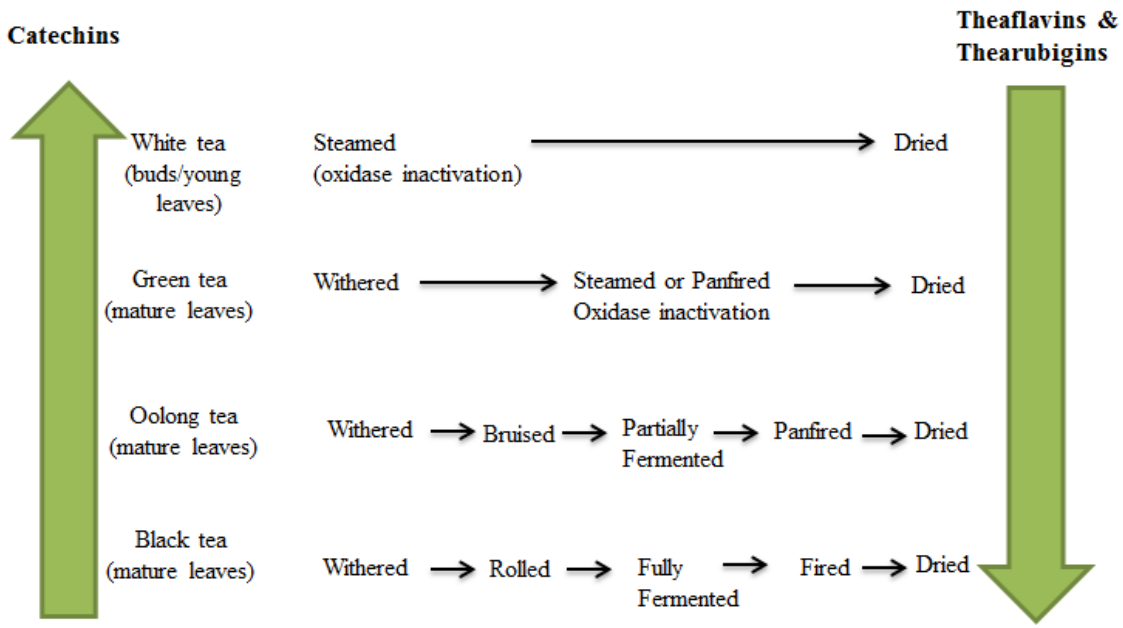

Figure 1: Schematic of the tea extraction processes adapted from reference [9]

The composition of tea can fluctuate depending on the age of tea leaf, the species, season, climate, and horticultural practices. For example, a study done by Yung-Sheng *et al.* showed that there was less overall catechins in manufactured green tea from young tea leaves versus old tea leaves. The level of caffeine was higher in the young tea leaves. The researchers also showed that young green tea leaves varied in the amount of catechins they contained depending on where the tea leaf was removed from the tea plant [1].

Green Tea Catechins (GTC)

Green tea and the major polyphenolic compounds it contains, catechins, have been investigated extensively. The bitter and astringent taste of the unfermented green tea is related to the colorless, water-soluble catechins within it. There are four main types of catechins found in fresh tea leaves including (-)-epicatechin (EC), (-)-epicatechin gallate (ECG), (-)-epigallocatechin (EGC), and (-)-epigallocatechin gallate (EGCG) (7). The major catechins are shown in figure 2 [10]. Catechins are differentiated by the meta-5, 7-dihydroxy substitution of the A ring and their di- or tri-hydroxyl group substitution of the B ring. The de novo biosynthesis of flavonoids like catechins are formed through condensation reactions of cinnamic acids and acetic acid. The synthesis of the flavonoids in tea requires enzymes of the shikimate/arogenate pathway, and the 5-dehydroshikimate reductase is a key regulatory enzyme in the process [7].

A typical cup of green tea contains 30-42% of catechins by dry weight. EGCG is the most abundant and widely studied catechin of green tea can account for 50% to 80% of

the total catechins in green tea [6,7]. Table 1 compares the polyphenol content of green and black tea. The tea brews are 1.25% water extracts (1.25 g of tea leaves per 100 ml of water) of green and black tea [11]. Catechins are found in other food sources as well as green tea. (+)-Catechin and EC are more commonly found in foods like fruits. Black grapes, apricots, strawberries, and beans have especially high levels of catechins. Apples, blackberries, black grapes, cherries, pears, raspberries, chocolate, and broad beans are all higher in EC [12]. The gallate and gallocatechins, including EGCG, are almost found exclusively in tea and particularly in green tea.

Table 1: Polyphenol Content by Tea Type [11]

Tea Polyphenols	Green Tea (µg/ml)	Black Tea (µg/ml)
Total polyphenols	1064	300
EGCG	444	128
EGC	411	42
ECG	90	73
EC	98	37
Catechin	21	20
Flavonols and flavonol glycosides	101	95
Total theaflavines	0	64
Undefined polyphenols	589	1466
Total tea polyphenols	1754	1925

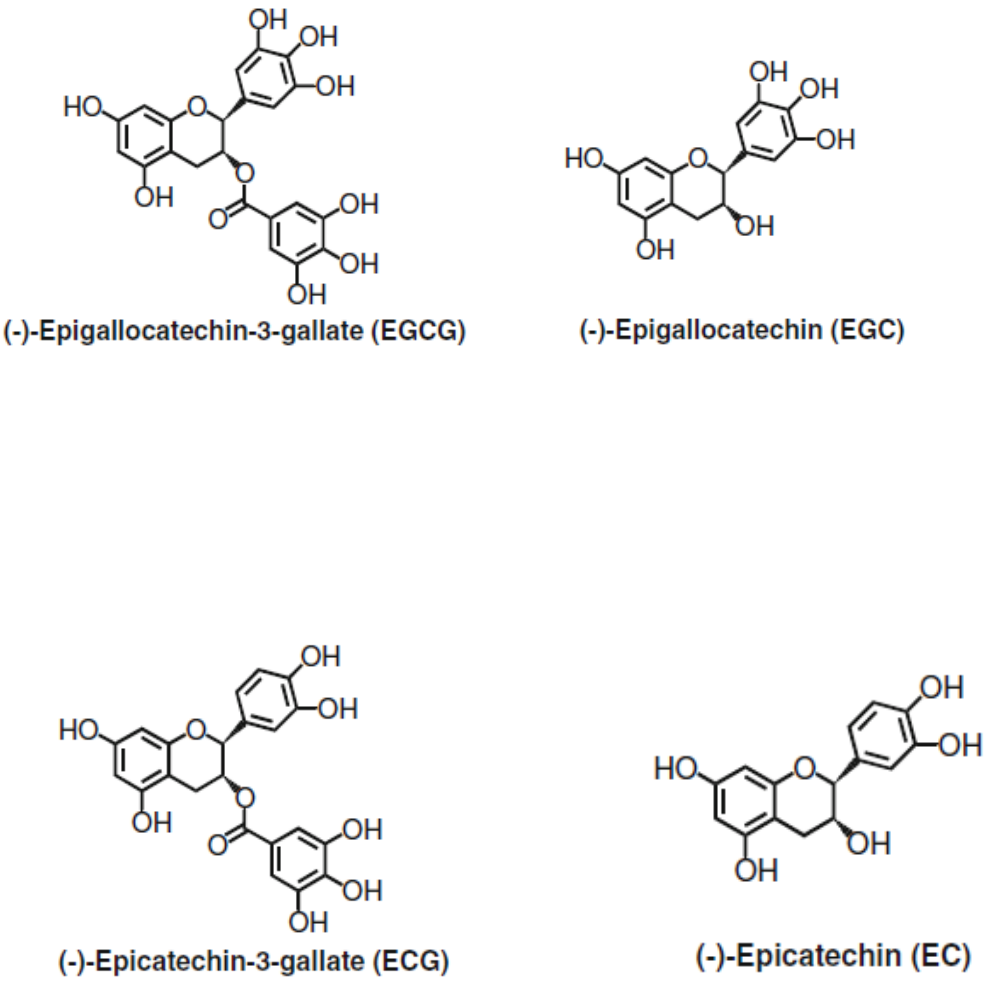

Figure 2: Major green tea catechins [10]

Tea & Health Benefits

Tea has been used as a medicinal drink in China since as early as 3000 B.C. and was written in the ancient Chinese pharmacopoeia during the Ming dynasty of the 16th century [1]. This long history of tea providing health benefits has led to the research of tea in more recent times. It has now been studied for its protective effects against cancer, cardiovascular, and other diseases. The polyphenolic compounds in tea have mostly been ascribed to these health benefits [13]. The biological functions of tea polyphenols are numerous and continue to be investigated.

Tea polyphenols are recognized for their antioxidant and antibacterial activity. In general, the antibacterial activity decreases the more the tea is fermented [14]. Strong antioxidant properties have been seen in both green and black tea[13]. A study by Chan *et al.* compared the role of non-polymeric phenolic and polymeric tannin constituents in tea and found that non-polymeric phenolic compounds were the major contributor to the antioxidant and antibacterial role in tea [15]. Green tea was shown to have higher antioxidant and antibacterial properties than black or herbal teas.

The health benefits of consuming green tea for a large variety of disorders have been reported. Many of the advantages regarding green tea are associated with its catechins, particularly the EGCG content. *In vitro* and animal studies have suggested the underlying mechanisms of GTCs and their biological actions [16,17]. GTCs have shown promising potential to treat common diseases through their ability to inhibit specific enzymes, to scavenge free radicals, and to simulate hormones and neurotransmitters [6].

Green Tea & Weight Loss

There is evidence that suggests that GTCs may reduce body weight and Body Mass Index (BMI). *In vitro* data indicates that GTCs, especially EGCG, have an anti-obesity effect through various mechanisms. A couple of these mechanisms include an inhibition of adipocyte differentiation and proliferation [18] and a reduction in fat absorption [19]. Animal studies have shown promising evidence that green tea may suppress the accumulation of body fat. In recent years, popular study designs yielding these results have induced obesity in the animal models with a high-fat diet and then followed with a period of green tea supplementation [20-22]. Bose *et al.* investigated EGCG on high-fat diet induced obese mice. EGCG was supplemented to a treatment group for either 16 or 4 weeks compared to the control group. The longer term of EGCG treatment attenuated the development of obesity and fatty liver. Shorter EGCG supplementation of 4 weeks decreased mesenteric fat weight in the mice and lowered the blood glucose compared to the control group [20].

Green tea contains caffeine, which can also lead to changes in anthropometric measurements by increasing energy expenditure. This factor needs to be taken into consideration when assessing the ability GTCs have on altering body weight. A recent meta-analysis analyzed 15 human trials noting if caffeine was either in the GTC or placebo supplement used in the trial [23]. The trials ranged from 8 to 24 weeks in length with 33 to 240 participants. Six of the trials used a GTC supplement that contained caffeine and a placebo that contained equal amounts of caffeine [24-29]. Statistical pooling of the data from these trials showed that consuming GTCs with caffeine at a dose ranging from 583 to 714 mg per day had a statistically significant beneficial effect on body

weight (-1.38 kg; 95% CI: -1.78, -1.06), BMI (-0.55; 95% CI: -0.65, -0.40), and waist circumference (-1.93 cm; 95% CI: -2.82, -1.04). No change was noted in waist-to-hip ratio. Six of the total trials in the meta-analysis used a GTC supplement dosage of 474 mg per day that contained caffeine, but the placebo used did not have caffeine [30-35]. Grouping the results from these studies together revealed that GTC ingestion significantly reduced body weight (-0.44 kg; 95% CI: -0.72, -0.15), but it did not have an effect on BMI, waist circumference, or waist-to hip ratio. Lastly, a trial using a range of 300 mg per day of GTC supplementation without any caffeine and a placebo without caffeine had no significant results for any anthropometric measurements [36]. The results from these studies could indicate that the caffeine contained in green tea may play a role in the effect green tea has on anthropometric measurements. More studies will need to be done using green tea supplements at different doses without caffeine to discover the true effect of green tea alone.

Green Tea & Diabetes

Type 2 diabetes is a major concern within many countries. One of the crucial factors leading to the onset of type 2 diabetes is impaired insulin sensitivity and glucose control. The underlying mechanisms in which GTCs may improve glucose control have been explored mainly through *in vitro* and animal based studies. *In vitro* data suggest that GTCs could reduce glucose absorption through the inhibition of gastro-intestinal enzymes involved in nutrient digestion. Kobayashi *et al.* showed that GTCs significantly inhibited the sodium-dependent glucose transporter and reduced glucose uptake from the intestines of rats [37]. Another study discovered the importance of the esterified functional

group in EGCG. This functional group can suppress the production of glucose from maltose by inhibiting the α-glucosidase enzyme [38]. Additionally, animal studies have shown that consuming GTCs leads to an increase in glucose uptake in the skeletal muscle. However, there is a decrease in glucose uptake in the adipose tissue [39]. GTCs may be activating or suppressing the expression of transcription factors related to adipogenesis.

Epidemiological studies have shown that green tea may decrease the risk for diabetes. A large epidemiological study conducted in Japan followed a total of 17,413 participants with no history of type 2 diabetes for up to five years. Subjects who habitually consumed greater than 6 cups of green tea per day had a decreased risk for diabetes with an odds ratio of 0.67 (CI: 0.47-0.94) compared with subjects who drank less than one cup of green tea per week [40].

Human intervention studies have studied this topic in more detail. One placebo-controlled study with 22 healthy volunteers had a treatment group that consumed 1.5 g of green tea powder containing 84 mg EGCG. Consumption of the green tea powder prior to an oral glucose challenge was found to result in smaller increases in the plasma glucose level at minute 30 ($p < 0.05$) and minute 120 ($p < 0.01$) during the oral glucose tolerance test of a participant [41]. Another study of 20 healthy individuals revealed that green tea containing 300 mg of EGCG reduced carbohydrate absorption following a carbohydrate rich meal by up to 25% ($p = 0.014$) by assessing breath hydrogen and carbon dioxide concentrations [42]. Fukino *et al.* studied the intake of 456 mg catechins in 60 Japanese participants with borderline diabetes. They found that consuming the high

dose of catechins for two months significantly reduced hemoglobin A1C ($p = 0.03$), a marker for long-term glucose control [43]. Participants in this study were allowed to continue drinking green tea while in the study, and although this was accounted for, can pose as a potential barrier to clear results.

Overall, the effects of green tea on glucose metabolism in type 2 diabetes appear to be mediated by several mechanisms. GTCs may decrease carbohydrate absorption and decrease hepatic glucose production. Other mechanistic ideas for the role of GTCs in type 2 diabetes include increased insulin secretion and insulin sensitivity, as well as an increased uptake of glucose into skeletal muscle [44].

Green Tea & Cardiovascular Disease

Cardiovascular health is another major concern and a field that has been investigated to a large degree. There are many *in vitro* and animal studies exploring the mechanisms by which GTCs may improve cardiovascular health. For example, a popular Chinese green tea called Dragon Well Tea has been shown to significantly suppress lipoxygenase activity and inhibit endothelial-cell induced LDL oxidation ($p = 0.001$) [45]. This type of green tea has also been shown to lower the plasma cholesterol in Sprague-Dawley rats that have been made hypercholesterolemic through their diet. This was achieved through increasing fecal bile acids and cholesterol excretion [46]. Also, several studies have shown that green tea slows down atherosclerosis in animals [47-49]. Possible mechanisms for slowing down the rate of atherosclerosis include reduced plasma lipid peroxides and lower aortic cholesterol and triglyceride contents in animals given green tea supplements.

A review by Cheng analyzing many *in vitro* and animal studies led to several suggested mechanisms in support of cardiovascular health through GTC intake. These mechanisms include the ability of green tea to exhibit antioxidant activity, anti-inflammatory properties, inhibit vascular smooth muscle growth, counteract vasoconstriction, as well as prevent hypertension and stroke [50].

Epidemiological studies have shown that green tea has a positive effect on cardiovascular health. A large prospective cohort study performed in Japan following 40,530 adults without a history of stroke or coronary artery disease for 11 years found that green tea consumption was inversely associated with mortality due to cardiovascular disease (men drinking 3 to 4 cups/day: OR 0.88; 95% CI, 0.79-.98 and women drinking 3 to 4 cups/day: OR 0.77 (95% CI: 0.67-0.89) [51]. Imai and Nakachi found an inverse relationship between green tea consumption and several markers for cardiovascular disease within 1,371 men. The incidence of heart disease was 26.0, 29.4, and 39.8 per 1000 in the population with a daily consumption of green tea greater than ten cups, 4 to 9 cups, and less than three cups respectively. Decreased serum concentrations for total cholesterol (p for trend < 0.001) and triglycerides (p for trend < 0.02) were found to be significantly associated with increased consumption of green tea. Increased proportion of high density lipoprotein cholesterol and decreased proportion of low and very low lipoprotein cholesterols were found to be associated with consumption of greater than 10 cups per day (p for trend < 0.02) [52].

Clinical trials have added to an understanding of the role green tea may play in cardiovascular health. A four-week study followed 12 healthy male volunteers. The

participants consumed 600 ml of green tea daily. The levels of oxidized low-density lipoprotein, a known biomarker to increase in the development of atherosclerosis, were significantly decreased after the four week time period of the study ($p = 0.006$) [53]. Another study using 14 healthy women found that consuming a green tea supplement containing 375 mg catechins led to a reduction in the concentration of oxidized low-density lipoprotein ($p = 0.017$) and modifications in vascular function ($p = 0.02$) [54]. A meta-analysis by Zheng *et al.* reviewing 14 randomized control trials studying green tea consumption and the lipid profile of adults concluded that green tea consumption lowered the total cholesterol concentration (-7.20 mg/dL; 95% CI: -8.19, -6.21) and low-density lipoprotein cholesterol concentration (-2.19 mg/dL; 95% CI: -3.16, -1.21) in the blood of the participants in the studies reviewed [55]. There are few clinical studies showing promising results with green tea and its relation to cardiovascular health. Larger and more in depth clinical trials will be needed to assess this multi-faceted disease.

Green Tea & Cancer

Perhaps the most well-known role of GTCs in human health is its role in cancer prevention. Both *in vitro* and animal studies have revealed the ability of green tea to prevent cancer cell metastasis [56,57]. Animal studies have shown inhibition of tumorigenesis in the oral-digestive tract, lungs, skin, prostate, bladder, and mammary tissue [58]. The primary actions of EGCG in the inhibition of carcinogenesis are thought to be its ability to directly bind to receptors and other target proteins to inhibit key enzyme activities or affect signal-transduction pathways. Also, the anti-oxidative functions of EGCG may be important to destroy reactive oxygen species at all carcinogenesis stages.

A large number of epidemiological studies have looked at the association between green tea consumption and the risk of cancer of several organs. Statistical analysis of these studies reveal that their results are not strong and neither confirm or deny what has been found in animal studies thus far. Epidemiological studies have suggested a moderate protective effect of green tea against oral cancer, while data is inconsistent in regards to esophagus and stomach cancer. The temperature of tea may play a role in this difference since the tea temperature has since been shown to be a factor in the development of these cancers [59]. Studies have also proved inconsistent in regards to colorectal, liver, pancreatic, lung, breast, prostate, and urinary bladder cancer making it hard to form a conclusion on the role of green tea in these organs. There has been some evidence from a small case-control study that green tea may reduce the risk of leukemia [60]. Because this was a small, hospital based study design, more studies are needed to validate these results.

There are many confounding factors within epidemiological studies such as diet, alcohol intake, smoking status, medications taken by participants, and activity level that can be more tightly controlled within a clinical trial leading to clearer results. One clinical trial researched the effects of EGCG in the form of ointment or capsule in patients with cervical lesions infected with human papilloma virus. An overall response rate of 69% to the treatment of green tea extracts in ointment or capsule form ($p < 0.05$) suggested that green tea extracts may be a potential therapy for human papilloma virus infected legions [61]. Lee *et al*. showed that green tea leaves were a useful source to slowly release catechins and theaflavins in the mouth. This may help explain green tea's potential role in the prevention of dental caries and oral cancer [62]. Clinical trials have been very limited

thus far in subject sample size, gender, and age. More research is needed in the area of GTCs and cancer.

Biotransformation of Catechins

Various studies have examined the bioavailability and biotransformation of GTCs. Fewer studies have investigated the biological activities of the metabolites of catechins [5]. It has been confirmed through research that tea catechins undergo considerable biotransformation. The extensive metabolism, including intestinal, microbial, and hepatic metabolism, of GTCs is most likely one of the reasons for its low bioavailability. Oral bioavailability of GTCs has been shown to be low in both animals [6,63] and humans [64,65]. Factors that may influence the low bioavailability and uptake of catechins include intestinal permeability and metabolism, transport inhibitors, efflux of catechins by multidrug resistance-associated proteins, and additional metabolism within the colon through microbial metabolism. The major metabolic pathways for catechins occur in the liver, kidneys, and gastrointestinal tract. These major pathways include methylation, glucuronidation, sulfation, and ring-fission metabolism and will be discussed in further detail in an upcoming section of this literature review [6]. The major metabolic pathways of GTCs are shown in figure 3.

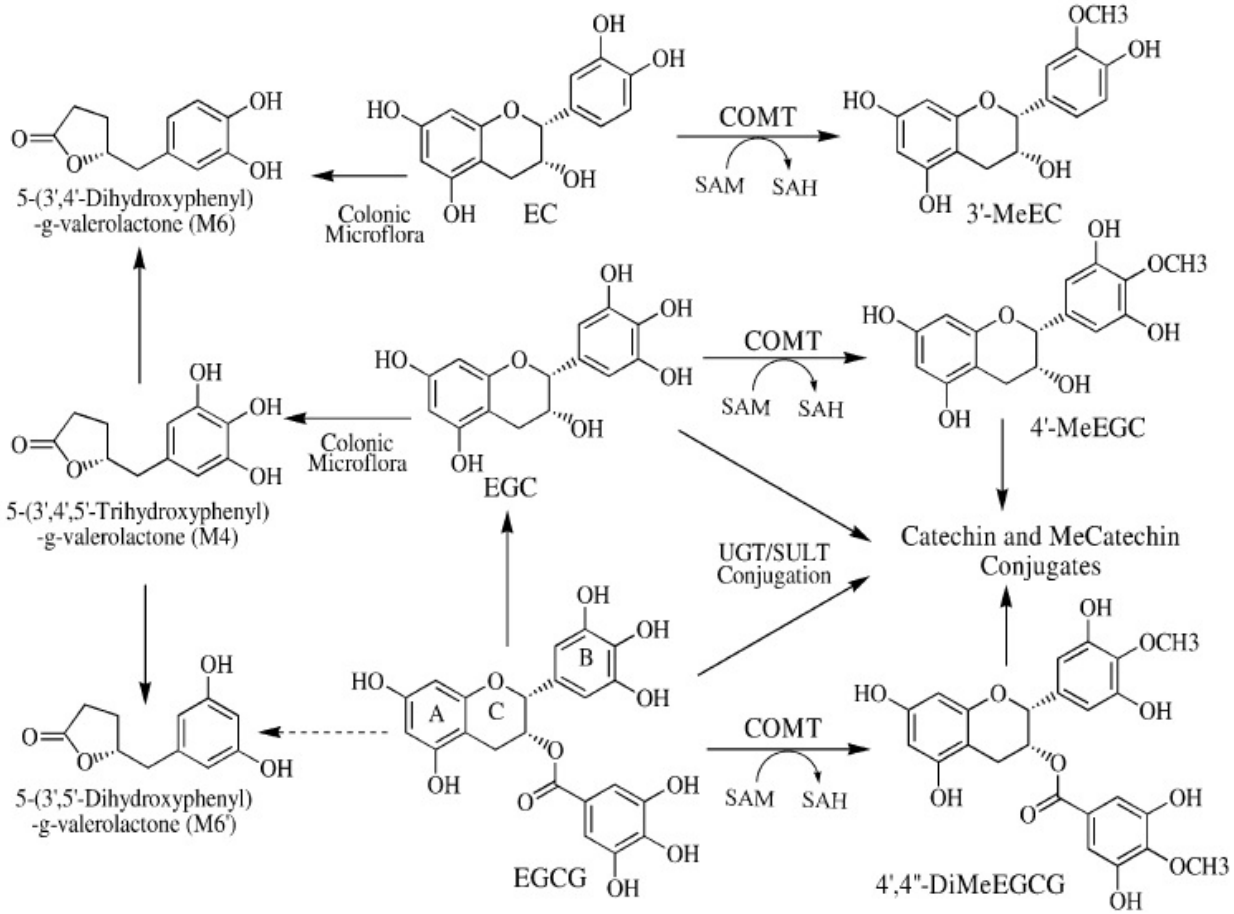

Figure 3: Major metabolic pathways of green tea catechins [5]

Methylation of GTC

Studies have shown that methylation of GTCs is the major Phase II metabolic pathway leading to a variety of metabolites [6]. The enzyme responsible for the methylation of GTCs is catechol-*O*-methyltransferase (COMT). COMT catalyzes the transfer of methyl groups from S-adenosylmethionine (SAM) to a hydroxyl group of a catechol or substituted catechols with Mg^{2+} present. This generates *O*-methylated catechol and S-adenosyl-L-homocysteine (SAH) [66]. Figure 4 shows the folate and methionine cycles where SAM is converted to SAH by COMT [67]. SAM is the methyl donor for COMT to methylate other substrates. COMT is found distributed throughout the tissues of mammals that have been investigated thus far. In rats and humans, the highest activity is found in the liver, and then the kidney and gastrointestinal tract [68]. COMT exists in both a soluble and membrane-bound form in mammals. The soluble form of COMT is present in most tissues, while a membrane-bound form is the main form found in the human brain [69]. The activity of COMT within erythrocytes has been shown to vary between species and is higher in rats and lower in humans [70,71]. COMT activity, specifically toward EGCG and EGC, has also been shown to be higher in rat liver cytosol than in human or mouse liver cytosol [72]. EC and EGC are good substrates for methylation by a placental cytosolic COMT, while ECG and EGCG are methylated at a far slower rate by the same enzyme [73]. Humans and mice are more similar, in general, than humans and rats in regards to COMT enzyme activity [5].

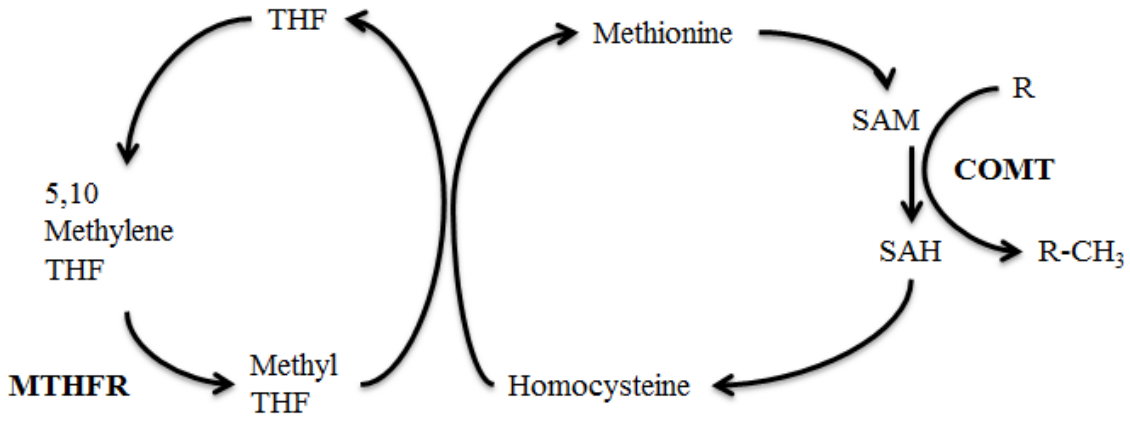

Figure 4: The folate and methionine cycles with COMT enzyme adapted from reference [67]

The overall function of COMT is to eliminate possibly noxious or active catechol structures of endogenous or exogenous compounds. The methylation of these structures decreases the hydrophilicity where it can then go on to be further sulfated or glucuronidated to be successfully eliminated from the body [68]. Physiological substrates for the COMT enzyme include 3,4-Dihydroxy-L-phenylalanine (L-DOPA), catecholamines, and catecholestrogens. Flavonoids, such as catechins, are dietary products that can also be a substrate for COMT [74]. Certain compounds can inhibit the COMT enzyme and significantly affect the metabolism of the compounds able to act as substrates with COMT. Selective COMT inhibitors, such as synthetic nitrocatechol compounds, are used in current therapies of Parkinson's disease (PD) in combination with other treatments [75]. These inhibitors reduce COMT activity and increase the bioavailability of L-DOPA to help PD patients. Flavonoids have been shown to inhibit

COMT [76]. EGCG and its metabolites were shown to be powerful inhibitors of COMT enzyme mediated *O*-methylation of both EGC and L-DOPA [68]. It has also been revealed that tea catechins inhibit the *O*-methylation of endogenous catecholestrogens further displaying tea catechins and their metabolites may be inhibiting COMT [77]. These inhibitory effects of tea catechins on the activity of COMT can alter the rate of metabolism and methylation of tea catechins.

There are various methylated catechin metabolites resulting from the COMT enzyme pathway. Researchers have detected methylated metabolites including 3'- and 4'-*O*-methyl-EC, 4'-*O*-methyl EGC, 4"-*O*-methyl EGC and EGCG, and 4' and 4"-di-*O*-methyl EGCG from observations *in vivo* and in incubations with rat liver homogenates [78]. The major metabolite found in the bile of the rats following oral EGCG administration was 4' and 4"di-*O*-methyl EGCG [5]. Enzymology studies have shown similar metabolites formed with EGC being easily methylated to form 4'-*O*-methyl-(–)-EGC and EGCG to 4"-*O*-methyl-(–)-EGCG or 4', 4"-*O*-dimethyl-(–)-EGCG by rat liver cytosolic COMT [68]. Sang *et al.* chemically synthesized several GTC metabolites in their lab and verified their structures in human plasma and urine samples using LC/MS. These metabolites included 4'-*O*-methyl EGC, 4"-*O*-methyl EGC, and 4', 4"-di-*O*-methyl-EGCG. The same researchers also confirmed that 4"-*O*-methyl EGC and 4', 4"-di-*O*-methyl-EGCG were the major metabolites of EGC and EGCG in human plasma and urine in their studies [78].

Factors affecting GTC methylation

There are factors that may affect the methylation of GTCs. Cytosolic protein concentration, concentration of S-adenosyl-L-methionine, incubation times, and incubation pH all affected methylation when investigating placental cytosolic COMT [73]. There is indication that glucuronidation on the B-ring or D-ring of EGCG will considerably inhibit methylation on the same ring while glucuronidation on the A-ring of either EGCG or EGC will not affect methylation when COMT was studied in rodents [68]. These studies also showed the concentration of the catechins were of importance. Lower concentrations of EGCG lead to the dimethylated EGCG metabolite being the major product, and higher concentrations of EGCG lead to more monomethylated products being formed.

Another factor that may affect methylation of GTC is a polymorphism in the gene coding for the COMT enzyme. The COMT enzyme is coded for by a single gene. This *COMT* gene encodes for both the membrane-bound COMT and the soluble COMT that are transcribed by the activation of two individual promoters. A single nucleotide polymorphism (G to A) in codon 108 of the soluble *COMT* gene or codon 158 of the membrane-bound *COMT* gene results in a valine to methionine substitution in both forms of the COMT enzyme [79]. This polymorphism produces a high-, intermediate, and low-activity form of COMT with genotypes of Val/Val (G/G), Met/Val (A/G), and Met/Met (A/A), respectively. The polymorphism results in thermostability differences leading to a thermolabile low-activity COMT enzyme. The high-activity enzyme is thermostable [80]. There is a three to four times decrease in enzymatic activity between the high- and low-

activity COMT enzymes.

Catechin Metabolism & *COMT* Genotype

Several studies have examined the relationship between the *COMT* genotype and GTC metabolism and excretion in humans. A cross-sectional analysis within the Shanghai Cohort Study evaluated whether urinary levels of GTC metabolites differed between participants with low-activity or high-activity genotypes among those who drank green tea daily [81]. A total of 18,244 men were enrolled in this study and interviewed upon enrollment to gain necessary information. A non-fasted blood sample and a single-void urine sample were also taken at this interview. Information regarding tea drinking was acquired at an in-person interview one year from the first interview held with each participant. These annual interviews were acquired from 14,210 of the 14,531 participants. It was found that 95% of participants were exclusive green tea drinkers and had a mean daily intake of 8.3g of dry green tea leaves (three to four cups) per day. The genotype distribution of *COMT* was 52.0% G/G, 40.6% A/G, and 7.4% A/A. The distribution was found to be unrelated to the consumption of green tea by participants. The low activity allele was found to be in Hardy-Weinberg equilibrium. It was discovered that the men with the homozygous A/A genotype of *COMT* had a statistically significant reduction in the levels of the urinary tea catechins EGC (p=0.047), 4'-MeEGC (p=0.049), M4 (p=0.037), and overall total tea polyphenols (p=0.007) in their spot urine compared with the men of A/G and G/G genotypes. EC (p=0.057) and M6 (p=0.099) were found to have borderline statistically significant lower levels in the spot urines of the men with the A/A genotypes. When stratified by level of green tea consumption, low

consumers (less than 5g per day of green tea leaves) carrying the A/G or A/A genotype had a 35-45% reduction in urinary levels of 4'-MeEGC and EC. However, urinary excretion rates of urinary catechins and their metabolites were similar between *COMT* genotypes of high consumers (greater than 5g per day of green tea leaves). This may indicate that at high doses of GTCs, the *COMT* genotype will not play a significant role in altering green tea metabolism.

A clinical, double-blind, placebo-controlled study supplemented participants with GTC and obtained 24-hour urine collections [82]. A total of sixty-four out of eighty-three subjects completed the study. Each participant was on a placebo supplement for a six-week period and a treatment supplement for a six-week period with a two week wash out period. The decaffeinated green tea treatment supplement contained 530 mg catechins (40.71% EGCG, 16.27% EGC, 8.74%EC, 6.02% ECG). A 24-hour urine collection was done by the participant approximately a week before starting and ending supplement. 24-hour urine collections were mixed into a homogenous solution and measured for EGC and 4'-*O*-methyl EGC. Urinary excretion of the catechins remained unchanged for participants at baseline and post-intervention of the placebo. The levels of EGC and 4'-*O*-methyl EGC were found to be lower in the men of the G/G genotype group compared to the A/G and A/A genotypes ($p<0.01$). The levels in the G/G genotype group were roughly half the amount found in the other genotypes. *COMT* distribution of participants was not reported. These results differ from the cross-sectional analysis from the Shanghai Cohort Study.

A recent clinical trial investigated fifty healthy, male subjects (25 G/G and 25 A/A) using a randomized, placebo-controlled, double-blind, crossover design [83]. 530 mg DGT (43.0% EGCG, 10.9% EGC, 8.3%EC, 8.9% ECG) was given to participants after 48 hours of a low flavonoid diet. A 24-hour urine collection was taken starting at the time of ingestion of study supplement to measure for EGC and 4'-*O*-methyl EGC in the urine. The urine collection was split into the first 5.5 hours and the last 18.5 hours. It was discovered that the A/A *COMT* genotype had significantly lower urinary concentrations of 4'-*O*-methyl EGC in the 5.5-hour urinary collections compared to the G/G genotype taking DGT. There were no genotype differences seen in the 18.5-hour collection. Dividing up the hours of the 24-hour urine collection could indicate that a difference in *COMT* genotype may be meaningful only in the first several hours of metabolism. These results suggest that the timing of sample collection may be crucial to give accurate insight on GTC metabolism.

***COMT*, Green Tea, & Breast Cancer Risk**

COMT could have the ability to reduce the cancer preventive effects of GTCs because of its varying activity due to the polymorphism. A case-control study of Asian-American women discovered a reduced risk of breast cancer (adjusted OR, 0.48; 95% CI; 0.29-0.77) in women who consumed green tea and were of A/A or A/G *COMT* genotype. However, there was no difference in breast cancer risk between green tea drinkers and non-tea drinkers for those that were homozygous G/G *COMT* genotype [84]. A larger, more recent epidemiological study looked at 3,454 breast cancer cases and 3,474 controls. It found that the *COMT* genotype did not significantly modify an association between green

tea drinking and breast cancer risk. Menopausal status did not change this insignificance [85]. The role COMT plays in catechin metabolism and disease is still unclear.

Glucuronidation & Sulfation of GTC

A second pathway in Phase II metabolism of GTCs is glucuronidation catalyzed by a group of enzymes called UDP-glucuronosyltransferases (UGT). UGT catalyzes the binding of glucuronic acid from UDP-glucuronic acid on structurally unrelated constituents with a carboxyl, hydroxyl, amine, or thiol group [78]. The glucuronidation of EGCG and EGC in human, mouse, and rat microsomes were studied by Lu and researchers. They also explored the glucuronidation of the catechins in nine different human UGT isozymes expressed in insect cells [85]. Four EGCG (EGCG-7-O-glucuronide, EGCG-4"-O- glucuronide, EGCG-3"-O- glucuronide, and EGCG-3'-O- glucuronide), and two EGC (EGC-3'-O- glucuronide and EGC-7-O-glucuronide) glucuronide metabolites were biosynthesized. EGCG-4"-O-glucuronide was shown to be the major EGCG glucuronide formed in all incubations. This major metabolite was most readily formed in mouse small intestinal microsomes, followed in decreasing catalytic efficiency order by mouse liver, human liver, rat liver, and rat small intestine. The glucuronidation of EGC was much lower than EGCG. The glucuronidation of EGC to EGC-3'-O-glucuronide had its greatest catalytic efficiency in the mouse liver microsomes followed by the human liver, rat liver, and then rat and mouse small intestine. In both glucuronidation reactions of EGC and EGCG, mice appear to be more similar to humans than rats [86]. EC has been reported to form only two glucuronide metabolites in rat liver

microsomes, while showing no signs of being glucuronidated in the human liver, small intestines, or colon microsomes [87].

It has been found that UGT1A1, 1A8, and 1A9 have the highest activity toward EGCG by using recombinant human UGT enzymes [6]. Human UGT1A1 is present in human liver and intestine, while UGT1A9 is present in human liver and kidney. UGT1A8 is only found in the intestine [6]. Intestinal specific UGT1A8 has the highest catalytic efficiency toward EGCG, however it has low activity with EGC. The activities of UGT1A1, 1A3, and 1A8 are much lower when the major glucuronidation site of EGCG is occupied by a methyl group. 4"-methylation can enhance glucuronidation of EGCG at this D-ring site with the UGT1A9 enzyme [78].

GTCs can also undergo sulfation. The enzymes responsible for this are sulfotransferases (SULT). These enzymes catalyze the transfer of a sulfate group from a donor molecule to an acceptor alcohol or amine [78]. EC undergoes sulfation catalyzed by both human and rat liver and intestinal enzymes in cytosol. The human liver is shown to be the most efficient [86]. SULT1A1 is mainly responsible for the sulfation of EC in the human liver cytosol, and both SULT1A1 and SULT1A3 contribute to sulfation of EC in the intestine. It has been shown that EGCG is sulfated by human, mouse, and rat liver cytosol. It is time and concentration dependent with the rat cytosol having the greatest activity followed by the mouse and human liver cytosol [72].

Microbial Metabolism of GTC

The human colon contains roughly 1000 bacterial species amounting to over 100 trillion bacteria in total in the large intestine. This microflora has vast catalytic and hydrolytic potential for dietary compounds including polyphenols [78]. Colonic microflora can catalyze the breakdown of polyphenols into simple compounds such as phenolic acids and their glycine conjugates. Studies have shown that the amount of GTCs absorbed in the small intestine is relatively small. This would imply that the majority of GTCs ingested, including those that have been absorbed and conjugated by other enzymes in the tissues, will reach the large intestine and be metabolized by the intestinal flora [6]. It has been reported that tea catechins are metabolized by the colonic microflora forming the following major ring fission products: 5-(3',4',5'-trihydroxyphenyl)-γ-valerolactone (M4), 5-(3',4'-dihydroxyphenyl)-γ-valerolactone (M6), and 5-(3',5'-dihydroxyphenyl)-γ-valerolactone (M6') [77]. These same ring fission products have been identified in both human urine and plasma after the ingestion of 20 mg/kg decaffeinated green tea approximately 13 hours after the consumption of tea [88].

Following consumption of 200 mg EGCG, peak urine concentrations of 8, 4, and 8 μM have been verified for M4, M6, and M6', respectively. M6 has been shown to form during anaerobic incubation of EGC and EC with human intestinal bacteria [78]. M4, M6, and M6' have all been shown to be produced as a result of anaerobic fermentation of EGC, EC, and ECG with human fecal microflora [72]. Further degradation of these ring fission products would result in the formation of lower weight phenolic acids by the intestinal flora [78]. Several other microbial metabolites have been identified in human

urine samples. These include 4-hydroxybenzoic acid, 3,4-dihydroxybenzioc acid, 3-methoxy-4-hydroxy-hippuric acid, and 3-methoxy-4-hydroxybenzoic acid [89]. These particular metabolites may need to be researched further to determine if they have any beneficial properties on human health.

Biological Activities of Catechin Metabolites

The metabolites of GTCs have been studied for their own biological activities. The radical scavenging ability of the glucuronide metabolites of EGCG and EGC has been investigated [86]. There were varying scavenging abilities depending on the metabolite. EGC had greater radical scavenging ability compared with both EGC-3'-glucuronide and EGC-7-glucuronide. However, the glucuronide metabolites of EGCG were more active than EGC and its glucuronide metabolites. EGCG-3'-glucuronide and EGCG-3"-glucuronide had equal radical scavenging abilities to EGCG, while EGCG-7-glucuronide and EGCG-4"-glucuronide were less active than EGCG. Studies have also examined the growth inhibitory activity of EGCG against cancer cell lines. There was a 40% growth inhibition when HT-29 human colon and KYSE150 human esophageal cancer cell were treated with M4. When these same cells were treated with EGCG, there was a 50% growth inhibition [90]. HCT-116 human colon cancer cells, INT-407 human, and IEC-6 rat immortalized intestinal cell lines were not all sensitive to M4 growth inhibitory effects. It has been reported that methylated metabolites 4'-*O*-MeEGCG and 4"-*O*-methylEGCG have decreased growth inhibitory and pro-apoptotic activity compared to EGCG [91]. The methylated metabolites also had reduced Fe (III)-reducing power, and 4'-*O*-MeEGCG had decreased H_2O_2-producing ability. These studies show that the various GTC

metabolites could play an important role in various biological activities but appears that the parent GTC compounds have an overall stronger activity.

GTCs and their metabolites have been studied for their difference in inhibitory effects on the COMT-mediated methylation of hydroxyestradiol. ECGC has been shown to be a potent inhibitor of COMT. Both 4"-*O*-methylEGCG and 4',4"-di-*O*-methylEGCG were less potent than EGCG at inhibiting COMT. EGCG-7-glucuronide was just slightly less potent at inhibiting COMT, while EGCG-3'-glucuronide, EGCG-3"-glucuronide, and EGCG-4"-glucuronide were significantly less potent. EGC was also significantly less potent than EGCG, and methylation at the 4'-hydroxyl group did not significantly affect the inhibitory activity of EGC [92]. Overall, these studies seem to indicate that EGCG has the strongest inhibiting qualities.

Pharmacokinetics of GTC

The pharmacokinetics and bioavailability of GTCs have been studied more extensively in rat and mice animal models than in humans. The plasma concentration-time curves of EGCG, EGC, and EC have been fit into a two-compartment pharmacokinetic model with elimination half-lives ($t_{1/2}$) of 212, 45, and 41 minutes, respectively, following intravenous administration of decaffeinated green tea in rats. The absolute bioavailability of EGCG, EGC, and EC were 0.1, 13.7, and 31.2%, respectively, following an intragastric administration of decaffeinated green tea [93]. Lambert *et al.* discovered the oral bioavailability of unchanged EGCG to be about 15.8% in mice [94]. Other studies have reported very low bioavailability of EGCG in rats [6,95]. Another related study found EGCG

levels in the plasma and tissues between 0.0003% and 0.45% of the ingested EGCG [96]. Both of these present the poor bioavailability of EGCG in rats. Excretion in the feces has been shown to be the major route of elimination of radioactive labeled EGCG. After the administration of 4-[4-^{3}H]EGCG intravenously to bile-duct-cannulated rats, only 2% of the radioactivity was excreted in the urine, and about 77% was excreted in the bile within 48 hours [93]. After the administration of [^{3}H]EGCG intragastrically to mice, about 6.5% of the radioactivity was excreted in urine and 35% in feces within 24 hours [97]. Several studies have investigated the pharmacokinetics of GTC in human participants. An earlier study used different amount of DGT to determine blood and urine levels of GTCs in humans [98]. Each gram of DGT contained 73 mg EGCG, 68 mg EGC, 22 mg ECG, and 25 mg EC. GTC reached peak plasma levels (T_{max}) in 1.5-2.5 hours after consumption of 1.5 g of DGT. The average maximum plasma concentration of the GTCs (C_{max}) was 0.71, 1.8, 0.65 μM of EGCG, EGC, and EC, respectively. C_{max} values increased 2.7- to 3.4- fold when the dosage was increased from 1.5 g to 3.0 g DGT. Increasing the dose to 4.0 g DGT did not increase the C_{max} values significantly. After oral administration of 1.5-4.0 g DGT, $t_{1/2}$ of EGCG, EGC, and EC were 4.9-5.5, 2.5-2.8, 3.2-5.7 h, respectively.

Chow *et al.* administered a single-dose of Polyphenon E or EGCG to twenty, healthy participants in a cross-over design study [99]. Polyphenon E is a DGT extract that contains 80-98% total catechins with EGCG accounting for 50-75% of total supplement material. Each Polyphenon E capsule in this study contained 200 mg EGCG, 37 mg EGC, and 31 mg EC. It was found equal doses of EGCG in pure form or in Polyphenol E led to similar pharmacokinetics of EGCG. **Figures 5 and 6** show the plasma concentrations of

unchanged EGCG in participants at a dose of 200, 400, 600, and 800 mg EGCG or EGCG in Polyphenon E. The average C_{max} after a 200, 400, 600, and 800 mg dose of EGCG were 0.16, 0.24, 0.37, and 0.96 μM, respectively. The average C_{max} after a 200, 400, 600, and 800 mg dose of Polyphenon E containing equivalent amounts of EGCG were 0.16, 0.27, 0.36, and 0.82 μM, respectively. Only EGCG was detected in the plasma after EGCG administration. When plasma samples were treated with β-glucuronidase and sulfatase deconjugating enzymes after a dose of EGCG, EGCG levels did not change significantly suggesting that EGCG is mostly in the unchanged form in the plasma. EGCG levels were detected, and EGCG and EC were low or undetectable in human plasma after a Polyphenon E dose. When plasma samples were treated with deconjugating enzymes after a dose of Polyphenon E, EGCG levels still did not change much, but EGC and EC level increased significantly. This suggests that EGC and EC are present mostly in the conjugated forms in the plasma and that the bioavailability of the GTCs are relatively low.

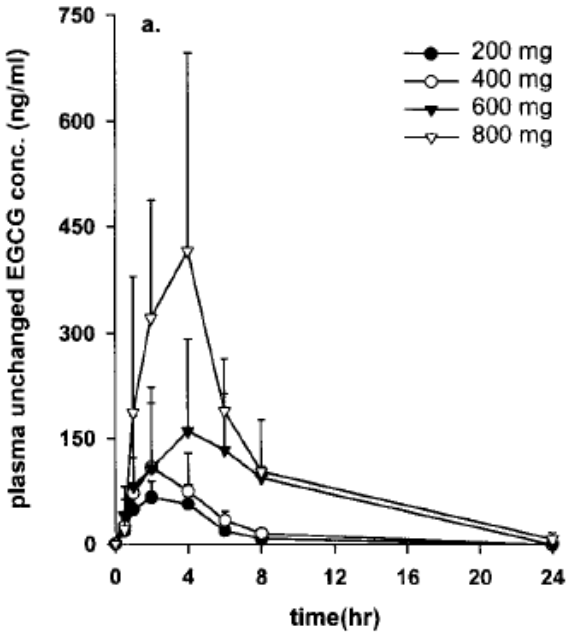

Figure 5: Average EGCG concentration (ng/ml) vs. time after oral administration of EGCG [99]

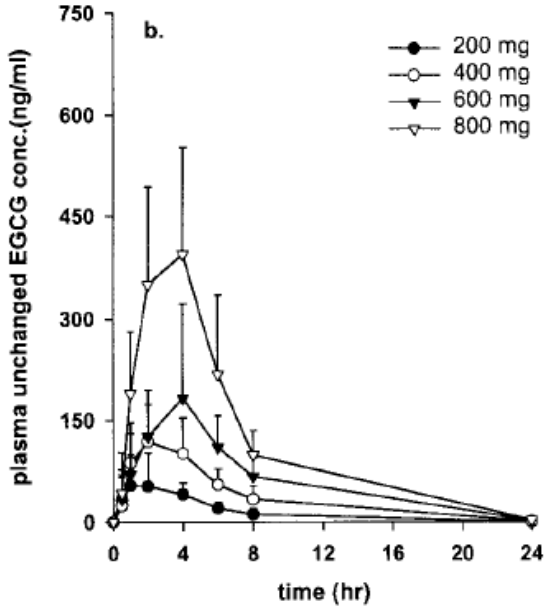

Figure 6: Average EGCG concentration (ng/ml) vs. time after oral administration[99] of Polyphenon E [99]

Another study administered 20 mg/kg green tea or decaffeinated green tea to eight healthy adult volunteers [100]. It was found that EGCG was mostly found in the free form in the plasma, similar to other studies. EGC and EC were mostly in the conjugated form. 77% of EGCG, 31% of EGC, and 21% of EC were found to be in free form in the one-hour plasma samples. After five hours, 64% of EGCG and 40-50% of EGC and EC were in free from. The catechins not in free form were conjugated and most were in the glucuronide form. In the 24-hour urine collections, tea catechins were found to be more than 95% in conjugated form. Greater than 90% of urinary catechins were excreted in the first eight hours, showing that extensive glucuronidation and sulfation occurs to these catechins similar to other findings. A higher percentage of EGC and EC appeared in the urine when green tea was taken in the decaffeinated form. 3.3% of EGC and 8.9% of EC was detected in the urine while only 2.3% of EGC and 4.6% of EC appeared when regular green tea was consumed. In all cases, EGCG was present in trace or non-detectable amounts in the urine [101]. This implies the significant metabolism EGCG undergoes within the body. The pharmacokinetics of conjugated metabolites of EGC and (-)-EC were not affected by repeated green tea administration.

Following the administration of one cup decaffeinated green tea or pure catechins in four healthy, male subjects, more than 80% of GTCs were found in the conjugate forms in the one-hour plasma samples [102]. In plasma samples, 58-72% of EGCG was in a conjugated sulfate form, 12-28% was in free form, and 8-19% was in glucuronide form. 57-71% of EGC was in the glucuronide form, 23-36% in sulfate form, and 3-13% was in free form. EC was almost found exclusively in the conjugated forms with roughly 33% glucuronided and 77% sulfated. ECG was not detected in the plasma. Maximum urinary

excretion of EGC and EC occurred three to six hours after administration of GTCs. The majority of EGC and EC occurred three to six hours after administration of GTCs. EGCG and ECG were not detected in urine. Sulfate and glucuronide forms of EGC and EC were present in about a 2:1 ratio. Free EC was not detected, and less that 1% of EGC was present in unconjugated form. Methylated metabolites were not assessed in this study.

Chow *et al.* assessed the pharmacokinetics in 40 healthy men and women supplemented daily with 400 mg or 800 mg of free EGCG or as part of a GTC supplement [103]. At the end of week 4, the area under the curve (AUC) was reported to be 145.6 min μg/ml with supplementation of 800 mg free EGCG. The average peak plasma concentration of the GTCs (C_{max}) was 390.3 ng/ml, the average time it took to reach the maximum plasma concentration (T_{max}) was 210.0 min, and the biological half-life ($t_{1/2}$) was 158.9 min at the end of week 4. It was discovered that there was a greater than 60% increase in the AUC of free EGCG after four weeks of supplementation compared to the first dose AUC in the group with an 800 mg dose ($p < 0.05$). There was no significant change in the subjects with a 400 mg EGCG dose. Inhibition of intestinal flora metabolism, intestinal efflux of EGCG, or methylation via the COMT enzyme may play a role in this effect.

One clinical trial has assessed the pharmacokinetic of GTCs and the effect of the *COMT* polymorphism [104]. Seven men and seven women each with the high-activity *COMT* genotype and three men and three women having the low-activity genotype were recruited. Two capsules of decaffeinated green tea extract totaling 448 mg EGCG were given to participants, a standardized breakfast was given one hour after supplementation

to avoid stomach upset. Blood samples were taken at 0, 30, 60, 90, 120, 150, 180, 240, 360, and 480 minutes after capsule consumption. A pharmacokinetic analysis was done for all participants for EC, EGC, ECG, EGCG, and 4'-*O*-methyl EGCG plasma concentrations. There were no significant differences found for C_{max}, T_{max}, AUC, EGCG half-life absorption, and EGCG half-life elimination between the two A/A and G/G genotypes. 4'-*O*-methyl EGCG was found to have a higher C_{max} and AUC in the A/A genotype, although these results were not statistically significant. No other significant parametric differences were found between the two *COMT* genotype groups.

Overall, studies in human have shown that GTCs are metabolized rapidly in humans. Average C_{max} were between one and three hours after oral consumption, reach T_{max} concentrations in the sub- or low μM range, and $t_{1/2}$ appear to be on average 2-4 hours [105].

Factors Affecting Bioavailability of GTC

The oral bioavailability of tea catechins have been shown to be quite low in rodents and humans in pharmacokinetic studies. The poor bioavailability of tea catechins can be a result of instability within digestive conditions, poor transcellular transport, and the rapid metabolism that is followed by excretion [6,106]. Almost total degradation of EGCG and roughly 80% total catechin loss has been observed in simulated digestion of simple tea infusions. Instability of catechins has been demonstrated in authentic intestinal juice and buffered systems above a pH of 7.4 [107]. EC and ECG have been shown to be less sensitive to simulated digestion. GTCs have been shown to be extremely unstable in neutral or alkaline conditions in general [6]. This degradation in the small intestine limits catechin

uptake and bioavailability. The low bioavailability of some catechins may be attributed to the comparatively high molecular weight and the large number of hydrogen-bond donating hydroxyl groups. This follows Lipinski's rule of 5 which states that compounds that have five or more hydrogen bond donors, or ten or more hydrogen bond acceptors, or a molecular weight greater than 500 will usually have poor bioavailability. This is because of the large size of the molecule and the formation of a large hydrogen shell [108]. Phase III metabolism is called active efflux and has been shown to limit the bioavailability of tea polyphenols. The multidrug resistance-associated proteins (MRPs) are the ATP dependent efflux transporters that carry out this active efflux and are expressed in many tissues. MRPs and active efflux may play an important role in restricting the bioavailability of tea catechins. MRP1 is located on the basolateral side of cells and is present in nearly all tissues. It transports compounds like tea polyphenols from the interior of a cell into the interstitial space. MRP2 is located on the apical surface of the intestine, liver, and kidney where it transports constituents from the bloodstream into the lumen, bile, and urine, respectively [72]. Studies have shown that the MRP inhibitor, indomethacin, causes an increase in the intracellular accumulation of EGCG, EGCG 4"-*O*-methyl-EGCG, or 4', 4"-di-O-mehtyl-EGCG by 10-, 11-, or 3-fold, respectively, in canine kidney MDCKII cells expressing MRP1. In the same way, an MRP2 inhibitor, MK-571, produced an intracellular increase in the levels of EGCG, EGCG 4"-*O*-methyl-EGCG, or 4', 4"-di-O-mehtyl-EGCG by 10-, 15-, or 12-fold, respectively, in MDCKII cells expressing MRP2 [109]. It has been demonstrated that ECG is a substrate for a monocarboxylate transporter, MCT, in Caco-2 cells. When MCT-1 is inhibited by benzoic acid or phloretin in these cells, the uptake of ECG is reduced [110].

There are factors that can help increase catechin bioavailability. Studies in Caco-2 cell models have shown that sugar, ascorbic acid, and even xylitol may modulate and increase catechin bioavailability [111,112]. Sugar and ascorbic acid together increased bioavailability greater than sugar or ascorbic acid alone in rats, and xylitol and ascorbic acid together increased bioavailability greater than xylitol or ascorbic acid alone in cells. It has been shown by Chow *et al.* that a greater oral bioavailability can be attained when free catechins are taken on an empty stomach after an overnight fast in humans. Human participants administered Polyphenon E on an empty stomach after an overnight fast resulted in greater plasma levels of free EGCG, EGC, and ECG. Fasting state did not affect total EGC levels but did significantly increase total levels of EGCG and decrease total levels of EC in the plasma [113]. These studies show that tea consumption practices and formulation factors could result in greater tea catechin bioavailability.

Overall, the gastrointestinal tract plays a crucial role in the bioavailability of GTCs as they metabolize. Metabolites that are formed in the small intestines and transported back to the intestinal lumen will make their way to the large intestine to be metabolized further by the gut microflora. The microflora of an individual therefore, is a factor that needs to be considered when addressing GTC metabolism. These metabolites may be reabsorbed or passed out through the feces. Figure 5 shows a summary of possible pathways of GTCs in a human body.

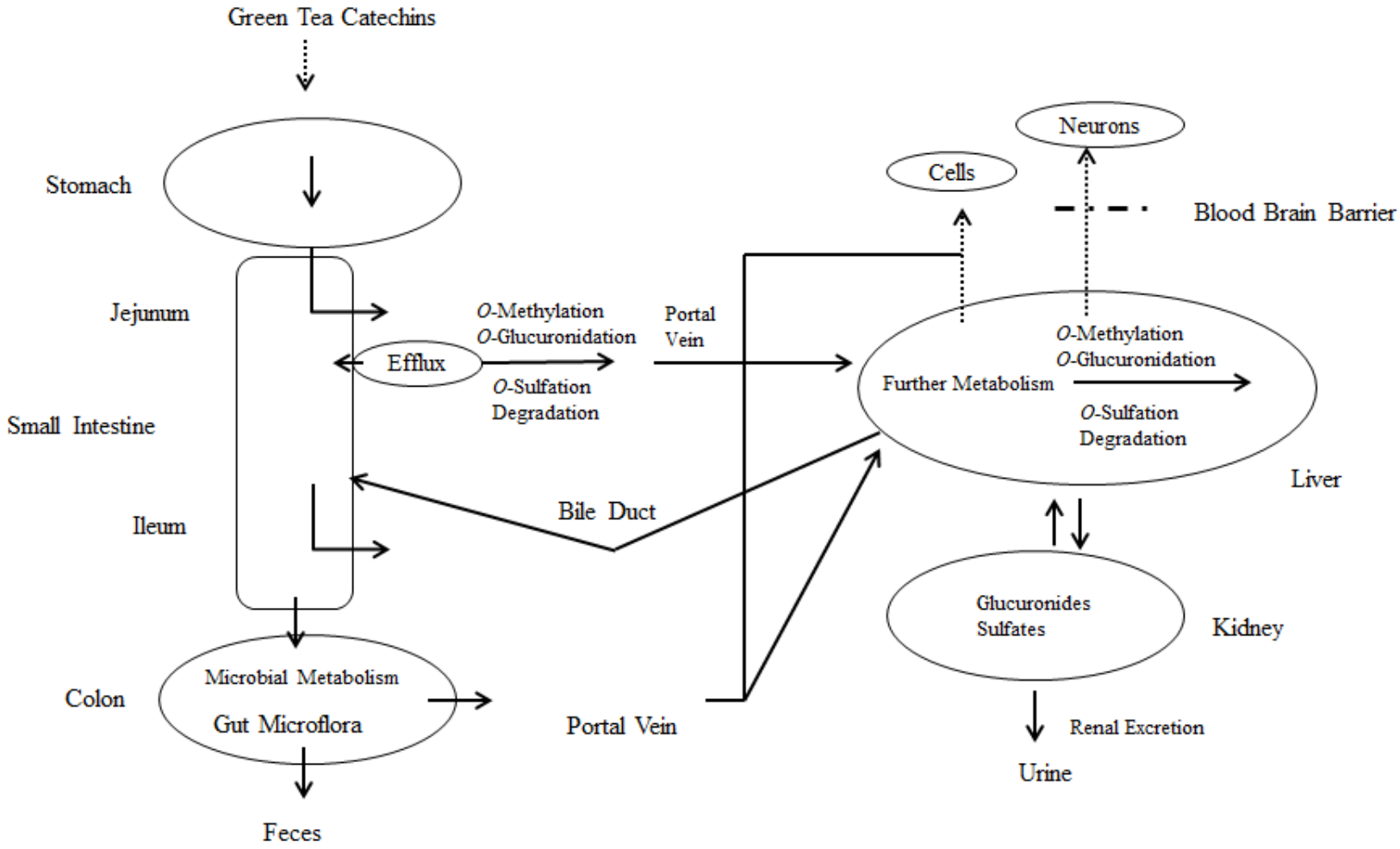

Figure 7: Suggested outcomes of GTC metabolism in humans adapted from reference [6]

Concluding Words: A Need for Research

There is a lack of research investigating the interaction of a green tea treatment, catechin metabolism, and the *COMT* genotype in humans. One epidemiological study with a larger sample size has assessed urinary catechin levels among differing *COMT* genotypes indicating COMT may play a role in catechin metabolism. Only a few clinical trials have investigated plasma or urinary catechin metabolism and have reported mixed results. All studies had small sample sizes and most did not assess the intermediate genotype.

The understanding of the GTC metabolism and biotransformation is essential for gaining knowledge into how green tea may protect against human disease. The complexities of green tea absorption and metabolism have yet to be fully understood, and addressing the *COMT* genotype may help further the understanding of catechin metabolism. A better experimental design with a larger sample size investigating both plasma and urinary catechin metabolism in all three *COMT* genotypes will be important to achieve this.

Manuscript

INTRODUCTION

Tea is one of the most commonly consumed beverages in the world. Brewed from the dried leaves of *Camellia sinensis* [113], green tea has been shown to have high levels of polyphenols due to minimal oxidation during the processing of fresh tea leaves. Catechins, the major polyphenolic compounds in green tea, have been investigated extensively in relation to the treatment or prevention of chronic diseases such as diabetes, cardiovascular disease, and cancer [72].

The four main types of catechins found in fresh tea leaves are (-)-epicatechin (EC), (-)-epicatechin gallate (ECG), (-)-epigallocatechin (EGC), and (-)-epigallocatechin gallate (EGCG) [7]. A typical cup of brewed green tea contains 30-42% of catechins by dry weight [8]. EGCG is the most abundant and widely studied catechin of green tea and could account for 50% to 80% of the total catechins in green tea [6]. Many of the biological effects of green tea consumption are associated with its catechin content, particularly EGCG.

Green tea catechins (GTCs) undergo considerable biotransformation and have been shown to have low bioavailability in both animals [6,63] and humans [64,65]. Therefore, understanding the efficacy of GTCs *in vivo* is quite complex. The major metabolic pathways for catechins include methylation, glucuronidation, sulfation, and ring-fission metabolism [6]. *O*-methylation is a major Phase II metabolic pathway of GTC metabolism and is catalyzed by the enzyme catechol-*O*-methyltransferase (COMT). The COMT enzyme is coded for by a single gene. A single nucleotide polymorphism (G to A

substitution) in codon 108/158 of the soluble COMT or codon 158 of the membrane-bound COMT results in a valine to methionine substitution in the enzyme. This polymorphism causes a three to four times decrease in enzymatic activity *in vitro* producing a high (G/G), intermediate (A/G), and low-activity (A/A) form of the COMT enzyme [79].

Interestingly, epidemiological studies have shown interactions between these polymorphisms in the COMT enzyme and the association of green tea consumption with breast cancer risk. A case-control study of Asian-American women [84] found green tea consumption to be associated with reduced risk of breast cancer in women who carried one low-activity *COMT* allele (adjusted OR, 0.82; 95% CI, 0.64-1.06) or both low-activity alleles (adjusted OR, 0.84; 95% CI, 0.54-1.30). A possible explanation for this observation is that individuals with a variant genotype resulting in decreased COMT activity would metabolize GTCs at a lower rate, and thus, receive more of the potential benefits from GTCs due to greater exposure. Consistent with this hypothesis, a recent cross-sectional analysis among men who drank green tea daily within the Shanghai Cohort Study [81] discovered that homozygous low-activity *COMT* individuals showed a 35-45% reduction in total tea polyphenol levels in spot urine samples compared with men with intermediate or homozygous high-activity genotypes ($p = 0.007$).

On the other hand, several human intervention trials have assessed green tea intake, metabolism, and *COMT* genotype with varying results [82,83,104]. The largest clinical trial performed to date with 71 subjects in an intention-to-treat design found 24-hour urinary levels of EGC and 4'-*O*-methyl EGC in the homozygous high-activity genotype to be

decreased by about half compared to the other genotypes [82]. These results differ from those found in the cross-sectional analysis of the Shanghai Cohort Study. Miller *et al.* discovered 25% lower urinary concentration of 4'-*O*-methyl EGC in the first 5.5 hours of 24-hour urine collections in the low-activity *COMT* genotype compared to the participants with high-activity *COMT* genotypes [83] A pharmacokinetic study on individuals consuming GTC and with different *COMT* genotypes found no differences in pharmacokinetic parameters in the plasma of subjects [104].

The role *COMT* genotype plays in GTC metabolism in humans remains unclear. There have been no large clinical trials performed to date assessing the impact of differing *COMT* genotypes on the metabolism and excretion of GTCs. The primary aim of this study was to determine if *COMT* polymorphisms will modify the excretion and plasma levels of GTCs in postmenopausal women at high risk for breast cancer after dietary supplementation of green tea extract (GTE; containing 1222 mg total catechins/day including 830 mg EGCG/day) for 12 months. It is hypothesized that total plasma and urinary GTCs, and urinary methylated GTCs will be reduced in individuals with the homozygous low-activity *COMT* genotype.

MATERIALS & METHODS

Study participants and recruitment

The study sample used for this research is a subsample of 180 participants from a larger parent study, "Green Tea and Reduction of Breast Cancer Risk," the Minnesota Green Tea Study (Clinical Trial Registration: clinicaltrials.gov identifier: NCT00917735). The

parent study is a large, randomized, double-blind, placebo-controlled, clinical trial investigating the effects of green tea supplementation on biomarkers of breast cancer risk. Mammographic density, reproductive hormones, insulin-like growth factor axis proteins, and oxidative stress will be assessed in over 800 healthy, postmenopausal women at high risk for breast cancer due to elevated breast density. A possible modifying effect of *COMT* genotype on all biomarkers will additionally be investigated.

All participants for this sub-study were recruited from the Breast Center at the University of Minnesota Medical Center, Fairview Southdale Breast Center, or Fairview Maple Grove Breast Center between July 2009 and September 2011 in the Minneapolis, MN metropolitan area. The study radiologist supervised the identification of women aged 50 to 70 years old with "heterogeneously dense" or "extremely dense" breasts as indicated by the American College of Radiology [114]. "Heterogeneously dense" breasts are approximately 51-75% glandular tissue, while "extremely dense" breasts have more than 75% glandular tissue. A recruitment letter approved by the Institutional Review Board (IRB Approval Code Number: 0806M36121) explaining the purpose of the study was mailed to prospective participants. Interested participants called a screening telephone number, and a screening questionnaire was administered to the potential participant over the phone. Potential subjects who met further eligibility criteria attended an in-person orientation. Potential participants then attended a screening visit where *COMT* genotype and final eligibility status was determined. Eligible participants were randomized into either the GTE or placebo group and scheduled for their baseline clinic visit. This study was approved by the University of Minnesota's Institutional Review Board and written informed consent was obtained from all subjects prior to the screening visit.

Inclusion criteria included: healthy women 50 to 70 years old; last menstrual period greater than one year ago; "heterogeneously dense" or extremely dense" breasts; and willingness to avoid consumption of green tea. Exclusionary criteria included positive serological markers for hepatitis B or C; elevated liver enzymes (above 1.5 times the upper limit of normal for the University of Minnesota-Fairview Hospital Laboratory); hormone modification therapy, hormone replacement therapy, selective estrogen-receptor modulator, or aromatase inhibitor usage in the last six months; current smoker; body mass index (BMI) less than 19 or greater than 40 kg/m^2; weight change greater than 10 pounds in the previous year; participation in weight gain or weight loss studies; history of breast cancer or proliferative breast disease; history of ovarian cancer; history of any cancer in the last five years; presence of breast implants; greater than seven alcoholic beverages consumed per week; greater than one cup of green tea consumed per week; or use of chemopreventative agents in the last six months.

For this sub-study, 180 participants were randomly chosen from those who had completed the study as of November 2012. From the treatment and placebo groups, 30 high-activity, 30 intermediate-activity and 30 low-activity *COMT* genotype participants were selected.

Study design

The parent study was a randomized, double-blind, placebo-controlled study with participants randomized based on their *COMT* genotype and treatment group (**Figure 1**). Randomization was performed through the Investigational Drug Services (IDS) at the University of Minnesota based on *COMT* genotype and treatment group. A total of 1,084

participants (289 high-activity, 451 intermediate-activity, and 344 low-activity) were randomized into the parent study with a withdrawal rate of 12.4%. The participants for this sub-study analysis were randomly chosen from completed participants by the study biostatistician in November 2012. At this time a total of 328 participants had completed the parent study (64 high-activity, 173 intermediate-activity, and 86 low-activity).

For one year, participants consumed two capsules twice daily (for total of four capsules). They were instructed to continue with their normal diets and lifestyles, and they came in monthly to the clinic for collection of biological samples.

For this study, participants provided a fasting blood sample and a 24-hour urine collection at baseline, six-month and twelve-month clinic visits. A health history questionnaire was given to the participants at the screening clinic visit and was completed and returned to study staff at the baseline clinic visit. Compliance was verified through study logs in which participants recorded capsules consumed and pill counts were also conducted on returned study supplement capsules at clinic visits.

Dietary Supplements

The GTE and placebo capsules were manufactured by Corban Laboratories (Plymouth, USA). They were encapsulated in a two-piece, green, opaque, size "0" cellulose capsule (V cap). High-performance liquid chromatography (HPLC) determined that each GTE capsule was comprised of EGCG (207.5 mg), ECG (62.4 mg), EC (23.0 mg), and EC (23.0 mg). **Table 1** shows the contents of the GTE supplement. Magnesium stearate (2 mg) was added as a flow agent. The placebo capsule appeared identical to the green tea

capsule but contained maltodextrin (50.0%), microcrystalline cellulose (49.5%), and magnesium stearate (0.05%). Participants received a three month supply of study supplement at their baseline clinic visit in three prescription bottles with corresponding prescription labels distributed by the IDS. Participants were asked to take two capsules in the morning with breakfast and two capsules in the evening with dinner for a daily dose of approximately 830 mg EGCG. Participants received a new supply of supplement every three months.

DNA isolation and COMT genotyping

COMT genotyping was performed at the University of Minnesota Molecular Epidemiology and Biomarkers Research Laboratory. The screening blood sample was centrifuged at 3,000 rpm for 10 minutes at 4°C. The buffy coat layer was transferred to an aliquot tube with 0.5 ml normal saline and stored at -80°C until analysis. DNA was extracted from buffy coats at time of analysis using a DNeasy Blood & Tissue Kit column method (QIAGEN Sample and Assay Technologies) according to manufactures protocol. DNA was eluted in 200μl nanopure water (Applied Biosystems) and quantified by nanodrop. Ten μl of the sample DNA was diluted to approximately 5ng/μl and added to the wells of a PCR plate. An assay mix consisting of PCR master mix and Genotyping assay mix (Qiagen) was also added to the wells. Genomic DNA (5.625μl per well) was used for PCR amplification. PCR was carried out on Applied Biosystem 7900 Real-Time PCR system. The initial thermal cycler step was 2 minutes at 50°C followed by 10 minutes at 95°C. Next, 50 cycles were carried out at 95°C for 15 seconds and at 60°C for 60 seconds. The plate was then analyzed by the Allelic discrimination protocol in the

same machine, and the output generated determined the genotype of the wells. A no template blank control and 3 wells of Corriell cell lines (GM15327, GM15080, and GM15334) were used as controls to be compared to the experimental samples to identify the genotype at each locus (G/G, A/G, and A/A) on each plate.

Urine collection and catechin analysis

Participants were instructed to discard their first early morning urine (start time) the day prior to their clinic visit and collect all urine for 24 hours including the urine produced on the following day at the same time as the start time (end time). Three grams of ascorbic acid were added to the three-liter collection jug at the start of urine collection. Participants were asked to keep the urine sample refrigerated and avoid alcohol consumption during collection. Collected urine samples were mixed into a homogenous solution and measured for total volume using graduated cylinders. Ten μl ascorbic acid-EDTA was added to aliquoted samples and stored at -80°C within 2 hours of receiving collection from participants until analysis. Urinary EC, EGC, methylated EGC, 5-(3',4',5'-trihydroxyphenyl)-γ-valerolactone (M4), and 5-(3',4'-dihydroxyphenyl)-γ-valerolactone (M6) were measured in the laboratory of C.S. Yang, Rutgers University, according to validated methods [98,102]. In brief, the thawed urine samples were mixed with sodium phosphate buffer, ascorbic acid, EDTA, and a mixture of β-glucuronidase and sulfatase. The mixture was incubated and the reaction stopped with1 ml of ethyl acetate. Seven hundred μl of organic phase was transferred to a microfuge tube after mixing and centrifugation. A second extraction was obtained with 700 μl of ethyl acetate, and the combined 1.4 ml extract was processed and analyzed on a HPLC system. The system

consisted of an ESA Model 465 refrigerated autosampler, two ESA-580 dual-piston pumps, and an ESA 5500 coulochem electrode array system. Standard tea catechins were used to assess the stability of the HPLC system. Duplicate measures were made for all samples as well as urinary creatinine concentrations. Every six samples included one intraday standard. Each set of samples also included an interday standard that was run in the middle of the set. A total of 22 sets were run, and coefficient of variations (CVs) were calculated from these samples. If the intraday CV was greater than 10% in the urine, samples were re-run. Intraday CV means for M4, EGC, M6, EC, and Me-EGC were 4.1%, 3.3%, 3.0%, 2.6%, and 3.4%, respectively. Interday CV means were 3.6%, 3.3%, 5.7%, 3.4%, and 4.3% respectively. Urinary tea catechin concentrations are expressed as the weight of analyte per gram of creatinine to adjust for the volume of urine.

Plasma collection and analysis

Fasted plasma samples were collected and aliquoted, and ascorbic acid-EDTA (100 μl) was added to aliquots that were stored at -80°C until analysis. Plasma EC, EGC, ECG, and EGCG were measured in samples according to the validated methods of Yang *et al* [115]. Briefly, the thawed plasma samples were incubated with a mixture of β-glucuronidase and sulfatase to determine total catechin content. The reaction was stopped by the addition of methylene chloride. To analyze free catechin content, the β-glucuronidase and sulfatase mixture was excluded from incubation. The reaction mixture was then extracted two times with ethyl acetate. Ten μl EDTA solution was added to these two ethyl acetate extractions and evaporated to dryness in a vacuum centrifuge

concentrator. Residues were re-dissolved in 100 μl acetonitrile aqueous solution and centrifuged. Prepared samples were analyzed on the same HPLC system as urinary catechin analysis. Duplicate measures were made for all samples. CVs were calculated similarly to urine. If the intraday CV was greater than 12%, samples were re-run. Intraday CV means for EGC, EC, EGCG, and ECG were 7.3%, 6.3%, 8.2%, and 8.4%, respectively. Interday CV means were 6.6%, 5.6%, 6.5%, 7.2%, respectively.

Statistical analysis

Urinary GTC metabolites were expressed relative to creatinine by weight (nmol/mg Cr). Plasma catechin concentrations were expressed as nmol/ml. Formal statistical testing was completed on logarithmically transformed values because GTC metabolites were not normally distributed.

ANOVA was used to compare catechin levels at baseline between treatment and placebo groups. Mixed model analyses were used to compare metabolite levels between groups at months 6 and 12 and between time points. ANOVA was used to compare levels of metabolites across discrete categories of demographic and lifestyle variables using age (year) as a covariate. For this analysis, measurements were done at the 12-month time point. The multivariate regression method was used when assessing the effect of *COMT* genotype on levels of urinary and plasma catechin metabolites at month 12. Adjustments were made for age (year), ever cigarette smoking (no, yes), alcohol drinking (no, yes), and tea drinking (no, yes).

Statistical analyses were carried out using SAS version 9.1 (SAS Institute Inc., Cary, NC) statistical software package. All reported *p* values are two-sided and $p < 0.05$ was considered statistically significant.

RESULTS

There were a total of 180 participants in this sub-study. Thirty subjects with the high-activity *COMT* genotype, thirty with intermediate-activity *COMT* genotype, and thirty with low-activity *COMT* genotype from each of the placebo and treatment groups were analyzed. **Table 2** shows baseline levels of urinary and plasma catechins in the treatment and placebo groups. Catechins levels did not differ between groups with the exception of plasma EC, which was significantly higher in the treatment group ($p = 0.044$). **Table 3** shows the effect of treatment and time on urinary and plasma catechins. All metabolites were significantly higher in the treatment group at both months 6 and 12 ($p < 0.001$), with the exception of plasma EGC, which did not differ between groups ($p = 0.10$). There were no significant differences in catechins between the 6 and 12-month time points with the exception of urinary M4, which was significantly higher at month 12 compared to month 6 ($p = 0.01$).

The remaining statistical analyses were performed in the 90 participants in the treatment group at month 12. **Table 4** shows the levels of urinary metabolites by selected demographic and lifestyle factors for the treatment group. **Table 5** shows the levels of plasma metabolites by selected demographics and lifestyle factors by treatment group. There was a statistically significant association between race and urinary EGC, urinary Me-EGC, and plasma EGCG. Urinary EGC and Me-EGC were significantly higher than

two participants that identified themselves as a race other than white. These same two participants had significantly lower levels of plasma EGCG. There was also a significant association between participants who had smoked previously in their lifetime and plasma EC levels, with decreased levels of plasma EC in subjects who had ever smoked compared subjects who had not. Level of education and alcohol consumption did not affect levels of GTC metabolites in plasma or urine.

No statistically significant differences were found in any urinary or plasma catechin metabolite measurements between homozygous high-activity *COMT* genotype and homozygous low-activity *COMT* genotype, although there may be trends toward higher urinary EGC and M6 in the homozygous low group ($p = 0.12$ and 0.10, respectively, **Table 6**). Additionally, no differences were found in any of the GTC metabolite between genotypes when the high-, intermediate-, and low-activity *COMT* genotype were compared, although there was a trend toward higher M6 in the intermediate and low groups ($p = 0.14$, **Table 6**).

Spearman correlation coefficients were calculated between urinary and plasma catechin metabolite levels (**Table 7**). A number of metabolites were strongly correlated within plasma and within urine, but few were correlated between plasma and urine.

DISCUSSION

This study investigated urinary and plasma catechin levels, and the modifying effect of differing *COMT* genotypes, in 180 postmenopausal women consuming either placebo or 1222 mg catechins/day (containing 830 mg EGCG/day) for one year. Participants

consumed 611 mg total catechins (containing 415 mg of EGCG) twice daily. This is similar to drinking approximately six to seven 250 ml cups of green tea per day [115]. Compliance was assessed in all participants through pill counts. There were 405 capsules distributed every three months and remaining capsules in bottles were counted at the next clinic visit. There was an average of 52 pills returned at the end of every three months with a calculated 97.2% average compliance rate.

The placebo and treatment groups were similar at baseline, with the only difference being that plasma EC was higher in the treatment group (Table 2). All metabolites were significantly higher in the treatment group at month 12, with the exception of plasma EGC, which did not differ between groups (Table 3). This is likely due to the low level of EGC in the GTC capsules.

Surprisingly, there was no effect of genotype on urinary or plasma catechins in the GTC group. This finding is not consistent with that of previous research. A clinical trial by Brown *et al.*[82] assessed catechin excretion in 24-hour urine samples in 71 male subjects taking roughly 430 mg EGCG per day in a 6-week cross-over study using a decaffeinated green tea (DGT) supplement. Urinary levels of EGC and 4'-*O*-methyl EGC were lower in the high-activity *COMT* genotype compared to the intermediate- and low-activity genotype ($p = 0.01$ and 0.03, respectively). Another study was reported in 25 homozygous low-activity *COMT* and 25 homozygous high-activity *COMT* healthy males who had followed a low flavonoid diet for 48 hours and then were given a one-time DGT supplement containing approximately 460 mg EGCG [82]. This study found significantly reduced levels of 4'-*O*-methyl EGC in the first 5.5 hours of 24-hour urine collections in

the low-activity *COMT* genotype ($p = 0.05$). Similar to our results, metabolite levels in 24-hour collections did not differ between groups [83]. It is possible that a full 24-hour urine collection may dilute GTC metabolites making differences undetectable. However, 24-hour collections are known for being a better representation than spot urine samples for metabolism in general. In a smaller study by Lee *et al*., one cup of DGT containing 88 mg EGCG, 82 mg EGC, 33 mg ECG, and 32 mg EC was administered to four, healthy males and 24-hour urine collections were obtained in three hour time increments [102]. It was discovered that the maximum level of EGC and EC metabolite urinary excretion happened three to six hours after ingestion of GTCs. Almost all EGC and EC metabolites were excreted within the first nine hours of the 24-hour collection. The timing of sample collection may be an important variable and incremental 24-hour urine collection may reveal more information.

One other recent epidemiological study investigated the association of *COMT* genotype with GTC metabolism. A cross-sectional analysis within the Shanghai Cohort Study [81] of 18,244 men who daily consumed brewed green tea revealed that subjects with the low-activity *COMT* genotype had a statistically significant reduction in overall total tea polyphenols ($p = 0.007$) in a spot urine compared to subjects with intermediate- and high-activity genotypes. Spot urine samples were taken an average of three hours after intake of a participant's last meal. Green tea is commonly consumed after meals allowing metabolites of GTCs to be at their maximum concentration in the urine samples. Further analysis showed low consumers of green tea (less than 5g/day of green tea leaves) carrying the intermediate- or high-activity genotype had a 35-45% reduction in urinary levels of -*O*-methyl EGC and EC. However, urinary excretion rates of urinary catechins

and their metabolites were similar between *COMT* genotypes in high consumers (greater than 5g/day of green tea leaves). Perhaps the COMT genotype only has a significant effect on GTC metabolism at moderate consumption levels of catechins.

Simple pharmacokinetic studies investigating variable dosing of GTC have been previously done without taking *COMT* genotype into account. GTC have been shown to have limited absorption and are in the nanomolar ranges in the plasma and urine [116]. Chow *et al.* assessed the pharmacokinetics in 40 healthy men and women supplemented daily with 400 mg or 800 mg of free EGCG or as part of a GTC supplement [103]. It was discovered that there was a greater than 60% increase in the calculated area under the curve (AUC) of free EGCG after four weeks of 800 mg supplementation EGCG, free or in GTC supplement, compared to the AUC from the first dose at week 0. Subjects taking only 400 mg per day of ECGC did not see a change in the AUC from week 0 to week 4. Inhibition of intestinal flora metabolism, intestinal efflux of EGCG, or methylation via the COMT enzyme could have caused the increase in AUC at a higher EGCG dosing. EGCG and its metabolites have been shown to be COMT enzyme inhibitors of *O*-methylation of EGC, [66] and higher doses of GTC could stimulate this effect to a larger degree.

There are a number of major differences between previous studies and the present study. Former clinical trials have only investigated male subjects, populations of different ethnicities, lower doses of GTC, fewer participants, and a shorter duration of green tea supplementation compared to the present study. The one epidemiological study was able to assess many participants, but intake of GTCs may not have been as accurately assessed

as in a clinical trial because diet and environment were not as tightly controlled. While there are a number of major differences between the present study and previous investigations, perhaps one of notable importance is the dosage of the green tea supplementation. The cross-sectional investigation within the Shanghai Cohort Study assessed tea intake based on dry tea leaves used per week for brewed infusions, while clinical studies used supplements with already known GTC contents. It is challenging to compare the catechin content in brewed tea infusions to GTC supplements due to variability in tea source and brewing time. Standardizing for a 1% infusion (1g tea leaves/ 100ml boiling water), there is approximately 350 mg EGCG in 5g of dried tea leaves. This is the level of tea ingested where an effect of *COMT* genotype was seen [81]. There is a possibility for recall bias in the cross-sectional study, and level of tea intake where *COMT* genotype may have an effect may be influenced by this. Adjustment for brewing time is not taken into account since the majority of tea flavonoids are extracted shortly after brewing [117]. The 350 mg dose of EGCG is similar to previous clinical trials detecting a difference in catechin excretion and varying *COMT* genotypes [82,83]. The 830 mg EGCG in the present study is far above previous amounts investigated. It is possible that there is a threshold of GTC intake in which the *COMT* polymorphism plays a role in altering the metabolism of GTC. Future studies may need to provide different dosages of GTC to their participants to investigate this further.

There were some limitations to this study. Health history questionnaires were completed by the participants alone. In-person interviews may have provided more accurate demographic data. Also, participants were not required to follow a low flavonoid diet due to the long period of this study. The analysis of 24-hour urines may have diluted any

increase in catechin excretion, compared with collecting urine in the few hours after supplement consumption. Similarly, fasting blood draws were taken 10 or more hours after consumption of the GTC.

In conclusion, there were no associations found between the *COMT* genotype status of postmenopausal women and the urinary and plasma levels of green tea catechins. The dosing of GTCs and the timing of biological sample collection are important factors that need consideration. Future trials may need to evaluate differing GTC dosing when assessing the effect of *COMT* genotype and GTC metabolism.

Figure 1: Parent Study (Minnesota Green Tea Study) Design

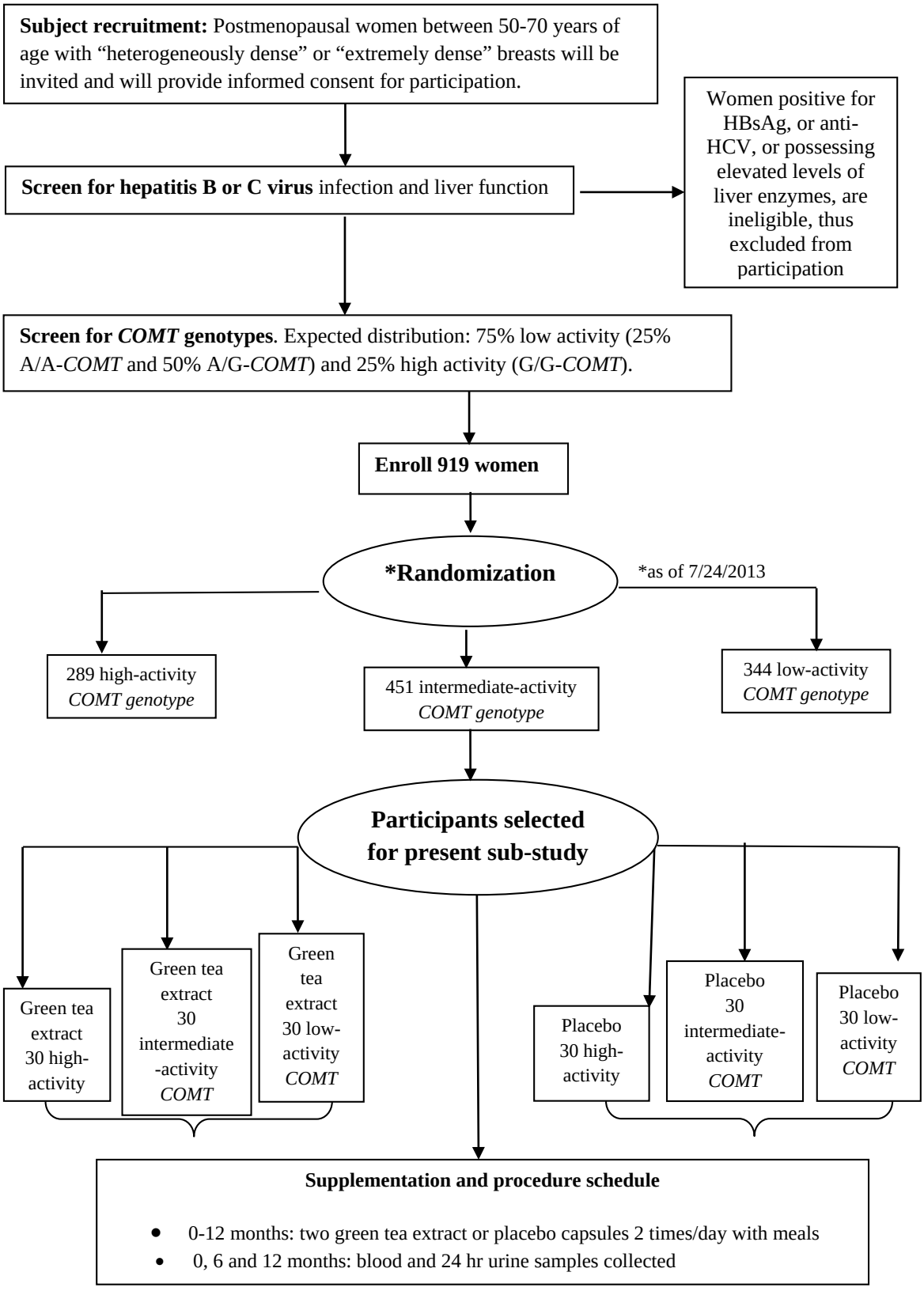

Table 1. Composition of Green Tea Extract Capsules

Compound	Percent of Extract	Content Per Capsule (mg)	Daily Catechin Dose * (mg/day)
Epigallocatechin gallate (EGCG)	53.0	207.5	830.0
Epicatechin gallate (ECG)	15.9	62.4	249.6
Epicatechin (EC)	5.88	23.0	92.0
Epigallocatechin (EGC)	1.6	6.2	24.7
Gallocatechin Gallate (GCG)	1.63	6.4	25.5
Catechin	<0.01	0.04	<0.16
Caffeine	<1.0	3.9	
Magnesium Stearate	<0.5	2	
Total Catechins		305.5	1221.8

* Study dose consisted of two capsules taken twice daily with breakfast and dinner

Table 2. Comparison of Baseline Catechins (Geometric Means (95% Confidence Interval)) between Treatment and Placebo Groups *

Catechin	**Treatment (n = 90)**	**Placebo (n = 90)**	***P* value**
Urinary Catechins			
M4, nmol/mg creatinine	0.06 (0.04-0.10)	0.06 (0.04-0.10)	0.89
EGC, nmol/mg creatinine	0.16 (0.10-0.22)	0.18 (0.12-0.24)	0.62
M6, nmol/mg creatinine	7.6 (5.6-10.3)	9.2 (6.8-12.4)	0.39
EC, nmol/mg creatinine	0.76 (0.56-0.98)	0.76 (0.56-0.98)	0.94
Me-EGC, nmol/mg creatinine	0.12 (0.06-0.20)	0.14 (0.08-0.22)	0.71
Plasma Catechins			
EGC, nM	1.3 (0.92-1.7)	1.2 (0.84-1.6)	0.73
EC, nM	1.7 (1.3-2.2)	1.1 (0.78-1.5)	0.04
EGCG, nM	3.5 (2.7-4.5)	3.9 (3.1-5.0)	0.52
ECG, nM	0.90 (0.70-1.14)	0.94 (0.74-1.18)	0.80

* Geometric means were derived from analysis of variance.

Table 3. Urinary and Plasma Catechins by Treatment Group and Time Point (Geometric Means (95% Confidence Interval)) *

Catechin	Treatment (n = 90)		Placebo (n = 90)		*P* Value for Overall Effect †	
	Month 6	Month 12	Month 6	Month 12	Treatment‡	Time
Urinary catechins						
M4, nmol/mg creatinine	1.4 (1.1-1.7)	1.9 (1.5-2.3)	0.10 (-0.02-0.24)	0.12 (-0.12-0.26)	<0.001	0.01
EGC, nmol/mg creatinine	0.48 (0.38-0.58)	0.48 (0.40-0.56)	0.18 (0.10-0.28)	0.18 (0.10-0.24)	<0.001	0.95
M6, nmol/mg creatinine	41.1 (31.7-53.1)	45.2 (34.9-58.4)	8.2 (6.1-10.8)	9.4 (7.1-12.4)	<0.001	0.11
EC, nmol/mg creatinine	10.9 (9.7-12.1)	10.9 (9.7-12.3)	0.68 (0.52-0.86)	0.7 (0.54-0.94)	<0.001	0.70
Me-EGC, nmol/mg creatinine	0.72 (0.56-0.88)	0.72 (0.56-0.92)	0.12 (0.02-0.24)	0.20 (0.08-0.32)	<0.001	0.37

Plasma measurements						
EGC, nM	2.0	2.0	1.4	1.6	0.096	0.585
	(1.5-2.6)	(1.5-2.7)	(1.0-1.9)	(1.1-2.2)		
EC, nM	18.3	17.2	1.2	1.5	<0.001	0.667
	(15.1-22.2)	(14.0-21.2)	(0.82-1.6)	(1.0-2.0)		
EGCG, nM	43.0	41.5	4.2	4.6	<0.001	0.806
	(33.4-55.2)	(32.4-53.0)	(3.1-5.6)	(3.4-6.2)		
ECG, nM	19.4	20.3	0.96	0.94	<0.001	0.858
	(16.0-23.4)	(17.1-24.0)	(0.64-1.4)	(0.64-1.3)		

* Geometric means were derived from analysis of variance.

† Based on mixed model analyses.

‡ 12 month time points were used in the comparisons between treatments.

Table 4. Urinary Catechins in Participants Consuming Green Tea Catechins, by Demographic and Lifestyle Factors (n=90, Geometric Means (95% Confidence Interval))*

	# Subjects	Urinary Catechin (nmol/mg creatinine) †				
	(%)	M4	EGC	M6	EC	Me-EGC
Race						
White	88 (97.8)	1.90	0.46	44.3	11.0	0.70
		(1.4-2.4)	(0.38-0.54)	(35.2-55.6)	(9.8-12.3)	(0.54-0.88)
Others	2 (2.2)	1.78	1.64	105.8	7.78	2.48
		(-0.1-7.6)	(0.78-2.9)	(22.7-480.9)	(3.3-17.0)	(0.78-5.8)
P value‡		0.95	0.004	0.27	0.40	0.04
Education						
High school	6 (6.7)	1.80	0.80	45.40	12.94	1.08
		(0.46-4.3)	(0.44-1.3)	(18.6-108.8)	(8.3-19.9)	(0.42-2.1)
College	84 (93.3)	1.90	0.46	45.14	10.78	0.70
		(1.4-2.4)	(0.38-0.54)	(35.7-57.1)	(9.69-12.1)	(0.54-0.88)

P value‡		0.91	0.08	0.99	0.44	0.32
Ever smoking						
No	60 (66.7)	1.74	0.50	40.32	10.7	0.72
		(1.2-2.4)	(0.38-0.60)	(30.5-53.0)	(9.3-12.4)	(0.52-0.94)
Yes	30 (33.3)	2.24	0.44	56.60	11.3	0.74
		(1.4-3.3)	(0.30-0.60)	(38.3-83.5)	(9.2-13.8)	(0.46-1.1)
P value‡		0.36	0.63	0.17	0.68	0.90
Alcohol Intake						
No	21 (23.3)	1.58	0.54	53.68	12.28	0.76
		(0.82-2.7)	(0.36-0.76)	(33.3-86.2)	(9.7-15.6)	(0.42-1.2)
Yes	69 (76.7)	2.00	0.46	42.84	10.54	0.72
		(1.5-2.6)	(0.36-0.56)	(33.6-55.5)	(9.2-12.0)	(0.52-0.92)
P value‡		0.46	0.44	0.42	0.28	0.83

Tea drinking						
No	35 (38.9)	1.78 (1.1-2.6)	0.46 (0.32-0.62)	48.90 (33.9-70.4)	11.42 (9.5-13.7)	0.84 (0.56-1.2)
Yes	55 (61.1)	1.96 (1.4-2.7)	0.48 (0.38-0.60)	42.92 (32.0-57.4)	10.62 (9.2-12.3)	0.66 (0.46-0.88)
P value‡		0.71	0.79	0.59	0.55	0.34

*All geometric means were derived from analysis of covariance with age (years) as a covariate.

†Measurements from 12 month timepoint was used in the analyses.

‡*P* values were derived from analyses of covariance with adjustment for age.

Table 5. Plasma Catechins in Participants Consuming Green Tea Catechins, by Demographic and Lifestyle Factors (n=90, Geometric Means (95% Confidence Interval))*

	# Subjects (%)	Plasma Catechin, nM†			
		EGC	EC	EGCG	ECG
Race					
White	88 (97.8)	2.0 (1.5-2.8)	17.8 (14.0-22.6)	43.3 (32.9-56.8)	20.8 (16.9-25.5)
Others	2 (2.2)	2.6 (-0.12-13.6)	3.7 (0.02-20.9)	5.9 (0.14-40.6)	6.3 (0.98-26.1)
P value‡		0.82	0.09	0.048	0.11
Education					
High school	6 (6.7)	4.5 (1.5-11.1)	39.8 (16.2-96.2)	57.9 (19.8-166.0)	23.8 (10.7-51.7)
College or above	84 (93.3)	1.9 (1.4-2.6)	16.2 (12.7-20.7)	40.5 (30.4-53.8)	20.0 (16.2-24.7)

P value‡		0.14	0.06	0.52	0.68
Ever smoking					
No	60 (66.7)	2.0	20.6	49.7	22.2
		(1.2-2.9)	(15.4-27.4)	(35.6-69.1)	(17.3-28.4)
Yes	30 (33.3)	2.1	12.0	28.9	16.9
		(1.2-3.5)	(7.8-18.2)	(17.9-46.4)	(11.8-24.1)
P value‡		0.90	0.04	0.07	0.23
Alcohol drinking					
No	21 (23.3)	1.9	12.3	41.3	19.4
		(0.9-3.5)	(7.3-20.4)	(23.0-73.5)	(12.6-29.8)
Yes	69 (76.7)	2.1	19.1	41.6	20.5
		(1.4-2.9)	(14.5-25.0)	(30.2-57.0)	(16.2-25.9)
P value‡		0.82	0.14	0.98	0.83

Tea drinking					
No	35 (38.9)	2.34	13.3	36.2	22.4
		(1.4-3.7)	(9.0-19.5)	(23.1-56.3)	(16.1-31.0)
Yes	55 (61.1)	1.88	20.3	45.3	19.0
		(1.2-2.8)	(15.0-27.4)	(31.8-64.3)	(14.6-24.7)
P value‡		0.49	0.10	0.44	0.45

*All geometric means were derived from analysis of covariance with age (years) as a covariate.

†Measurements from 12 month timepoint was used in the analyses.

‡*P* values were derived from analyses of covariance with adjustment for age.

Table 6. Effects of *COMT* Genotype on Urinary and Plasma Catechins in Participants Consuming Green tea Catechins at month 12 (n=90, Geometric Means (95% Confidence Interval)) *

	***COMT* genotype activity**			***P* value†**	***P* value‡**
	High (n=30)	**Intermediate (n=30)**	**Low (n=30)**	**High vs Low**	**H vs I vs L**
Urinary					
M4, nmol/mg creatinine	1.9 (1.1-2.9)	1.7 (1.0-2.8)	2.0 (1.1-3.9)	0.88	0.96
EGC, nmol/mg creatinine	0.40 (0.24-0.56)	0.58 (0.40-0.78)	0.52(0.34-0.70)	0.32	0.12
M6, nmol/mg creatinine	39.2 (25.7-59.5)	58.5 (38.3-89.1)	61.3 (38.8-96.5)	0.14	0.10
EC, nmol/mg creatinine	11.0 (8.9-13.6)	12.5 (10.1-15.5)	11.0 (8.7-13.9)	1.00	0.58
Me-EGC, nmol/mg creatinine	0.66 (0.36-1.0)	0.84 (0.52-1.2)	0.78 (0.44-1.2)	0.60	0.42

Plasma					
EGC, nM	2.5 (1.4-4.1)	1.6 (0.78-2.9)	2.1 (1.0-3.7)	0.66	0.38
EC, nM	15.2 (9.8-23.5)	13.6 (8.6-21.2)	12.2 (7.4-19.7)	0.48	0.54
EGCG, nM	40.6 (24.2-67.5)	35.4 (20.9-59.5)	35.9 (20.3-63.0)	0.74	0.67
ECG, nM	18.2 (12.3-26.5)	18.2 (12.3-26.8)	22.0 (14.4-33.2)	0.49	0.70

*Geometric means were derived from analysis of covariance with adjustments for age (year), ever cigarette smoking (no, yes), alcohol drinking (no, yes), and tea drinking (no, yes).

†*P* values were derived from multivariate regression models that compared the high *COMT* activity group with low activity groups with the adjustments for all variables listed above.

‡*P* values were derived from multivariate regression models that compared the high *COMT* activity group with intermediate and low activity groups combined with the adjustments for all variables listed above.

Table 7. Spearman Correlation Coefficients between Urinary and Plasma Catechin Metabolites in Participants who Consumed Green Tea Catechins (n = 90)*

		Urinary Catechins					Plasma Catechins			
		M4	EGC	M6	EC	Me-EGC	EGC	EC	EGCG	ECG
Urinary Catechins	M4	1.00	-0.11	0.23[1]	-0.04	-0.23[1]	-0.15	-0.05	0.07	0.05
	EGC		1.00	0.37[3]	0.43[3]	0.61[3]	0.05	0.22[1]	0.05	0.07
	M6			1.00	0.45[3]	0.39[3]	-0.06	-0.03	-0.03	-0.004
	EC				1.00	0.42[3]	0.11	0.27[2]	0.08	0.15
	Me-EGC					1.00	0.03	0.19	0.08	0.12
Plasma Catechins	EGC						1.00	0.35[3]	0.16	0.10
	EC							1.00	0.74[3]	0.57[3]
	EGCG								1.00	0.80[3]
	ECG									1.00

*12 month time points were used in the analyses.

[1]2-sided P < 0.05; [2]2-sided P < 0.01; [3]2-sided P < 0.001.

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Appendix

Name of caller _____ Subject ID# _____

Phone (h) _____ (w) _____Email _____

Interviewer _____ Date _____/_____/_____

Phone Interview Questions: Green Tea Study

1. To verify your identity, what is your date of birth? _____/_____/_____
2. Is this your first time contacting the Green Tea Study? _____
3. What is your age? _____ *(50-70)*
4. Do you use tobacco products? _____ **Includes nicotine cessation products containing nicotine**
5. When was your last menstrual period? _____/_____/_____ *(must be at least 1 yr ago)*
6. What was the location and date of your last mammogram? _____/_____/_____
   _____

7. Have you ever had breast cancer or proliferative breast disease? _____
   Proliferative breast disease, also called hyperplasia, is an overgrowth of breast cells diagnosed with a needle or surgical biopsy. It is not a cancerous condition but usually requires more frequent breast exams to examine the breast tissue.

8. Have you ever had ovarian cancer? _____
9. Have you been diagnosed with any other form of cancer in the last 5 years? _____
   Basal and Squamous cell skin cancer are acceptable

10. Have you been on any of the following medications at any time during the past 6 months?
    <u>The individual qualifies for the study if NONE of these drugs have been taken in the past 6 months.</u> Y/N
    - **Hormone Replacement Therapy**

      (For menopause symptoms) _____

    - **Tamoxifen**
      (For breast cancer treatment or risk reduction) _____
    - **Raloxifene**

      (Osteoporosis treatment or risk reduction, or breast cancer risk reduction)

      _____

    - **Aromatase Inhibitors**

Such as Arimidex, Aromasin, or Femara *(For breast cancer treatment)*

_____

- **Methotrexate or Enbrel**

  (*For rheumatoid arthritis*) _____

11. During the past 6 months, have you taken any medications regularly? _____
    If so, what are they?

    _____
    _____

12. Do you have any chronic health problems? _____
    If so, what are they?

    _____
    _____

*****Per Protocol: if a subject has any of the conditions below they are not eligible to participate in the study. *****

- Diabetes? _____
- Hyperthyroidism? _____
- Uncontrolled high blood pressure (hypertension)? _____
- Crohn's or Ulcerative Colitis: ___

13. How tall are you? _____ What do you weigh? _____

    Calculate BMI _____ *(19-35)* *BMI = m/kg^2 (1 lb. = 2.2 kg; 1 in. = 0.0254 m)*

14. Has your weight changed in the past year? _____

    If so, how much? _____ *(<10 lbs past year)*

15. Do you consume alcohol? _____ If so, how much? _____
    (< 7 drinks/week; 1 drink = 5 oz. wine, 12 oz. beer, or 1.5 oz. 80-proof distilled spirits)

16. Do you consume tea? _____ If so, what kind?
_____
If you consume green tea, how often? _____ (*Less than one cup/wk*)

17. Are you able to come to the Human Nutrition Research Clinic on the St. Paul campus of the University of Minnesota, first thing in the morning, before breakfast? YES NO

18. If you are interested in the study, can you commit to monthly visits to the Human Nutrition Research Clinic over the 12 months of the study? For most visits, you will be able to come at the time of your choosing. Three visits (at months 0, 6, and 12) will require you to have fasted since 10:00 the night before. YES NO

19. What is the best time to reach you? _____

20. Would you prefer to be contacted by phone or email? _____

Congratulations! You have met the preliminary requirements to participate in our Green Tea and Breast Cancer Risk Reduction Study.

~OR~

Thank you very much for your time and interest. Unfortunately, you do not qualify for our study for the following reasons:

_____

_____

Thank you again, and have a great day.

- If caller meets requirements, describe the study to them.

Our study will look at breast cancer risk through the use of biological markers such as breast density. We will be providing women that meet our criteria with either a green tea extract or placebo (inactive capsule) every day for one year. We will be measuring several biological factors from your blood and urine that can help indicate your risk for breast cancer.

Would you like to hear more information? _____

Another variable involved in our study is the genetic difference between women. Differences in specific genes may affect how a woman responds to the green tea extract. We will be conducting a genetic test at the screening visit to confirm your eligibility for this study. We will also test you for exposure to the hepatitis B and C viruses and measure your liver function before you can participate in the study. These are routine tests that require about 2 to 4 tablespoons of blood withdrawn from your arm. The blood will be drawn by nurses at the Human Nutrition Research Clinic at the University of Minnesota in St. Paul. The results will be available in 2-4 weeks, and you will be notified if you meet the criteria for our study. At that point, you will be able to schedule appointments at the clinic for the entire year of the study at your convenience, keeping in mind that 3 appointments—at months 0, 6, and 12—will require you to have been fasting since 10 o'clock the night before, so these appointments will be in the morning (between 7:30 and 10). If you do not meet the criteria for our study, you will be informed and compensated for your time.

Would you like to hear more information? _____

I will give you a brief description of the study. If you would like to participate, we can schedule an orientation session to explain the study in more detail and answer all the questions you may have. As part of a clinical trial, you will be randomly assigned to consume either a green tea extract or placebo capsule twice a day for one year. During one year, biological markers from your blood and urine associated with breast cancer risk will be evaluated throughout fourteen clinic visits at the Human Nutrition Research Clinic (HNRC) of the University of Minnesota. Each clinic visit can take 0.5-1.5 hours. During each clinic visit, your weight, blood pressure, heart rate, and respiratory rate will be measured. Your waist and hip circumferences will also be measured at the second and last (fourteenth) clinic visits. Each clinic visit involves drawing a little more than 1 teaspoon to 4 tablespoons blood depending on the clinic visit. For 3 visits, at the month 0, month 6, and month 12, you will need to have been fasting since 10 pm the night before your clinic visit. You will collect your entire 24 hour urine at the baseline, month 6, and

12 and will bring collected urine in provided jugs to the HRNC for the clinic visits 2, 8, and 14.You will also complete a food frequency questionnaire, which is a survey about your eating habits that takes approximately 60 minutes. Your first routine mammogram will be compared with the mammogram that you are normally scheduled for during the following year, at the end of study. Finally, you will be asked to avoid drinking green tea during the study period so we will be able to assess the exact effect of consumed green tea extract.

- Would you like to come to an orientation to learn more about participating in our study?

Circle one of each of the following:

Status:	**Preferred contact:**	**Orientation session:**
ELIGIBLE **_____/_____/_____**	**EMAIL**	
INELIGIBLE **_____**	**PHONE**	

Telephone Screening Questionnaire Eligibility Information

<u>Question 7:</u> Restricted Medications
Eligible if subject uses local cream, ring, or suppository no more than 3 days per week, & plans to continue therapy throughout the year of the study.
(<u>NO oral estrogens</u>).

ELIGIBLE:
Cream: Estrace
Ogen
Ortho Dienestrol
Premarin
(Vaginal ONLY. No oral).
Tablet: Vagifem
Ring: Estring
Femring
IUD: Mirena

INELIGIBLE:
Patch: Alora
Climara
Esclim
Estraderm
Vivelle
Vivelle-Dot
Gel: Estrogel
Cream: Estrasorb

<u>Question 8:</u> Regular medications
INELIGIBLE:
- Warfarin (Coumadin)
- Methotrexate
- Enbrel
- Anti-coagulation meds
- More than 10 different medications regularly.

Less common medications; for reference:

INELIGIBLE: Oral estrogen/estrogen-progestin pills

Activella
Cenestin
Estinyl
Estrace (pill form)
Estratab
Femhrt
Menest
Ogen (pill form)
Ortho-Est
Ortho-Prefest
Premarin (pill form)
Premphase
Prempro

ELIGIBLE: Progestin-only pills

Aygestin
Cycrin
Norlutate
Prometrium
Provera

<u>Question 13:</u> Tea Consumption
ELIGIBLE:
- Subject drinks more than one cup of green

tea per week, but is willing to avoid drinking green tea while participating in the study (<1 cup per week).

- Subject may still consume Black, Oolong, white, and herbal teas during study.

CONSENT FORM

Study Title: Green tea and reduction of breast cancer risk

You are invited to be in a research study of how green tea extract consumption affects levels of biological factors (biomarkers) that may influence breast cancer risk. We ask that you read this form and ask any questions you may have before agreeing to be in the study. This study is being conducted by Mindy Kurzer, Ph.D., Jian-Min Yuan, M.D, Tim Emory, M.D., Carolyn Torkelson,M.D. of the University of Minnesota and Karen Swenson, Ph.D of Park Nicollet.

The purpose of this study is to determine the effect of green tea consumption on breast cancer biomarkers, such as mammographic density and sex hormone levels, to further understand how green tea might reduce the risk for breast cancer. Although there is research that indicates that green tea reduces risk for breast cancer, not much is known about how green tea reduces risk. We think that green tea might change the way women metabolize estrogen, a sex hormone. We also think that green tea may reduce oxidative stress. Both of these physical changes have been shown to reduce breast cancer risk. If we can show that green tea changes these factors for the better, it will help us to better understand how green tea reduces risk for breast cancer. In addition, we are going to evaluate specific genetic variations to find out whether these genetic variations influence your physiological responses to the protective effects of green tea on biomarkers of breast cancer risk.

The genetic testing done in this study will measure genetic markers that are not related to breast cancer risk or risk of any other disease. We will simply be examining genetic variations that may influence your physiological response to green tea consumption. Catechol-O-methyltransferase (COMT) is the main enzyme responsible for breakdown and excretion of the active compounds in green tea that we think are responsible for the cancer-preventive effects. Previous studies have shown that people with the low-activity COMT (which is more common) gene benefit more from possible anti-carcinogenic properties of green tea than people with the high-activity COMT gene (which is less common). We will also test two other genes that help break down these green tea compounds: SULT and UGT genes. You will not receive any results or counseling regarding the genetic testing. No genetic markers related to disease risk will be evaluated.

Procedures:

We anticipate that we will screen up to 8,000 women to find the required 800 participants and place them into either the treatment or control group according to a process that will not be under your control or the study investigators'. First, we will perform blood tests to confirm that you meet the study criteria and to evaluate your genetic variations in the COMT gene. The results of these tests will determine whether or not you can continue with the study. Once we have determined that you are eligible to continue, the process used to place participants into groups will be random (like the flip of a coin). Half the participants will be placed in the treatment group and will consume two green tea extract capsules twice per day (two in the morning and two in the afternoon) for one year. The other half will be placed in the control group and will consume two placebo capsules twice per day for one year. Capsule assignments will be made by the University of Minnesota Medical Center/Fairview Investigational Drug Services (IDS) Pharmacy. Green tea extract and placebo capsules will be identical and will be administered to the subjects by a research staff member or nurse at the HNRC blinded to the contents in the capsules. Once you are placed into the treatment or control group it will not be possible to change groups. Neither you nor the investigators will know which group you are in**.**

Please note that even though you may initially qualify for participation, you may not be invited to participate in the study after the first blood tests are performed.

If you agree to be in this study, we would ask you to do the following things:

1. Go to the Human Nutrition Research Clinic (HNRC) at the Food Science and Nutrition Department of the University of Minnesota in Saint Paul, MN 10 times during a 12-month time period. All ten clinic visits will involve a blood draw. At five clinic visits, urine samples will also be collected. Clinic visits 3, 4, 6 and 7 have the option of being completed at Fairview Crosstown, Fairview Jonathan, Fairview Oxboro, Fairview Maple Grove and Fairview Farmington. The visits are described in detail below.
2. At the beginning of the study and at the 6^{th} and 12^{th} month, collect all urine for 24 hours in jugs that will be provided.
3. Go to the University of Minnesota Medical Center (UMMC)/Fairview Breast Center, Fairview Southdale Breast Center, or Fairview Maple Grove Breast Center for your routine annual mammogram at the end of the study.
4. Allow a portion of the blood drawn at the first clinical blood draw (about 1 tablespoon) to be used for DNA analysis. DNA will be isolated from your blood sample and stored. We will then analyze the gene variations, which will allow us to determine if these gene variations influence your response to green tea extract consumption.
5. Keep your body weight stable during the study, and do not participate in any weight loss or weight gain studies or programs.
6. Consume four capsules per day for one year, containing either green tea extract or placebo, as decided by the researchers on a full stomach only, two in the morning and two in the afternoon.
7. Refrain from drinking more than one cup of green tea per week while participating in the study.

8. Refrain from drinking more than 7 alcoholic beverages per week while participating in the study.

Here is the list of measurements to be made in this study

- Body weight
- Height
- Waist and hip circumferences
- Blood pressure, heart rate, respiratory rate and body temperature
- Completing a Food Frequency Questionniare
- Completing a Health History Questionnaire
- Completing a Menopause-Specific Quality of Life questionnaire
- Blood collections (a little more than 1- 4 tablespoon(s) depending on the clinic visit, 14 times) for evaluation of plasma F2-isoprostanes (marker of oxidative stress), insulin like growth factor –1 (IGF-1) and its binding proteins (these are biomarkers for breast cancer), reproductive hormones, liver enzymes, vitamin D, glucose, insulin, HbA1c (a blood test for determining your blood glucose over prolonged periods of time), C-peptide (a factor useful in assessing insulin function and secretion), HDL-Cholesterol, LDL-C, Total-C, TG (lipid factors), oxidized LDL-C (a risk factor for heart disease), hsCRP, IL-1β, IL-6, IL-8, TNF-α (proteins in blood involved in immune system regulation), prolactin (a hormone that affects growth of the mammary glands), adiponectin (a protein that regulates glucose and lipids metabolism), osteocalcin, pyridinolines, osteoprotegerin, CTX and NTX (biomarkers for bone metabolism), ghrelin and leptin (hormones involved in appetite and weight regulation), catechins (green tea bioactive compounds), HBsAg, anti-HBc, anti-HCV (markers for hepatitis B and C), assessing DNA repair capacity and specific changes in the following genes that are related to metabolism of the green tea bioactive compounds: COMT, GSTM1, GSTT1, UGT, SULT, IGF-1, IGFBP-3, PIK3CB and HSD3B1
- Urine collection (two spot urines at the clinic in 10% of the subjects and three 24-hour complete collections for all subjects) for measurement of creatinine (a muscle metabolite), estrogens and catechins
- Mammogram to evaluate the changes in your breast density from baseline visit to the end of the study. Breast density changes measured by the mammograms will be calculated by aid of a computer program.

Detailed Description of Clinic Visits:

Clinic visit 1
This clinic visit will take place at the Human Nutrition Research Clinic (HNRC), Food Science and Nutrition, University of Minnesota Saint Paul, MN. Measurements taken at this visit (hepatitis B and C virus infection, liver function and COMT gene variations) will be used to make the final assessment of eligibility. A trained medical professional will weigh you, measure your height, take your blood pressure while you are resting and then draw 45mL (about 3 tablespoons) of blood. You will be sent home with a urine collection container and instructions should you meet all inclusion criteria and return for Visit 2 to be randomized into the study. If you are found to be ineligible due to not fulfilling the criteria for inclusion in the study after this visit, you will be notified within one month, released from the study and thanked for your time. This visit will take approximately 30 minutes.

Clinic visit 2
This clinic visit takes place at the HNRC after checking your eligibility at the first clinic visit. You should not have had anything to eat or drink other than water for 10 hours prior to your clinic visit. A trained medical professional will weigh you, measure your height, take your blood pressure while you are resting and will draw 65mL blood (about 4 tablespoons). After that, your waist and hip circumferences will be measured using a tape measure. You will also complete a health survey, a quality of life questionnaire and a food frequency questionnaire as part of this visit. At the end of this visit, you will be given your first 3 month supply of capsules and a study log for recording pills which you have taken. You will also bring the first 24-hour urine collection to the HNRC at this visit. This urine was collected the day before and kept refrigerated until delivery to the HNRC. The visit will take approximately 30 minutes.

Clinic visit 3
This visit will take place at the HNRC or a Fairview clinic mentioned in part 1, page 2, approximately one month after your clinic visit 2. A trained medical professional will draw 5mL (about one teaspoon) of blood. This visit will take approximately 30 minutes.

Clinic visit 4
This visit will take place at the HNRC or a Fairview clinic mentioned in part 1, page 2, approximately one month after your clinic visit 3. A trained medical professional will draw 5mL (about one teaspoon) of blood. This visit will take approximately 30 minutes.

Clinic visit 5
This clinic visit takes place approximately one month after clinic visit 4. A study staff member will weigh you and measure your blood pressure, body temperature and heart rate while you are resting. The trained medical professional will draw 5mL of blood

(about one teaspoon), and you will provide a urine sample. You will be asked to bring your empty or partially empty bottles of your capsules and study log to this visit. At the end of this visit, you will be given your next 3-month supply of capsules and materials to complete your 24 hour urine collection for clinic visit 8 in several months. This visit will take approximately 30 minutes.

Clinic visit 6
This visit will take place at the HNRC or a Fairview clinic mentioned in part 1, page 2, approximately one month after your clinic visit 5. A trained medical professional will draw 5mL (about one teaspoon) of blood. This visit will take approximately 30 minutes.

Clinic visit 7
This visit will take place at the HNRC or a Fairview clinic mentioned in part 1, page 2, within one month after clinic visit 6. A trained medical professional will draw 5mL (about one teaspoon) of blood. This visit will take approximately 30 minutes.

Clinic visit 8

This visit takes place at the HNRC approximately one month after your clinic visit 7. You will be asked to not eat or drink anything but water for 10 hours prior to your clinic visit and to bring your empty or partially empty bottle of capsules and study log. A study staff member will weigh you and measure your blood pressure, body temperature and heart rate while you are resting. A trained medical professional will draw 65mL blood (about 4 tablespoons). You will also bring your 24-hour urine collection from the previous day. This urine should have been collected the day before and kept refrigerated until delivery to the HNRC. You will be asked to bring your empty or partially empty bottles of your capsules and pill diary. As part of this visit, you will complete a quality of life questionnaire, and you will be given your next 3 month supply of capsules. This visit will take approximately 30 minutes.

Clinic visit 9

This visit takes place at the HNRC approximately three months after your clinic visit 8. This visit repeats the tests and measurements taken in clinic visit 5. A study staff member will weigh you, measure your blood pressure, body temperature and heart rate while you are resting. A trained medical professional will draw 5mL of blood (about one teaspoon). At this visit, you will also provide a urine sample. You will be asked to bring your empty or partially empty bottles of your capsules and study log. At the end of this visit, you will be given your last 3 month supply of capsules and materials to complete your 24 hour urine collection for clinic visit 10 in several months. This visit will take approximately 30 minutes.

Clinic visit 10

This is your last clinic visit. This visit will be scheduled at the HNRC approximately three months after your clinic visit 9, during month 12 of your participation. You will be asked to not eat or drink anything but water for 10 hours prior to this visit and to bring your empty or partially empty bottle of capsules, study log and 24-hour urine collection. This urine should have been collected the day before and kept refrigerated until delivery to the HNRC. A study staff member will weigh you, measure your blood pressure, body temperature and heart rate while you are resting. Your waist and hip circumferences will be measured as well. A trained medical professional will draw 65mL of blood (about 4 tablespoons). You will also complete a quality of life questionnaire and a food frequency questionnaire as part of this visit. This visit will take approximately 30 minutes.

Specific procedures to be performed:

Food Frequency Questionnaire

At clinic visits 2 and 10 you will complete a questionnaire about your eating habits over the past year. This survey is given in a web-based format and should take about 60 minutes.

Menopause-Specific Quality of Life Questionnaire

At clinic visits 2, 8, and 10 you will answer questions regarding your experience of certain physical, psychosocial, and sexual symptoms over the previous week. These questions are designed to assess your quality of life in association with your menopausal experience. The required time to complete this questionnaire will be less than 15 minutes.

Collection of 24-hour urine samples

The day before clinic visits 2, 8 and 10, you will collect all urine for a 24-hour period in jugs that we provide to you. You will keep them refrigerated and bring them to the clinic at the time of your visit.

Mammogram
As part of your routine medical checkup, you will undergo one mammogram within one week of finishing the study. Also, you might need to wait for your next annual mammogram until the Green Tea Study is over, which could be up to 15-16 months from your initial mammogram.

Risks of Being in the Study:

Participating in this study has the following risks:

First, liver toxicity has been seen in a few subjects who used green tea extract as a weight reduction aid. The risk of toxicity from taking manufactured green tea extracts has been estimated to be about 1 case out of 83,812 treatments, although no toxicity has been reported in any clinical trials performed to date. To be cautious, we will measure liver

enzymes 9 times throughout our study for possible toxicity and tolerance at each visit at the HNRC or a Fairview clinic. If your liver enzymes are elevated, you will be informed and released from the study.

Second, as with any dietary supplement or pharmaceutical, there is a slight risk of stomach upset, nausea, vomiting, and diarrhea. To prevent any of these digestive problems, we advise that you take the study supplement on a full stomach, after breakfast and after dinner. There is also a slight risk of headache from consuming the study supplement. If discomfort persists, you may contact the study coordinators.

You may experience discomfort from hunger and feel inconvenienced by having stop eating 10 hours before the blood draws at visits 2, 8 and 10 of the study.

Also, there is a small risk of infection and bruising at the needle puncture site when blood is taken. The risk is minimal as all needles and equipment are sterilized and the procedures are performed by trained phlebotomists: registered nurses and certified medical assistants at the HNRC or a Fairview clinic. You may also feel some pain, dizziness, or feel faint lasting a few seconds upon insertion of the needle used to draw the blood.

Lastly, screening mammography is the best way to detect early breast cancers. You are currently getting your mammograms approximately every 12 months. If you participate in this study, your mammogram may be delayed by at most 3-4 months. Some experts (U.S. Preventive Services Task Force, 2009) have suggested that this type of delay has little effect on the benefits of mammography. If you wish to have your regular mammogram on a yearly basis, we will ask you to have a second limited view research mammogram after you have been on the study for 1 year. The additional limited view mammogram would be at no cost to you.

Benefits of Being in the Study:

There may be no direct reduction of breast cancer risk as a result of participation in this study. Additionally, upon your request we can send the first liver function test results conducted at your screening visit to you or your primary care physician.

Costs:

No charges will be made for the Human Nutrition Research Clinic (HNRC) any Fairview clinic visits while you are a participant in this study.

Compensation

You will also receive financial compensation of up to $450 for study participation: $20 for completing the first clinic visit, $70 for the next four clinic visits (clinic visits 2, 3, 4 and 5), $100 for the next three clinic visits (6, 7, 8), $60 for clinic visit 9, and $100 for completing the last clinic visit (clinic visit 10). Finally, upon completion of all clinical

research endpoints (visits, questionnaires and mammogram), you will receive another $100 at the end of the study.

Participants found ineligible after completing the first clinic visit will receive $20.00. Participants who become ineligible during the study will receive pro-rated compensation.

Care in the case of injury

In the event that this research results in an injury, treatment will be available, including first aid, emergency treatment, and follow-up care as needed. Care for such injuries will be billed in the appropriate manner, to you or your insurance company. If you think that you have suffered a research-related injury, let the principal investigator or a study coordinator know right away Dr. Mindy Kurzer: (612-624-9789) or study coordinators: (612-624-3412).

Your participation in the study may be terminated by the investigator without regard to your consent in the following circumstances:

1. Failure to come to clinic visits after one reschedule
2. Circumstances change so that you are no longer eligible

Confidentiality:

The information provided by you and the information taken from the measurements of your body will be held strictly confidential and used for the purposes of research only. The HNRC, whose staff has completed the federally required training with regard to confidentiality of health information in research, will maintain medical records with your name on them for the purposes of scheduling and billing procedures only. Any/all medical information gathered, test results, lab samples will NOT have your name on them. Instead, they will be labeled with a study ID number only.
Laboratory results and other test results will not be included in the medical record. Your name will be associated with your study ID number on one list, to be kept in a locked file cabinet. Your study ID number will appear on all other study records. Representatives of the University of Minnesota or the National Institutes of Health may be given access to your records to assure that the study is conducted properly.

All your study records will be kept private, in locked storage according to HIPAA standards. None of your information will ever be given to anyone, and your name will never be associated with your records on paper or on computer. In any sort of report we might publish, we will not include any information that will make it possible to identify you as a subject of this study.

With regard to your blood and urine samples:

- We will send samples of your blood with only a code number on it to the University of Southern California (USC) to analyze it for biomarkers of breast cancer called IGF-1, binding proteins for IGF-1, as well as reproductive hormones. USC will be paid to do these tests .We will NOT tell USC researchers your name or give them any identifying information about you. Any excess blood will be destroyed when researchers have completed these tests.
- We will send samples of your blood and urine with a code number on it to Rutgers University to analyze it for plasma and urine catechin levels. Rutgers University will be paid to do these tests. We will NOT tell Rutgers University researchers your name or give them any identifying information about you. Any excess blood and urine will be destroyed when researchers have completed with these tests.
- We will store any remaining blood and urine in a freezer in the Food Science and Nutrition Building at the University of Minnesota (St. Paul campus). The vials will have your study ID on them and the date on which the blood was drawn. Your name will NOT be stored with your blood. We will store these vials for up to 5 years after the entire study is over. The freezer in which they are stored is kept behind a locked door. The only people who have access to this freezer are paid research staff members who have completed the federally required training with regard to confidentiality of health information in research. The purpose for storing these samples is to enable us to conduct additional tests regarding green tea health effects. The principal investigator will maintain ownership of these samples while they are stored. Samples will be destroyed within five years after the completion of the study. You will not receive any results from future tests conducted with these stored samples.
- USC and Rutgers University labs do NOT have access to your name. There is one confidential list and file that links your ID to your name. These files will be kept in the locked file cabinet as described above. The only people who will have access to this list are the principal investigator (Dr. Kurzer) and her research staff, who have completed the federally required training with regard to confidentiality of health information in research.

Protected Health Information (PHI)

Your PHI created or received for the purposes of this study is protected under the federal regulation known as the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Refer to the accompanying HIPAA authorization for details concerning the use of this information.

A new federal law (2009), called the Genetic Information Nondiscrimination Act (GINA) generally makes it illegal for health insurance companies, group health plans, and employers of 15 or more persons to discriminate against you based on your genetic information. Health insurance companies and group health plans may not request your genetic information that we get from this research. This means that they may not use your genetic information when making decisions regarding insurability. Be aware that this

new federal law will not protect you against genetic discrimination by companies that sell life insurance, disability insurance, or long-term care insurance.

Voluntary Nature of the Study:

Your decision whether or not to participate will not affect your current or future relations with the University. If you decide to participate, you may withdraw at any time without affecting those relationships. In addition, you may request your blood and urine samples to be destroyed following your withdrawal from the study. The procedure to withdraw is to call Mindy Kurzer, Ph.D. at (612) 624-9789 or the study coordinators at (612) 624-3412 and inform them that you wish to withdraw.

New Information:

If during the course of this research study, there are significant new findings discovered that might influence your willingness to continue, the researchers will inform you of those developments.

Contacts and Questions:

The primary researcher conducting this study is Mindy Kurzer, Ph.D. If you have questions, you may contact her at the Department of Food Science and Nutrition, University of Minnesota, 1334 Eckles Ave., St. Paul, MN 55108. Phone: (612) 624-9789; email: mkurzer@umn.edu. You may also contact the study coordinators in the Department of Food Science and Nutrition, University of Minnesota, 1334 Eckles Ave., St. Paul, MN 55108. Phone: (612) 624-3412; email: greentea@umn.edu. If you have any questions or concerns regarding the study and would like to talk to someone other than the researcher(s), you are encouraged to contact the Fairview Research Helpline at telephone number (612)672-7692 or toll-free at (866) 508-6961. You may also contact this office in writing or in person at University of Minnesota Medical Center/Fairview Riverside Campus, 2200 Riverside Avenue, Minneapolis, MN 55454.

Statement of Consent:

I have read the above information. I have asked questions and have received answers. I consent to participate in the study.

_____

Signature of Participant Date

_____

Name of Participant (printed)

_____

Street Address City State Zip code

_____

Signature of Person Obtaining Consent Date

You will be given a copy of this form for your records.

Participant handbook

For

The green tea study

PLEASE NOTE:

If at any time you have questions or concerns regarding the study, please contact any of the GREEN TEA STUDY staff immediately.

Contact information is included in this handbook and on the website (www.greenteastudy.umn.edu).

GREEN TEA STUDY staff members are here to assist you throughout the study process. Please do not hesitate to call for any reason (612) 624-3412 ext. 1

Table of Contents

Clinic Visit 1 (screening visit):

- After receiving your consent at the orientation session, your first clinic visit will be scheduled.
- Measurements taken during this visit will be used to make the final judgment of your eligibility.
- This clinic visit must take place at the *Human Nutrition Research Clinic* (HNRC) located at the University of Minnesota in Saint Paul campus.

What to expect at your Screening Visit:

Our trained Staff will collect several measurements which include:

- Weight (in light clothing, no shoes),
- Height
- Blood pressure
- Body temperature
- Pulse
- Respiratory rate
- Draw 2 tubes of blood

- You will be notified ***within one month*** if you do or do not qualify for the study.

At this visit you will be given the following materials for your assumed Clinic Visit 2:

- 3-liter urine jug
- 1-liter travel jug
- 1-cooler bag
- 2-collection "hats"
- 1-small bag of foil-wrapped vitamin C
- 2-Ice-packs for the 24-hr urine collection.
- 1-Health History Questionnaire

You will need to return the completed Health History Questionnaire and urine jug(s) containing your 24-h urine at the clinic visit 2 if you are found eligible for the study.

Clinic Visit 2: Will be scheduled after confirming your eligibility after Clinic Visit 1.

Clinic Visit 2 (baseline visit):

This clinic visit must be conducted at the HNRC on the St. Paul Campus.

- Clinic Visit 2 should be scheduled on weekdays between 6:30 am-10:00 am.
- Do not eat anything or drink anything but water 10 hours before this clinic visit.
- You must be FASTING for this clinic visit.

What to do to prepare for this visit:

· DO NOT eat or drink anything (except water) for 10 hours prior to the clinic visit.
· DO NOT take any medications by mouth during this time.
· DO NOT drink coffee or tea the morning of your visit.

· Please bring all months of your study log.
· Get a good nights' sleep and drink plenty of water.
· Be prepared to give about 3 tablespoons of blood.
· Bring your refrigerated 24-hour urine collection from the previous day.

Measurements at this clinic visit include:

- Weight (in light clothing, no shoes)
- Blood pressure
- Body temperature
- Pulse
- Respiratory rate
- Collection of your 24-hour urine specimen

- Prior to coming to your second visit you will complete the Health History Questionnaire that was given to you at your screening visit.

- In addition to your Health History Questionnaire you will need to complete the: Dietary History Questionnaire (DHQ) and the Menopause-Specific Quality of Life questionnaire (MENQOL). These may be completed at your home or HNRC. It is important to note that completing these questionnaires at the HNRC will extend you appointment time up to 2 hours.
- You will receive your first 3 month supply of the capsules and study log. Start taking supplements the day of this clinic visit.
- This appointment should take about 30 minutes.

At the end of this clinic visit the study staff will schedule you for your next appointment to the St. Paul Campus/ HNRC.

A study staff member will contact you several days prior to your next visit as a reminder.

Clinic Visit 3:

This visit will take place approximately one month after your last or most recent clinic visit.

This visit may be completed at a partnering Fairview clinic or the HNRC on the St. Paul Campus. Please note that at your second visit you had made the decision of which location you would prefer to go to for this visit.

What to do to prepare for this visit:

- Get a good nights' sleep and drink plenty of fluids.
- Be prepared to give about 1 teaspoon of blood.
- You DO NOT need to fast.

Measurements at this clinic visit include:

- Blood drawing for assessment of your liver function (1 tube)

If you are choosing to use the Fairview Clinics for part of your study visits, please schedule your next month's clinic visit and notify Green Tea Study Staff of this time.

A study staff member will contact you several days prior to your next visit as a reminder.

Clinic Visit 4:

This visit will take place approximately one month after your last or most recent clinic visit.

This visit may be completed at a partnering Fairview clinic or the HNRC on the St. Paul Campus. Please note that at your second visit you had made the decision of which location you would prefer to go to for this visit.

What to do to prepare for this visit:

- Get a good nights' sleep and drink plenty of fluids.
- Be prepared to give about 1 teaspoon of blood.
- You DO NOT need to fast.

Measurements at this clinic visit include:

- Blood drawing for assessment of your liver function (1 tube)

Your next clinic visit is *required* to be completed at the St. Paul Campus. Several days before your next visit you will be contacted by study staff to remind you of this appointment.

Clinic Visit 5:

This visit will take place approximately one month after your last or most recent clinic visit.

This clinic visit must be conducted at the HNRC on the St. Paul Campus.

What to do to prepare for this visit:

- Please bring all months of your study log.
- The bottles which you have been taken your pills from and any remaining capsules.
- You will need to provide a spot urine sample.
- Get a good nights' sleep and drink plenty of fluids.
- Be prepared to give about 1 teaspoon of blood.
- You DO NOT need to fast.

Measurements at this clinic visit include:

- Weight
- Blood pressure
- Body temperature
- Pulse
- Respiratory rate

- Blood drawing for assessment of your liver function (1 tube)

Study Supplement and Supplies:

- At this visit you will receive 3 new bottles of our study supplement. Please return any pills that you may still have along with the bottles they came in.
- You will receive 24-hour urinary collection materials for your 8th clinic visit.
    - This includes: one 3-liter urine jug, one small bag of foil-wrapped vitamin C. Of other supplies are needed please let study staff know and they will provide it to you.

At the end of this clinic visit the study staff will schedule you for your next appointment that is required to take place at the St. Paul Campus/ HNRC.

You will be responsible for scheduling your next month appointment if not coming to St. Paul Campus/ HNRC.

A study staff member will contact you several days prior to your next visit as a reminder.

Clinic Visit 6:

This visit will take place approximately one month after your last or most recent clinic visit.

This visit may be completed at a partnering Fairview clinic or the HNRC on the St. Paul Campus. Please note that at your second visit you had made the decision of which location you would prefer to go to for this visit.

What to do to prepare for this visit:

- Get a good nights' sleep and drink plenty of fluids.
- Be prepared to give about 1 teaspoon of blood.
- You DO NOT need to fast.

Measurements at this clinic visit include:

- Blood drawing for assessment of your liver function (1 tube)

If you are choosing to use the Fairview Clinics for part of your study visits, please schedule your next month's clinic visit and notify Green Tea Study Staff of this time.

A study staff member will contact you several days prior to your next visit as a reminder.

Clinic Visit 7:

This visit will take place approximately one month after your last or most recent clinic visit.

This visit may be completed at a partnering Fairview clinic or the HNRC on the St. Paul Campus. Please note that at your second visit you had made the decision of which location you would prefer to go to for this visit.

What to do to prepare for this visit:

- Get a good nights' sleep and drink plenty of fluids.
- Be prepared to give about 1 teaspoon of blood.
- You DO NOT need to fast.

Measurements at this clinic visit include:

- Blood drawing for assessment of your liver function (1 tube)

Your next clinic visit is *required* to be completed at the St. Paul Campus.

Several days before your next visit you will be contacted by study staff to remind you of this appointment.

Clinic Visit 8:

This clinic visit must be conducted at the HNRC on the St. Paul Campus.

- Clinic Visit 8 should be scheduled on weekdays between 6:30 am-10:00 am.
- Do not eat anything or drink anything but water 10 hours before this clinic visit.
- You must be FASTING for this clinic visit.

What to do to prepare for this visit:

· DO NOT eat or drink anything (except water) for 10 hours prior to the clinic visit.
· DO NOT take any medications by mouth during this time.
· DO NOT drink coffee or tea the morning of your visit.

· Please bring all months of your study log.
· Get a good nights' sleep and drink plenty of water.
· Be prepared to give about 3 tablespoons of blood.
· Bring your refrigerated 24-hour urine collection from the previous day.

Measurements at this clinic visit include:

- Weight (in light clothing, no shoes)

- Blood pressure
- Body temperature
- Pulse
- Respiratory rate
- Collection of your 24-hour urine specimen

Study Supplement and Questionnaires:

- At this visit you will receive 3 new bottles of our study supplement. Please return any pills that you may still have along with the bottles they came in.
- You will need to complete an online Menopause-Specific Quality of Life questionnaire (MENQOL) prior or the day of your clinic visit. This questionnaire may be completed at home or at the HNRC.

At the end of this clinic visit the study staff will schedule you for your next appointment to the St. Paul Campus/ HNRC.
A study staff member will contact you several days prior to your next visit as a reminder.

Clinic Visit 9:

This visit will take place approximately three months after your last or most recent clinic visit.

This clinic visit must be conducted at the HNRC on the St. Paul Campus.

What to do to prepare for this visit:

- Please bring all months of your study log.
- The bottles which you have been taken your pills from and any remaining capsules.
- You will need to provide a spot urine sample.
- Get a good nights' sleep and drink plenty of fluids.
- Be prepared to give about 1 teaspoon of blood.
- You DO NOT need to fast.

Measurements at this clinic visit include:

- Weight
- Blood pressure
- Body temperature
- Pulse
- Respiratory rate
- Blood drawing for assessment of your liver function (1 tube)

Study Supplement and Supplies:

- At this visit you will receive 3 new bottles of our study supplement. Please return any pills that you may still have along with the bottles they came in.
- You will receive 24-hour urinary collection materials for your 8th clinic visit.
    - This includes: one 3-liter urine jug, one small bag of foil-wrapped vitamin C. Of other supplies are needed please let study staff know and they will provide it to you.

At the end of this clinic visit the study staff will schedule you for your next appointment to the St. Paul Campus/ HNRC.

A study staff member will contact you several days prior to your next visit as a reminder.

Clinic Visit 10 (Final visit):

- Clinic Visit 10 should be scheduled on weekdays between 6:30 am-10:00 am.
- Do not eat anything or drink anything but water 10 hours before this clinic visit
- You must be FASTING for this clinic visit.

WHAT TO DO TO PREPARE FOR THIS VISIT:

· DO NOT eat or drink anything (except water) for 10 hours prior to the clinic visit.
· DO NOT take any medications by mouth during this time.
· DO NOT drink coffee or tea the morning of your visit.
· Get a good nights' sleep and drink plenty of water.
· Be prepared to give about 3 tablespoons of blood.
· Bring your 24-hour urine collection from the previous day.

· Complete the following questionnaires: Menopause-Specific Quality of Life Questionnaire (MENQOL) and Dietary History Questionnaire (DHQ)

What to expect at your Final Visit:

Our trained Staff will collect several measurements which include:

- Weight (in light clothing, no shoes),
- Height
- Blood pressure
- Body temperature
- Pulse
- Respiratory rate
- Waist and hip circumferences

- You will need to complete your final: Dietary History Questionnaire (DHQ) and the Menopause-Specific Quality of Life questionnaire (MENQOL). These may be completed at your home or HNRC. It is important to note that completing these questionnaires at the HNRC will extend you appointment time up to 2 hours.

- This appointment should take about 30 minutes.

Summary of Clinical Measurements

Clinic Visit #:	1	2	3	4	5	6	7	8	9	10
Measurements										
Weight/vital signs	**X**	**X**			**X**			**X**	**X**	**X**
Height	**X**									**X**
Measure waist and hip circumferences		**X**								**X**
Sample Collection										
Blood Draw	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
24 Hour Urine Collection		**X**						**X**		**X**
Collect Spot Urine					**X**				**X**	
Supplement & Questionnaires										
Distribute Supplement		**X**			**X**			**X**	**X**	
Collect Bottles & Remaining Supplement					**X**			**X**	**X**	**X**
Health History Questionnaire (HHQ)	**X**									
Dietary History Questionnaire (DHQ)		**X**								**X**
Menopause-Specific Quality of Life Questionnaire (MENQOL)		**X**						**X**		**X**

Green Tea Study Staff

Principle Investigator:

Mindy S. Kurzer, Ph.D.
Dept. of Food Science and Nutrition- University of Minnesota
Telephone: (612) 624-9789
Fax: (612) 625-5272
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Study Coordinators:

Jane Mobeck Wilson, RN
Research nurse

Hamed Samavat
Ph.D. Nutrition Student

Allison Dostal
Ph.D. Nutrition & Epidemiology Student

Alyssa Perry
M.S. Nutrition Student

Sarah Bedell
Regulatory Support and Coordination

April Rose
Junior Scientist and Laboratory Coordinator

Study Contact Information

Address: 1334 Eckels Avenue
Saint Paul, MN 55108

Telephone: 612-624-3412

Fax: 612-626-3037

E-mail: greentea@umn.edu

Scheduling Questions and Study Concerns:

PLEASE NOTE: If at any time you have questions or problems arise, please contact any member of the Green Tea Study staff immediately.

The Green Tea Study staff members are here to assist you through the study process; do not hesitate to call for any reason.

The website may also provide you with study information and details:
www.greenteastudy.umn.edu.

If leaving a message via telephone or email please provide:

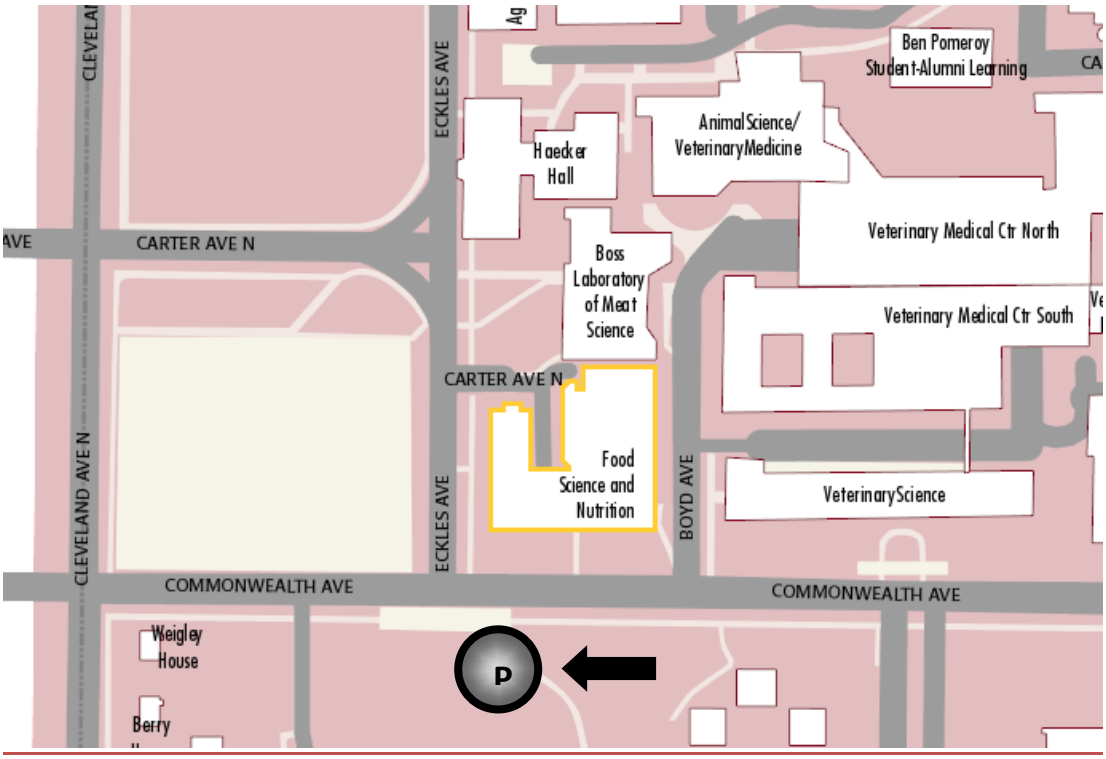
- Your name and
- Contact information
- Brief description about the reason for your contacting the study.
- If you have a preferred time for us to contact you.

If we do not hear from you:

PLEASE NOTE: If a participant does not set up a clinic appointment when it is expected to occur, a Green Tea Study staff member will make three (3) attempts to contact the participant, at various times, via phone and email, over 7 business days, inquiring of her interest in continuing participation and advising of the possibility of termination of her study participation. The third message will state that the participant has 72 hours to contact Green Tea Study staff to reschedule or to communicate that the participant has chosen to end her participation.

HNRC Location, Transportation, and Parking

Human Nutrition Research Clinic (HNRC)



Location

This building is located on the St. Paul University of Minnesota campus.

The HNRC is located on the 1st floor of the Food Science and Nutrition Building, Room 116, 1334 Eckles Ave.

FREE PARKING AVAILABLE TO STUDY PARTICIPANTS

There are two meters on Commonwealth Ave. between Cleveland Ave. N and Eckles Ave. that have been bagged and are reserved for study participants.

Public Transportation

Several city bus routes service the St. Paul campus, including 3, 87, and 272. Additionally, the U of MN has campus shuttles—routes 121 and 124—that run frequently between campuses. Information on specific routes, times, and fares may be obtained by calling the Metro transit office at (612)373-3333 or going to their website at www.metrotransit.org.

Directions to HNRC from I-35W

These directions will take you to the intersection of Eckles Ave and Commonwealth Ave.

Exit S Cleveland Ave and go south on Cleveland Ave N for about 1.8 miles. Make a left (east) on Commonwealth Ave. Go one block to Eckles Ave. The HNRC will be ahead and to the left; the Dept. of Food Science and Nutrition will be directly to your left.

Directions to HNRC from I-94

These directions will take you to the intersection of Eckles Ave and Commonwealth Ave.

Take exit 236 for MN-180 toward University Ave. Once on MN-180, take the Energy Park Drive exit and go right (east) on Energy Park Drive. Take your first left, Raymond Ave, and continue onto Cleveland Ave N. Make a right (west) onto Commonwealth Ave. Go one block to Eckles Ave. The HNRC will be ahead and to the left; the Dept. of Food Science and Nutrition will be directly to your left.

Parking near HNRC

Besides the free parking discussed above, you have other options for parking if you choose to drive to the HNRC.

(1) Metered parking is available on Eckles Ave.

(2) There is a lot directly across from the HNRC for $4.00 (daily).

Fairview Clinics

- As a Green Tea Study participant, you have the option to complete eight clinic visits at one of our partnering Fairview Clinics.

  You may utilize Fairview Service only for clinic visit # 3, 4, 6, and 7.

- Green Tea Study staff will assist in reminding for these appointments but will not be present at the clinic for these visits.

- In order to have all study documents present for a visit to a Fairview Clinic it is the subject's responsibility to inform the Green Tea Study of the upcoming appointment.

  - When informing the staff of your visit(s) please let them know: What date, time and clinic you will be attending for you visit.

Current Participating Fairview Clinics

- Crosstown Clinic/ Southdale Medical Center
  6545 France Ave, Edina, MN 55435
  Hours: Monday - Friday: 8 AM - 5 PM
  Appointment Line: (952)- 848 - 5600

- Maple Grove Family Medical Center

  14500 99th Ave N, Maple Grove, MN 55369
  Hours: Monday – Friday 7 AM - 5 PM & Saturday: 8 AM - 5 PM
  Appointment Line: (763) – 898-1000

- Jonathan Clinic
  1447 White Oak Drive, Chaska, MN 55318
  Hours: Monday – Friday 8 AM - 5 PM & Saturday: 8 AM - 12 PM
  Appointment Line: (952) – 448-3500

Current Participating Fairview Clinics - Continued

- Oxboro Clinic

  600 W. 98th St., Bloomington, MN 55420
  Hours: Monday, Wednesday, Thursday & Friday: 8 AM - 5 PM
  Tuesdays: 8 AM - 12 PM
  Appointment Line: (952) – 885-6150

- Farmington Clinic

  19685 Pilot Knob Road, Farmington, MN 55024
  Hours: Monday, Wednesday & Thursday: 8 AM – 4:30 PM
  Tuesday: 8 AM – 6:30 PM
  Friday: 8 AM – 4 PM
  Appointment Line: (651) – 463-510

Blood Draws

- During 10 clinic visits, you will have about 1 or up to 12 teaspoons of blood drawn each time.

- The amount of blood drawn is dependent on which clinic visit that you will be attending.
    - Visit Numbers: 1,3,4,5,6,7 and 9, Approximately 1 teaspoon of blood will be drawn.
    - Visit Number: 2, 8 and 10 - Approximately 12 teaspoons of blood will be drawn. These visits are also fasting visits and are preferred to be seen between the hours of 6:30-10am Monday-Friday.

What are we drawing your blood for? :

Blood is drawn to measure the following biomarkers during the aforementioned clinic visits: sex hormones (estrogen, estradiol, androstendione hormones), oxidative stress biomarkers, green tea catechins, and enzymes related to liver function.

Are there any risks? :

There is a small risk of infection when blood is taken, but the risk is minimal as all needles and equipment are sterilized and the procedures are performed by trained clinical staff at the human Nutrition Research Center (HNRC) or a partnering Fairview Clinic.

You may experience some mild to moderate pain lasting a few seconds upon insertion of the needle used to draw the blood. You may also get a bruise from the blood draw. Please inform the clinical staff if you feel faint or nauseous.

24-Hour Urine Collection

Throughout this Study you will be asked to collect your urine a total of 3 times at home.

These collections take place at visit 2, 8 & 10.

Collection supplies:

To assist you in y our 24 hour urine collection, the study will provide you the following supplies:

- 1-3 Liter plastic jug
- 1 Liter brown jug
- 1 Baggie containing 3 grams powdered ascorbic acid (Vitamin C)
- 1 thermal/lunch bag
- Ice packs
- Urine collection hats

Upon completion of the study you may dispose of all remaining study materials at home

At each of the clinic visits 1, 5, and 9 we will provide you with one large 3-liter container and one 1-liter container. If you have these visits at a Fairview Clinic, the materials will be delivered to your residence. These jugs will be used **for the collection of 24 hours urine during the day before clinic visits 2, 8, and 10.**

You will be provided with one small bag containing foil-wrapped vitamin C. The contents of this bag of powdered vitamin C must be put in the large 3-liter jug at the beginning of the collection to help preserve the urine. The extra 1-liter collection container and accompanying cooler bag with the ice packs are meant to accommodate your active lifestyles of work, school, and other commitments.

You will also be provided with two collection hats. They are designed to make the collections easier. You can keep one at work and one at home if you like. Place the hat near the front of the toilet bowl before you urinate. After you urinate, pour the urine into the container. Please wipe the hat with a damp paper towel between each use.

Please follow the instructions below to complete the 24-h urine collection:

1. On the collection day (the day before your visit), do not collect your first void in the morning. That urine was produced in your body overnight and therefore is representative of the previous night's urine. **At this point, with the 3 liter jug still empty, add the vitamin C powder to the container.**

2. **Record the time of that first void of the day as your starting time on the label. Urine is being produced in your body from that time on and will be collected in the day's collection.** So although you are not collecting this first void, it is the start time for the 24 hours of the collection period.

3. Throughout the day, whenever you are away from home, carry the 1-liter container and collection hat with you in the cooler bag with ice packs to ensure a full day's collection.

4. When you arrive home, immediately transfer the urine to the large 3-liter container labeled for that day. Label your 3-liter urine container with the start and end times as well as the appropriate dates. You will be provided with labels to attach to all your containers. Keep all containers filled with urine in a refrigerator or cooler with ice. DO NOT STORE OUTSIDE DURING WINTER MONTHS.

5. Before coming to the HNRC the next day, void and transfer the last urine into the 3-liter jug. **Note that your start and end time for the 24 hour urine collections will depend on what time you get up that first morning.** You will need to get up at the same time for the days of the 24 hour urine collections.

EXAMPLE: On the day before your clinic visit, you wake up and void at 7:00 a.m., but you do not collect since this urine was produced in the previous 24 hours. This is your START TIME—7:00 a.m.. On the next day, you wake up again at 7:00 a.m., void and transfer the urine into the 3-liter container labeled for the complete 24 hours urine collection.

6. Keep your 3-liter container of the urine collections in the refrigerator.

7. You will bring the 3-liter container to the HNRC on your designated clinic visits.

8. You will receive your month 6 and 12 urine collection jugs at the end of clinic visits 5 and 9 in the HNRC.

What if…

- If you happen to urinate enough in one day to more than fill the 3-liter container, simply use the 1-liter container. Make sure to label both containers and indicate there are two containers for that day.

- If you find while collecting urine that you produce more than 3 liters on the first day (and this was a normal day for you), please contact the study coordinators immediately, and we provide you with additional collection containers for visit 8 and 10.

- NOTE: Do not fill the container to the top, as it will increase the chance of leaking. Instead, once a 3-liter container is ¾ full, begin collecting into the second 3-liter container. Always make sure you have one extra 3-liter container at home if you normally produce more than 3 liters urine within 24 hours.

- We do not need to know each time you use the restroom. We are concerned with the time frame for the day you collected, which should be 24 hours.

- Make sure you place all labels on the 3-liter jug and extra containers (if any), and check on the labels for dates and times, and check the box that says “Vit C added”.

EXAMPLE OF : CORRECT URINE LABEL

24 Hours Collection	**Clinic Visit 2**
Green Tea Study Protocol # 10039	
Study ID #	
VIT C ADDED	
Baseline	
Start Date _____ End Date_____	
Start time ____:_____ End time _____:_____	

Completing Questionnaires

All participants in the Green Tea Study will be required to complete three (8) questionnaires throughout the study. The questionnaires ask about your health history, dietary habits, and menopause symptoms and can be completed either at the clinic or at your home.

HEALTH HISTORY QUESTIONNAIRE (HHQ)

One health survey will be administered at the screening visit (clinic visit 1) to be turned in at clinic visit 2. The survey includes a physical activity interview along with a survey asking questions regarding the following: exercise, life events, emotional state, body image, menstrual cycle and reproductive history characteristics, and demographics.

DIETARY HISTORY QUESTIONNAIRE (DHQ)

You will be completing an online diet history questionnaire (DHQ) at the beginning (clinic visit 2) and the end (clinic visit 10) of the study. This survey consists of questions about consumption of 144 food items, and it takes approximately 1-2 hours to complete it.

Generally, you can complete this questionnaire either at home or at the HNRC with the aid of a research staff member.

Menopause-Specific Quality Of Life (MENQOL) Questionnaire

The Menopause-Specific Quality of Life questionnaire assesses the effects of menopausal symptoms on health-related quality of life. The collected information from this questionnaire will help us to study the possible effects of the supplement on the quality of

your life. This is a self-administered questionnaire including 32 questions, which ask about your experience of certain physical, psychosocial, and sexual symptoms over the previous week. If you have experienced any of the questioned items, answer “Yes” and then rate the experience from zero (not at all bothered) to six (extremely bothered), otherwise check the “No” box and proceed to the following item. This questionnaire will be online at the beginning, month 6, and the end of study, and can be accomplished in less than 15 minutes.

Taking the Study Supplement

Instructions for Taking the Green Tea Supplement

You will be provided the Green Tea supplement or placebo during your visits to the HNRC by study staff.

To remain compliant with our study, please take 2 capsules in the AM hours and 2 capsules in the PM hours.

What if:

- **You miss a pill:** If it is within the same day as the missed dose, consume the missed pill(s) as soon as you remember (with a meal or snack). If the missed pills were on a previous day, do not consume the missed dose and record this in your study log.

- **You feel nauseous after taking the pills:** Try taking the pills with a snack such as crackers or toast. If your symptoms don’t improve or get worse, contact the study coordinators.

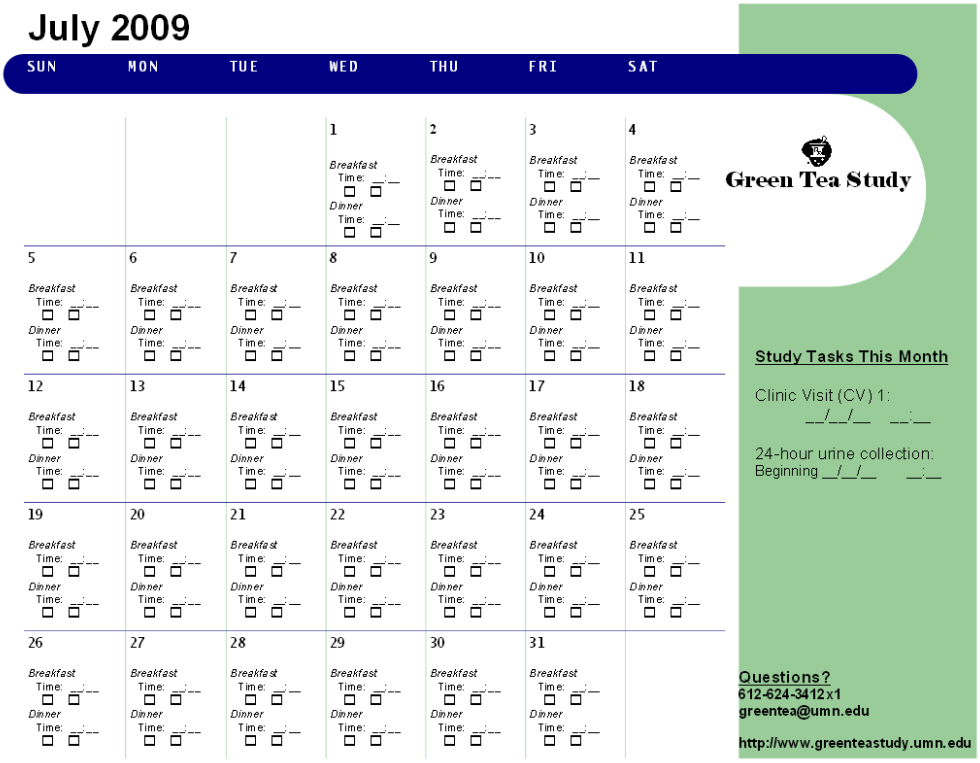
- **You lose your pills:** Contact the study coordinators ASAP to get the new bottle.

- **A child or animal consumes the pills:** If it is just one capsule, there should be no problem; but if you have no idea about the number of taken pills, or if you think it is more than 2, induce vomiting and call the Poison Control Center at: 1-800-222-1222.

- **You begin or discontinue a medication while in the study:** Let the study coordinators know ASAP.

- **You leave home for more than one day and you forget your capsules:** Call the study coordinators ASAP, and record the number of missed pills on your study log.

Example of Study Log:



July 2009

SUN	MON	TUE	WED	THU	FRI	SAT
			1	2	3	4
5	6	7	8	9	10	11
12	13	14	15	16	17	18
19	20	21	22	23	24	25
26	27	28	29	30	31	

Each day: Breakfast Time: __:__ ☐ ☐ Dinner Time: __:__ ☐ ☐

Green Tea Study

Study Tasks This Month

Clinic Visit (CV) 1: __/__/__ __:__

24-hour urine collection: Beginning __/__/__ __:__

Questions?
612-624-3412x1
greentea@umn.edu

http://www.greenteastudy.umn.edu

If you need to change your appointment time, you can contact Green Tea Study staff at (612) 624-3412 or at greentea@umn.edu.

If leaving a message, give:

- Your name
- Contact information (phone number, etc),
- Available date and times you are able to come in for your visit.
    - The clinic is open 6:30am-2:30pm Monday-Friday.

Thank you for your Participation.