# Effects of Green Tea Catechin Extract on Serum Lipids in Postmenopausal Women: A Randomized, Placebo-Controlled Clinical Trial

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## Dedication

I dedicate this thesis to the most important and wonderful people in my life…who from the beginning have fostered my curiosity in the world, provided me with strength and faith along the way, and who always believed in me- my parents and family.

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## Abstract

**Objective:** To test the efficacy and impact of a concentrated green tea extract to improve lipid profile in postmenopausal women.

**Methods:** 886 postmenopausal women were recruited in to the study and randomized to consume either 1200 mg of a green tea catechin extract ("GTE", 800 mg as EGCG) or placebo, daily. Fasting serum samples were drawn for measure of lipid panel at baseline, midpoint (month 6), and endpoint (month 12) of study, to be analyzed.

**Results:** After one year on treatment, total cholesterol ("TC", -4.6 mg/dL, $P<.0001$), LDL-C (-5.0 mg/dL, $P<.0001$), and non-HDL cholesterol (-4.4 mg/dL, $P>.0001$) were significantly reduced in the GTE group, both compared to placebo and overall. The largest reductions in TC, LDL-C, and non-HDLC occurred in participants with baseline total cholesterol > 200 mg/dL. HDL-C decreased slightly in the GTE group, both after 6 months on treatment ($P=.0016$), and overall ($P=.0038$). Triglycerides increased significantly after one year in the GTE group compared to placebo (+3.5 mg/dL, $P=.023$) as well. A similar trend was seen in participants who took statins during the trial, had a baseline TC $\leq$240 mg/dL, or had a baseline of BMI $\leq$ 24.9 or $\geq$ 30 kg/m$^2$.

**Conclusion:** Daily supplementation of GTE at 1200 mg (800 mg as EGCG) for one year resulted in significant reductions in TC, LDL-C, and Non-HDLC in a population of postmenopausal women. Further research in to proper dosing guidelines, in particular for hyperlipidemic populations, would be beneficial to increase potential therapeutic use of these findings.

# Chapter One: The Literature Review

## 1. Green Tea and Catechins

Green tea comes from the plant *Camellia Sinensis.* It is one of the most popular and widely-consumed beverages in the world, and has been a source hydration, medicine, and tradition for centuries. The antioxidant[1], antiatherosclerotic[2], antihypercholesterolemic[3], and anticarcinogenic[4] properties of green tea demonstrated through *in vivo, in vitro* and animal studies, have fueled further interest and research in this field. A growing body of evidence suggests the many beneficial applications of green tea (**sections 3-7**, in this review), and active research in to the extent of these benefits in ongoing. However, in order to understand the potential of this plant and compounds, it is important to understand its composition, origin, and preparation.

Green tea is the least fermented tea of the *Camellia Sinensis* varieties (green, black, and oolong teas). Because of this, the high polyphenolic content of the virgin plant is preserved. The catechins, a type of polyphenol, are the biologically active compounds believed to be responsible for the health-promoting properties of green tea[5]. Chung S. Yang *et al*. report there are around 620-880 mg of water-extractable solids in an average tea bag-sized tea and water infusion (~two grams tea leaves in eight ounces water). The catechins account for about 30% of the dry weight of the tea leaves, with epigallocatechin gallate (EGCG) comprising on average 45-80% of that weight[1,6]. Three of the other primary catechins present in green tea include epicatechin (EC), epicatechin gallate (ECG), and epigallocatechin (EGC):

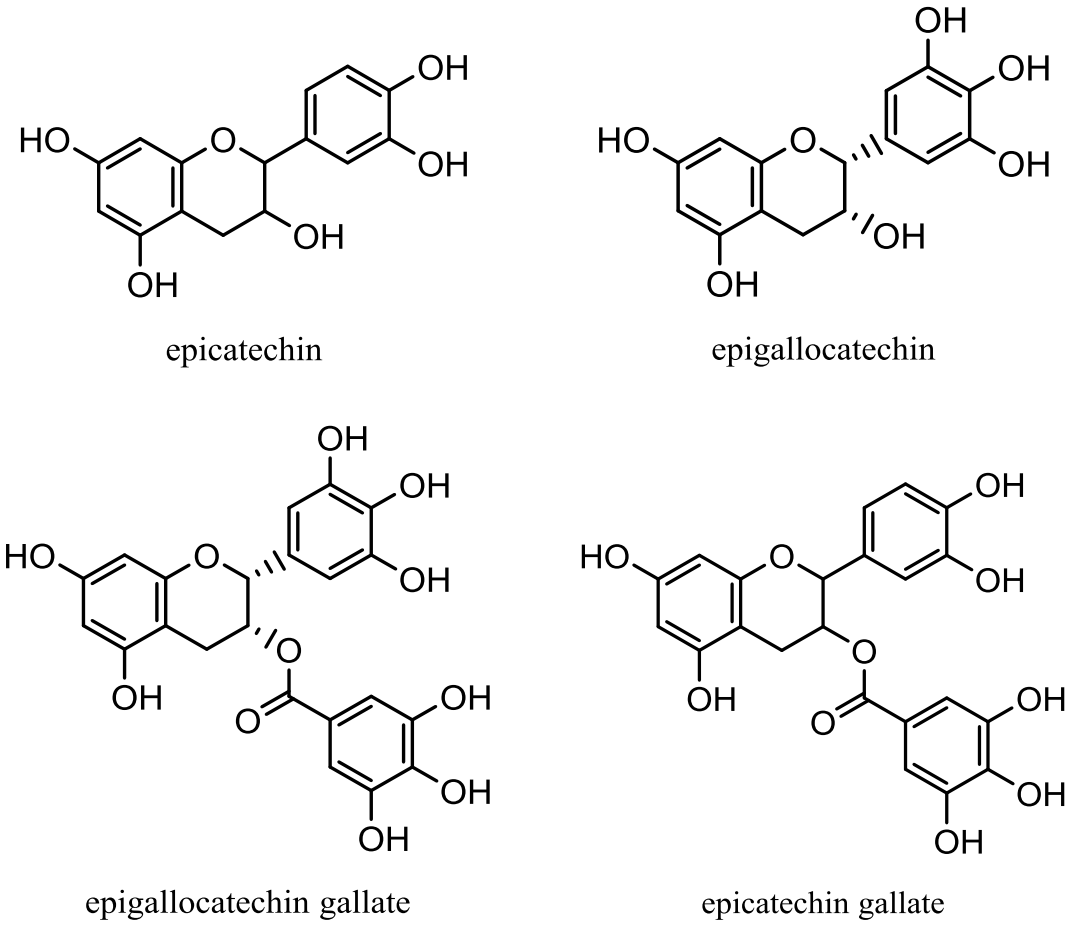


**Figure 1.** Chemical structures of the major catechins in green tea.

Green tea catechins are primarily absorbed in the small intestine, and metabolized by Phase II enzymes including sulphotransferases (SULTs), UDP-glucuronosyltransferases (UGTs), and catechol-*O*-methyltransferase (COMT), with further metabolism occurring in the liver. EC and EGC appear to be preferentially metabolized in enterocytes during absorption, since their conjugated forms are found more in plasma, versus EGCG and ECG being found in their free form[7].

The overall bioavailability of the catechins in humans has been questioned however, since catechins (especially EGCG) are rapidly degraded in the intestine upon being consumed[7]. Large catechins that cannot be absorbed in the small intestine often pass on to the large intestine, where they can be broken down into smaller metabolites by microbiota in the colon[8]. A paper by Calani *et al*. investigated the contribution of these colon-derived metabolites to overall catechin bioavailability, finding they greatly increased bioavailability of catechins absorbed by the human body when compared to intestinal absorption alone. This contribution of colon-derived catechin metabolites may account for some of the inter-individual variability of absorption seen in tea consumers[8].

Green tea catechins are relatively stable compounds. They are most stable in acidic conditions (pH <4), and degrade quickly in alkaline solutions. Relative to brewing in water alone (average pH ~7), there is a 20% increase in EGCG content at pH 3, which is comparable to a cup of tea with 10 mL of lemon juice added during steeping[9]. One theory for the low bioavailability of orally ingested catechins is the change (increase) in pH from the stomach to the intestine, with EGCG and EGC being more readily degraded in alkaline solution than EC and ECG[7]. Aside from being pH-dependent, catechins are quite heat-stable. The concentration of EGCG and related catechins in a tea infusion is highest after ~7 minutes of steeping in 100 degree Celsius water[9], and exhibit only ~15% reduction in catechin concentration after being boiled in water for up to 7 hours[10].

Tea is a natural plant native to certain parts of the world, most notably China and Japan. Like tea flavor, from mild and mellow, to fruity, bold or grassy, there are various factors that affect tea catechin content. Season of harvest, leaf age, and sun exposure

during growth are all associated factors[11]. A study by Song *et al.* found a slight inverse association between EGCG content and sun exposure, with content increasing from bud to first leaf ($P$=.01), as long as the plant was also partially shaded (receiving 60% shade, $P$<.001). Without partial shade cover, this association was not observed. Interestingly, inter-catechin variations may also exist, given the same growing conditions. In the same study for example, EGC was strongly correlated with leaf age (increasing with leaf age, $P$<.0001), with less dependence on shade level ($P$<.05)[11]. It is logical to consider the temperature and photosynthetic differences in shaded vs. exposed plants, however the direct effect of shade versus other environmental factors on catechin content in teas is still unclear[11]. Aside from environmental factors, catechin content in commercial teas may be influenced by steeping temperature and length, manufacturing process (highest with air-tight packaging), cultivar and origin (immense variety), and pH of infusion. Differences in catechin content have even been shown between lots of the same type and brand of tea, highlighting some susceptibility to variation at many points during the growth and production chain[12].

## 2. Lipids and Lipid Metabolism

### *2.1 Digestion and Absorption*

In order to understand how catechins from green tea may affect lipid profile, a brief overview of lipid and lipoprotein metabolism is provided. Triglycerides are the most common lipid found in the diet. A triglyceride molecule consists of a glycerol backbone esterified to three fatty acids (FAs), which can vary in length (short, medium,

or long-chain), as well as degree of saturation (saturated, monounsaturated, and polyunsaturated)[13]. Digestion of lipids begins in the stomach by gastric lipase, but occurs primarily in the small intestine where lipids are absorbed[14]. Partially-digested lipids from the stomach assemble in to small droplets and enter the duodenum of the small intestine, stimulating the gall bladder to express bile into the lumen. Bile functions to make the hydrophobic lipid droplets soluble in the aqueous lumen, a feat made possible by its amphipathic components, including bile salts and phospholipids[14]. The churning of the bile and lipid droplets in the duodenum result in the production of tiny aggregates called mixed micelles, composed of partially hydrolyzed triglycerides, phospholipids, bile salts, cholesterol, and fat-soluble vitamins. Pancreatic lipase and colipase act upon these micelles, hydrolyzing any remaining full triglycerides in to free fatty acids and monoglycerides, which enter the enterocyte via simple diffusion or fatty acid transport proteins[15,16]. Cholesterol can only be absorbed in the free form, so dietary cholesterol esters must be hydrolyzed by pancreatic cholesterol esterase prior to absorption[17].

It is known that about 50% of cholesterol consumed in the diet is actually absorbed[18], however clarifying the exact metabolic pathway is still an active area of research. In 2004, Altmann and Davis, *et al.*, reported their identification of a transmembrane protein vital for intestinal absorption of cholesterol, the Niemann-Pick C1 like 1 (NPC1L1) protein[19]. Prior to the discovery of NPC1L1, the mechanism by which cholesterol entered the enterocyte from micelles was poorly understood. The generally accepted mechanism was free cholesterol could enter intestinal cells via passive diffusion, but the presence of NPC1L1 challenges this theory. Further research has

supported a central role for NPC1L1 protein in cholesterol homeostasis as well. Harry R. Davis Jr. and colleagues used NPC1L1 knockout mice to demonstrate its influence on *in vivo* cholesterol regulation. In NPC1L1 (-/-) mice, intestinal absorption of cholesterol was inhibited. A compensatory increase in intestinal and liver expression of cholesterol biosynthetic enzymes and an upregulation of low-density lipoprotein (LDL)-receptor proteins was also observed. The result was increased LDL cholesterol (LDL-C) clearance from the plasma and increased biosynthesis of cholesterol. In wild type mice, cholesterol balance is in part controlled by monitoring cholesterol absorption from the intestine. In the current study, cholesterol feeding in healthy wild-type mice caused a down-regulation of NPC1L1 protein transcription, which is believed to act as a primary barrier against over-absorption of cholesterol[20].

As with cholesterol, the prevailing theory for triglyceride absorption was passive diffusion of hydrolyzed monoglycerides and free fatty acids via the apical membrane of enterocytes. Though this method is feasible for short and medium-chain fatty acids, growing support for the use of facilitated diffusion is strong, particularly for long chain fatty acids (LCFAs). Min Chen *et al*., explored this theory in rat enterocytes, and found substantial evidence to suggest a key role of the multifunctional enzyme CD36 (Fatty Acid Translocase in rat enterocyte) in LCFA intestinal absorption[15]. Once in the enterocyte, the free fatty acid or monoglyceride molecule is bound by an intracellular fatty acid binding protein (FABP), which chaperones the lipid to its active site of function[21]. Most FAs will be reassembled in to a triglyceride, to follow one of four main pathways: incorporation in to chylomicrons, storage within the cell, incorporation in to

fatty acid products (ie: cholesterol esters), or used for energy via beta oxidation. A significant number of dietary triglycerides will be packaged in to chylomicrons with fat-soluble vitamins, cholesterol esters and phospholipids, and released in the lymph to eventually enter portal circulation via the thoracic duct and distribute their contents to peripheral tissues[22].

### *2.2 Cholesterol Biosynthesis*

Humans are able to synthesize enough cholesterol to meet physiological needs. Cholesterol is an essential building block of cell membranes, and functions as a precursor for steroid hormone synthesis and other compounds important for cell function. Due to its key role in membrane and hormone synthesis, cholesterol balance is a highly regulated and monitored process. If a cell needs cholesterol and dietary cholesterol is not sufficient, the cell will upregulate transcription of cholesterol-synthesizing enzymes, in particular 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase[23]. In summary, cholesterol begins its synthesis by merging molecules of acetyl-CoA to form acetoacetate. Acetoacetate is converted in to HMG-CoA through the action of HMG-CoA synthase, then in to the cholesterol precursor mevalonate by HMG-CoA reductase. Mevalonate then undergoes a series of reactions that result in formation of an isoprene which continues on to be become cholesterol, or can branch in to synthesis of other nonsterol products, such as ubiquinone[24,25] (**Figure 2**). The action of HMG-CoA reductase to form mevalonate is the rate-limiting step of cholesterol biosynthesis, and its function is regulated by the presence and active status of sterol regulatory element

binding protein-2 (SREBP2), a transcription factor central to the regulation of many metabolic pathways[23]. When intracellular cholesterol is low, SREBP2 translocates to the nucleus and increases transcription of HMG-CoA reductase mRNA, which is translated in to the active enzyme as needed. When intracellular cholesterol is high, SREBP2 is deactivated and any SREBP2 in the nucleus is degraded[25]. Cholesterol not immediately needed for synthesis of new cell membranes enters one of four main pathways: esterification and storage within the cell (forming a cholesterol pool to be used later), conversion in to bile salts, synthesis of steroid hormones, or is added to bile for fecal excretion. The average person synthesizes about 700-900 mg of cholesterol per day, with the liver and ilium being the primary organs of synthesis[24].

Diet and genetics appear to interact in determining how a person metabolizes cholesterol[18]. With only half of the cholesterol consumed in food being actively absorbed[18,19], much of the cholesterol in our body is of endogenous origin. Enterohepatic circulation is the process of cholesterol being incorporated in to bile to aid digestion, then its re-absorption in the distal intestine (ileum) and recycling in the liver, to be used again in bile, excreted, or for the synthesis of other compounds. Composed of bile acids (derived from cholesterol), phospholipids, and free cholesterol, bile is a primary route for excretion of excess cholesterol in humans, responsible for up to 500 mg of excreted cholesterol per day[26].

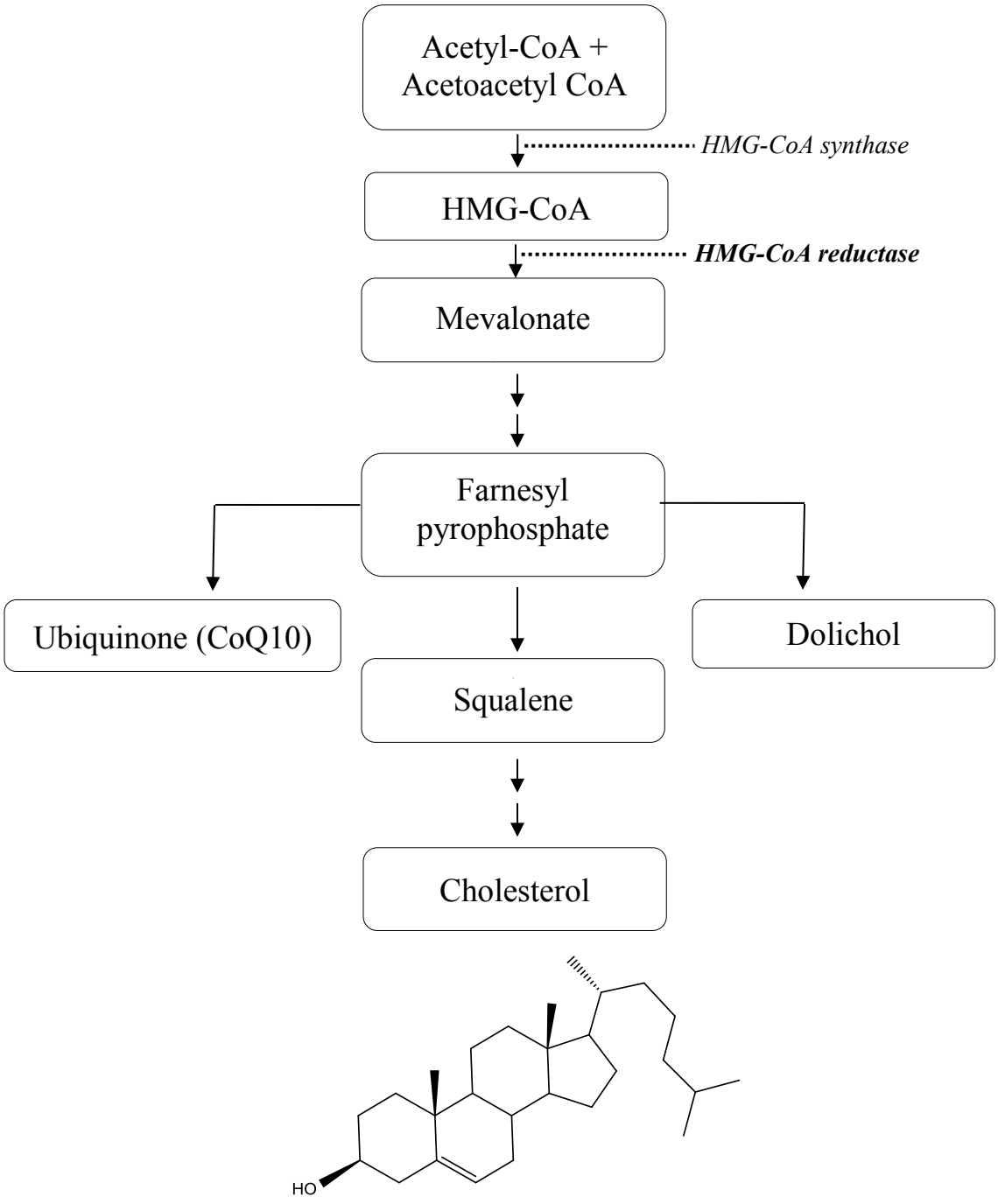


**Figure 2.** Cholesterol biosynthetic pathway (overview)[27].

*2.3 Lipoproteins and Apolipoproteins*

The method of classifying lipoproteins by density, based on the composition of their lipid core, has helped to define the distinct roles of these compounds. Lipoproteins are protein-lipid complexes made water-soluble by the presence of a phospholipid-enriched membrane, with the primary function of transporting and distributing lipids throughout the body. The apolipoproteins are type-specific functional accessory proteins located on and around the surface membrane, and are largely responsible for determining the physiological activities of each lipoprotein.

Two primary classes of apolipoproteins define the lipoprotein density categories: apolipoprotein A-containing (apoA), and apolipoprotein B-containing (apoB). The apoA-containing lipoproteins have the highest proportion of phospholipids, and are generally associated with high density lipoprotein cholesterol (HDL-C). The apoB-containing lipoproteins have a higher percentage of cholesterol esters and triglycerides in their lipid core, reducing the density of their core to the level of low-density (LDL-C) or very low-density (VLDL-C) lipoproteins[28]. The apolipoproteins act as ligands for binding to necessary receptors, such as LDL-receptor and scavenger receptor B1 (SR-B1), and serve as identifiers on the lipoprotein surface. The apolipoprotein composition of the lipoproteins shifts as their contents do. During the lipid distribution process, the ratio of triglyceride to cholesterol ester shifts, converting the VLDL-C first to IDL-C, then to LDL-C. As it releases triglycerides to peripheral tissues, VLDL-C loses its E and C apolipoproteins (two apoproteins which appear in various forms on many lipoproteins), making B-100 the primary apolipoprotein of LDL-C [29].

## 3. Green Tea and Health

### *3.1 Cardiovascular Disease*

Having elevated serum levels of triglycerides, total cholesterol (TC), and LDL-C are associated with an increased risk for developing cardiovascular diseases (CVD). This risk is further compounded by having low levels of serum HDL-C, a central component of reverse cholesterol transport and cholesterol recycling. Though there is some inconsistency in results regarding green tea and improvement of CVD risk factors, there is growing evidence to support an association [30,31], via various pathways including elevation of HDL-C, increased antioxidant activity, and reduction of blood pressure[32]. Sulibursk*a et al.* reported a positive change in HDL levels in obese patients receiving a moderate dose of green tea catechins (379 mg green tea extract, "GTE") daily for three months. Though no difference was seen between treatment groups, patients within the GTE group experienced significant increases in HDL-C ($P<0.044$)[33]. A 2012 randomized, placebo-controlled double-blind study conducted by Bogdanski and colleagues investigated the antiatherogenic potential of green tea catechins as well. Three months of daily supplementation (379 mg, 208 mg as EGCG) of green tea extract to 56 obese, hypertensive patients significantly reduced blood pressure (SBP: $P=0.004$, DBP: $P<0.001$), fasting glucose ($P=0.016$), insulin ($P<0.001$), and inflammatory factors ($P<0.001$), and increased antioxidant status. Additionally, a 15% increase in HDL-C was observed in the green tea group ($P<0.001$), as well as significant reductions in TC ($P=0.009$), LDL-C ($P=0.011$), and triglycerides ($P=0.004$)[32]. The large increase in HDL-C observed in this treatment group is interesting due to great inconsistencies reported in

previous green tea lipid trials looking at HDL-C. All participants were asked to retain their diet (monitored by questionnaire every 14 days, plus 3 days before lab visits) and physical activity habits in an attempt to isolate the influence of the intervention alone, but it is possible differences in fat quality of diet, age, and varying compliance may have yielded unintended influence on results[32].

The initiation of fatty streak development is atherosclerosis is dependent on the action of inflammatory cytokines. In LDL-receptor knockout mice, an animal model for human hypercholesterolemia, four weeks of daily 50 mg/kg supplementation of green tea extract (GTE) significantly decreased plasma levels of monocyte chemotactic protein-1 (MCP-1), an inflammatory cytokine, in atherosclerotic mice. GTE at 50 mg/kg per day also promoted relaxation of the aorta, reduced plasma triglyceride levels, and reduced atherosclerotic lesion size ($P<0.001$) as compared to control hypercholesterolemic mice[34]. Kumaran *et al.* demonstrated a high cholesterol diet induces more fatty changes in hepatic tissue in aged rats, as opposed to young rats. Daily administration of 100 mg/kg GTE for 30 days significantly reversed these changes, including hepatic cholesterol, triglycerides, serum cholesterol, LDL-C, and VLDL-C[35]. An *in vitro* study by these same researchers also showed improved resistance to lipid peroxidation, a known proliferator of atherosclerosis and cardiovascular disease, in tissues treated with EGCG[35]. Furthermore, Friedrich *et al.* found in mice fed a high-fat diet supplemented with EGCG, post-prandial plasma triglycerides were reduced dose dependently, as well as liver triglycerides in the post-prandial state[36].

### *3.2 Obesity and Type II Diabetes*

Regular green tea consumption has been loosely associated with improving symptoms of obesity, type 2 diabetes, and metabolic syndrome, conditions exacerbated by the high-calorie diets and sedentary lifestyles of present day. The metabolic syndrome is a cluster of adverse anthropometric and biochemical symptoms, including dyslipidemia, hypertension, and elevated fasting glucose ($\geq$100 mg/dL)[37]. Aside from being associated with development of type 2 diabetes, obesity can also induce chronic inflammation in the body. Adipose tissue functions not only as lipid storage, but also as an endocrine organ, secreting hormones related to appetite, metabolism, and inflammation (cytokines). Adipose tissue accumulation has been linked to a constant state of inflammation, leading to many of the complications associated with this disease[33].

Though this syndrome can affect people of all ages, the loss of the protective effects of estrogen in menopause can be conducive to developing this condition in postmenopausal women. Suggested lifestyle interventions for these symptoms include emphasis of a plant-based diet (for phytoestrogens and catechins), increased physical activity, and weight loss[37]. Obesity is a growing problem in the United States, making research in to its treatment and prevention of great interest. The anti-obesigenic potential of green tea appears to be multi-dimensional, including interaction with catecholamine metabolism[38], increased beta oxidation[39], and suppression of lipogenic enzymes[40], among others.

Some catecholamines (epinephrine, norepinephrine) are stimulators of lipolysis in the body. By inhibiting the enzyme responsible for catecholamine breakdown (catechol-*O*-methyltransferase), catechins are thought to increase the duration of the catecholamines presence in plasma, prolonging their effects[38]. Some evidence in human studies also shows green tea to potentially increase energy expenditure through enhanced thermogenesis and fat oxidation. A metabolic study in 10 healthy men reported daily consumption of 270 mg EGCG for 5.5 weeks was found to increased total 24-hour energy expenditure as compared to placebo or caffeine-only (150 mg/day) treatment, by 3.5% and 2.8% respectively[39]. These same interactions with tea may not be seen in children however. A 2008 study in Japanese school children by Matsuyama *et al.* investigated the effect of a catechin-enriched (576 mg, 102 mg as EGCG) green tea beverage on fat mass, cholesterol, and blood pressure. Study participants were randomized in to treatment groups, then provided either a 340 ml can of catechin-rich test beverage (base beverage of tea leaf-water infusion, with catechin extract added), or a can of control beverage, to consume once per day for 24 weeks. The control beverage in this study also contained catechin extract (75 mg) to help blind the beverages so they would not be discernable by appearance or taste. Interim and post-treatment analyses showed no significant changes in major endpoints (blood pressure, fat mass, BMI) other than LDL:HDL ratio[41]. A randomized, double-blind, placebo-controlled clinical trial in 78 obese women showed significant reductions in LDL-C ($P<0.001$) and a slight increase in HDL-C ($P=0.01$-$0.05$) after 12 weeks of daily 1200 mg GTE supplementation. These changes were only significant for within-group comparison however, and did not

maintain that significance in cross-group comparisons[42]. A significant reduction in weight and total percent body fat was found in mice fed a high-fat diet, who consumed 3.2 g/kg supplemental EGCG every day for 16 weeks. In the green tea extract-treated group, weight gain was 33-41% lower than in the untreated mice consuming a high fat diet. The amount the rats were consuming would equate to about 10 small cups of green tea per day[43].

Favorable results in green tea and lipid research have led to the development of more specific trials to explore its effects in patients with type 2 diabetes as well. A number of animal and *in vitro* studies provide evidence toward the ability of green tea catechins to help ameliorate complications with diabetes, and impact insulin sensitivity. Bose *et al.* reported mice fed a high-fat diet supplemented daily with 3.2 g/kg EGCG for 16 weeks had 25% lower blood glucose, a 61% reduction in plasma insulin, and a lower homeostasis model assessment of insulin resistance score ($HOMA_{IR}$: a calculation used to estimate insulin resistance), than untreated mice fed the same high fat diet[43]. Waltner-Law *et al.* demonstrated *in vitro* the ability of EGCG to decrease hepatic glucose production, via downregulation of the gluconeogenic enzymes phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase[44].

Despite promising results in animal studies, human trials have failed to follow suit in consistency. An epidemiological study in Japan reported that those who habitually drank six or more cups of green tea per day decreased their risk of developing type II diabetes, when compared to those who consumed less than one cup per week[45]. Han *et al.* reported one potential mechanism to explain this observation. The *in vitro* study

reported EGCG exerted a protective effect over pancreatic beta cell death, in part by reducing the expression of nuclear factor kappa-B (NFkB), a potent transcription-factor for many pro-inflammatory molecules[46]. A study by Nagao *at al.* reported a significant increase in insulin and adiponectin in type II diabetic patients, after 12 weeks of daily 582.8 mg GTE consumption, though these changes were significant for within treatment group only[47]. In contrast, a study by Brown *et al.* explored the potential of isolated EGCG to improve insulin resistance in 46 overweight or obese non-diabetic males. Participants consumed 400 mg of EGCG twice daily for 8 weeks, with no resulting impact on insulin resistance as measured by $HOMA_{IR}$[48]. Though the proposed hypoglycemic effects of green tea are more persuasive in animal trials, the dose of catechins given to animals tends to be higher than what would be given to humans, complicating dosing guidelines for future human studies.

### *3.3 Antioxidant Function, Cancer*

A large cohort study published in 2004 ($n$ = 29,199) suggests breast cancer risk in postmenopausal women may be increased in those who are overweight or have a history of metabolic syndrome, and a slight inverse association of this trend was associated with HDL-C quartile[49]. Though reports on the interaction of catechins and HDL-C metabolism have been conflicting, there is a lack of large-scale, placebo-controlled studies investigating this relationship. Greater mechanistic support for the anti-carcinogenic properties of green tea and tea catechins often come from studies conducted in animals[50]. An example of this is a 2013 study by Gu and colleagues which found

EGCG (50-100 mg/kg/day) to be protective against induced breast cancer development in 16 female mice, by inhibiting the activation of NFkB and vascular endothelial growth factor (VEGF) in breast tumors. A cross-section of mouse tumors after two weeks of supplementation showed tumor area to be 65% less in the EGCG group ($P<0.01$), as compared to controls[4]. A number of systemic reviews and meta-analyses have been conducted using case-control and cohort data to analyze the chemopreventive potential of green tea and catechins[51]. Well-controlled intervention studies may shed greater light on any cause-effect relationship between catechin consumption/supplementation and cancer risk.

Oxidative stress has been implicated in tumor progression as well[4]. Kumaran *et al.* documented increased levels of lipid peroxidation, and decreased levels of glutathione, in aged rats as compared to young control rats. Antioxidants can function by donating an electron to unstable compounds to help protect against peroxidation and radicalization, or by chelating bioactive metals that can produce oxidation products in the body (ex: copper, iron). Although antioxidant ability reduces with age and cell turnover, their experiment showed green tea extract helped restore function, or increase levels, of these cellular antioxidant enzymes[52]. Suliburska *et al.* also reported significant increases ($P<0.01$) in total antioxidant status in obese participants consuming 379 mg green tea catechins (208 as EGCG) per day[33], and though similar findings have been reported relative to the antioxidant function of catechins, their potency *in vivo* may be questioned by a weak bioavailability[50].

## 4. Epidemiological Studies: Green Tea and Lipids

Before any clinical investigation, reports on the health benefits associated with consumption of green tea came from observational studies. One of the largest and most widely recognized of these is a 2002 study conducted by Tokunaga *et al.* which looked at the effects of daily consumption of brewed green tea in 13,916 healthy Japanese workers[53]. The researchers reported a significant inverse association between green tea consumption and serum cholesterol, which appeared to plateau at around 10 cups per day. An interesting aspect of this study is that the researchers broke down the cholesterol-lowering ability to a "per cup" level, reporting approximately a -0.39 mg/dL reduction of TC in men ($P$=.03), and a -0.46 mg/dL reduction in women ($P$=.03) associated with each cup of green tea drunk per day[53]. Though this claim could be influenced by many factors as well great interindividual variability, therefore complicating its direct usability, it makes for an interesting inclusion of the authors to suggest a "per cup" benefit. These values remained statistically significant, even after adjusting for age, BMI, and dietary factors, which was an important consideration as 86.7% of their study population reported being average daily tea drinkers[53]. Intriguing findings such as the association reported by Tokunaga and colleagues, has sparked further scientific exploration in green tea's hypolipidemic potential. A cross-sectional study conducted by Kono *et al.* also reported an association between green tea intake and serum total cholesterol in 1,306 Japanese men. Total cholesterol was found to be approximately 8 mg/dL lower in the serum of men who consumed >9 cups of green tea per day, with no reported association with HDL-C and triglycerides[54]. Conversely, a cross-sectional analysis of self-reported

green tea consumption in 630 Japanese men and their 370 wives found no association between green tea consumption and serum lipid levels[55]. Though the large population of these studies provides strength for observational evidence, randomized, placebo-controlled intervention trials are often a stronger indicator of any cause-effect relationship that might be present.

## 5. Clinical Trials: Green Tea Catechin Extract and Lipids

Centuries of cultural literature and anecdotal evidence attest to the favorable impact of green tea on health. The biomedical inquiry into these effects however, including lipid homeostasis, is relatively new. Existing studies have tried to quantify the impact of green tea on lipid metabolism, through use of green tea beverage, extract, or purified catechins. Though differences may exist between methods of catechin administration (expanded in **section 6**), all of these methods have been tested to some degree. Trials have been conducted in various human populations, encompassing a mix of ages, race, gender, and groups with co-morbidities (ie: diabetes, obesity, metabolic syndrome). Though there are studies that do not correlate green tea consumption (beverage, extract, or purified catechin) with improvement in lipid parameters[48,55,56], there are a number of trials contributing to the literature that suggest otherwise.

A randomized, double-blind, placebo-controlled intervention published in 2009 investigated the effects of a 400 mg daily GTE supplementation on lipid biomarkers in healthy men and women. A significant decrease was seen for total (-5.60%, $P$=0.011) and LDL-C (-6.79%, $P$=0.037) in male participants in the GTE group after 3 weeks on

supplementation. Additionally, sub-analyses in participants (both male and female) showed significant decreases in LDL ($P$=0.019) in those with baseline LDL-C >99 mg/dL. No change was reported for HDL-C or triglycerides[57]. In contrast, a different 3-week study in healthy men consuming 714 mg catechins per day found no significant impact on lipid profile, except for a slight reduction in total:HDL cholesterol ratio[58].

Other clinical trials have focused on the impact of green tea supplementation in hypercholesterolemic or obese patients, including those with metabolic syndrome. Suliburska and colleagues conducted a double-blind, randomized trial in 46 obese men and women, to test the effect of 379 mg GTE per day on blood and anthropometric biomarkers[33]. After three months, significant reductions in BMI ($P$=0.03), total cholesterol (TC, -6.9%, $P$<0.01), LDL-C (-12.21%, $P$=0.02), and triglycerides (-15.75%, $P$<0.01) were seen. Though there was a 14% increase in HDL-C in the treatment group after intervention, there was an unexplained 5% increase in HDL-C in the placebo group as well, despite both groups being instructed to maintain isocaloric diets and their typical exercise habits. The drastic reduction in triglycerides seen post-treatment in the GTE group is uncommon, and may have been a secondary factor to the weight loss rather than a direct catechin interaction. Thus the question may remain if similar results would be seen in a cohort of healthy-weight individuals[33]. Similar effects were not seen in obese men. Brown *et al.* reported no significant change in lipid biomarkers was observed in overweight or obese men after 800 mg daily consumption of isolated EGCG for eight weeks[48].

Two studies also included a dietary intervention component. A 2009 prospective, placebo-controlled crossover study by Batista and colleagues found after daily consumption of 250 mg GTE for 16 weeks, participants experienced a significant reduction in TC (-3.9%, $P$=0.006), LDL-C (-4.5%, $P$=0.026), and BMI (-1.7%, $P$=0.002)[59]. Non-significant reductions in HDL, and non-significant increases in triglycerides and apolipoprotein B were also observed. The concomitant dietary intervention alone resulted in a significant decrease in daily calories (about -500 calories per day), percent total fat, percent saturated fat, and cholesterol, as well as an increase in protein ($P$<0.0001 for all). All of these dietary changes would have an impact on lipid profile, so discerning the actual impact of the GTE versus that of the diet is difficult[59]. Similarly, a 2005 study by Erba *et al.* was designed to investigate effects of a catechin supplement in amounts that could be consumed by an average tea drinker, while also implementing a dietary modification (participants consumed ~4 servings of fruits and vegetables per day, along with normal diet)[60]. 24 healthy women participated in the trial, 12 consuming two GTE beverages per day (250 mg total catechins), and 12 consuming control diet alone. After 6 weeks, no significant change in lipid profile was observed, except a slight reduction in LDL-C within the GTE group ($P$<0.05)[60]. Though the researchers attempted to control for outside dietary catechins by giving participants a list of restricted foods, there was no placebo or blinding associated with the treatment. Additionally, fruits and vegetables are a valuable source of fiber in the human diet, and soluble fiber in particular is known to have a favorable impact on cholesterol metabolism and balance in itself[61].

Most green tea and lipid trials have used serum drawn from fasting patients. In contrast, Unno *et al.* tested the effects of catechins on postprandial lipid metabolism[62]. In a triple-crossover design, nine male subjects were enrolled and asked to consume a test "meal" (one slice of bread spread with butter) and a green tea mixed catechin beverage, to assess the effects of the catechins on postprandial triglyceride levels. Blood was taken at baseline (fasting), then at hours 1, 2, 3, 4, and 6 post-meal. Participants were given a control (10 mg catechins), moderate (224 mg), or high (674 mg) dose of catechins to consume with each test meal, with a 1 week washout period between treatments. Postprandial triglycerides peaked 3 hours after the meal, averaging a 62 mg/dL rise. The high dose of catechins (674 mg total, 243 mg as EGCG) significantly reduced triglyceride levels after the test meal, peaking at just over 37 mg/dL ($P<0.05$)[62]. This study differs from other green tea and lipid trials because it measures the immediate effects of catechin consumption. This interesting concept, if replicated and validated in a larger trial, could perhaps lead to guidelines for green tea application in control of postprandial triglyceridemia, a known and independent risk factor for atherosclerosis[63]. The small sample size however ($n = 3$ per treatment arm), does not protect against the variability expected if this test were to be repeated again using different volunteers or a different population, thus highlighting the need to repeat a larger trial of this theory.

Along with many other metabolic processes, lipid metabolism also shifts with increasing age. This could perhaps increase the impact of a low-cost, easily attainable lipid-modifying intervention for this particular group. A study by Miyazaki *et al.* investigated the potency of a GTE supplement with a simultaneous walking program in

older adults, measuring changes in lipid profile and other anthropometrics[64]. Though TC (-6.10%) and LDL-C (-8.66%) were significantly reduced in the GTE group after supplementation for 14 weeks ($P<0.05$), these were only observed as within-group changes. Between group comparisons of GTE versus placebo did not show any significance[64]. A potential reason for this could be the underlying study design, as a walking program, would increase exercise in both the treatment and placebo groups if compliance was maintained. Increasing physical activity is a common recommendation for people with dyslipidemia, as it can exert beneficial effects on lipid panel (increased HDL-C, reduced triglycerides) and other biomarkers of health and disease[64]. If the walking itself improved lipid parameters in the placebo group, this may have contributed to the lack of significance seen in the between-group analyses. Additionally, participants were asked to continue their typical diet, with no restrictions on food or beverages containing catechins or polyphenols (including green tea), making it difficult to assess the actual total dose of catechins consumed per day by the treatment group.

In a study in adult women without the exercise component, however, similar improvements in lipid profile were still seen. A 2012 randomized, placebo-controlled study by Wu *et al*. supplemented 103 postmenopausal women with either 400 mg or 800 mg of GTE daily for two months[65]. Within-group comparisons showed significant reductions in TC (-5.0%, $P=0.012$) and LDL-C (-7.9%, $P=0.007$) in the 400 mg group, as well as in the 800 mg group (TC: -3.1%, $P=0.045$; LDL-C: -6.6%, $P=0.012$). Overall, only the reduction in LDL was significant when compared to placebo group ($P=0.021$), though TC was borderline ($P=0.072$). No treatment effect was seen in HDL-C or

triglyceride levels[65]. Postmenopausal women represent a population that may especially benefit from improvements to lipid profile, due to natural anthropometric and metabolic shifts that occur during menopause. The baseline values of TC and LDL-C in the Wu and colleagues study designate these subjects as borderline hypercholesterolemic, which previous trials suggest may be associated with a more favorable response to a green tea intervention. A 2009 study by Derby and colleagues compared the impacts of both menopausal transition and advancing age on lipid biomarkers in women. The researchers determined this by monitoring lipid changes over the natural (uterus and at least one ovary intact) pre- to post-menopausal transition in 2,659 American women. Baseline lipid panels were performed on fasting serum samples, and follow-up occurred at one year, then once annually in years 3-7. Both estradiol and follicle-stimulating hormone (FSH) were monitored in participants to verify menopausal status. Total and LDL cholesterol increased significantly during the pre- to postmenopausal transition ($P<0.001$ for both), peaking during early postmenopause[66].

Overall, in studies reporting significant reductions in TC and LDL-C[33,57,59,60,64,65], the average reduction was around 6.3% and 9.54%, respectively. Though very suggestive in their findings, existing studies in green tea and lipid profile vary considerably in population, design (length, treatment, control of outside factors), and dose or type of treatment administered. Also, variation in sub-analyses, within- versus between group comparison reporting, alternate p-values, and confusing or dissimilar discussions of findings, make the overall interpretation of these results difficult. The tendency to conclusively state a significant (or lack thereof) effect of GTE consumption after a short

duration trial, or in small sample sizes, might add to the discrepancies seen as well. Of the above-mentioned studies, most end their conclusion with the call for a larger, placebo-controlled clinical trial to confirm or contest previously stated findings.

### 6. Green Tea Beverage and Lipids

In discussing the interaction of green tea and lipid metabolism, it is important to acknowledge that the type of intervention may impact the results observed. A traditional hot water infusion of the green tea leaf contains numerous compounds including proteins, amino acids, carbohydrates, vitamins, minerals, chlorophyll and flavonoids (polyphenols/catechins)[67], that are lost when the catechins are separated from the whole tea leaf. The potential for a synergistic (or antagonistic) effect is quite possible between these other constituents and the catechins *in vivo*, though generally trials relating green tea to lipids using beverage and GTE have produced similar results[68]. Additionally, the overall health-promoting properties of green tea are supported by a wide spectrum of studies, especially observational studies, which helps demonstrate the effects of whole leaf brewing versus catechin supplementation alone.

Many studies, particularly clinical trials intent on determining a cause-effect relationship, use a catechin extract intervention. This is advantageous for a few reasons. Catechin dose can be better monitored and controlled when delivered as a pre-determined amount in encapsulated form. The added convenience of ingesting a few capsules, as opposed to being asked to brew 1-10 cups of tea per day, likely increases compliance to a protocol as well. An example of this comes from a study conducted in 2010 by Arpita

Basu and collegues. In this randomized, controlled trial, the researchers compared the effects of green tea consumption on biomarkers of health in obese men and women with metabolic syndrome. The treatments were brewed green tea (928 mg catechins, 440 as EGCG; tea bags from a commercially available brand), a green tea catechin extract ("GTE": 870 mg catechins, 460 as EGCG), or control (4 cups water), consumed daily for 8 weeks. The researchers recognized the potential for inconsistencies in asking participants to brew and consume 4 cups green tea per day on their own. To circumvent this, subjects in the green tea beverage group were asked to come to the testing center each day to receive their morning dose of tea (2 cups), and were given a canister filled with 2 cups of tea to consume later with dinner. The control and GTE groups only returned to the center every few weeks for compliance measurements and/or to receive more supplement (GTE group)[69].

Interestingly, in contrast to many of the clinical trials looking at GTE and lipid parameters, no significant association was found between the extract group and changes in lipid profile. In the beverage group, a trend toward significant decreases in LDL-C and LDL/HDL ratio were observed ($P<0.1$). Also unlike most of the extract trials, participants in the green tea beverage group showed trends toward increasing levels of HDL-C ($P=0.08$)[69]. A notable component of this study was the researcher's decision to also measure changes in lipoprotein size and composition. Though a reduction of small LDL-C (atherogenic) particles was seen in the GTE group when compared to age and gender-matched controls, this change did not reach statistical significance. The researchers reported green tea beverage improved biomarkers of oxidative stress, which

was not seen in the catechin extract group. Whether this disparity is due to different sample size or bioavailability of the catechins (in beverage versus extract) is not known, but it does present a question of their differing biokinetics[69]. Consuming 800 mg of catechins per day, via extract or capsule form, is comparable to consuming 8-10 cups of moderate-strength brewed green tea per day[48].

A randomized, placebo-controlled study by Bertipaglia *et al.* also administered a brewed green tea beverage (3 grams of green tea leaves in 500 mL water; ~145 mg EGCG per day) daily to hypercholesterolemic patients and monitored changes in lipid profile. After 90 days, non-significant increases in TC, LDL-C, HDL-C and triglycerides were seen in the green tea group compared to controls[70]. The unexpected upward shift of lipid parameters post-intervention, though not statistically significant, is of interest because the amount consumed in the study is a volume widely achievable by the general public, equal to ~2 cups of green tea per day. One potential explanation of this is the control group was actually prescribed a diet low in saturated fats and cholesterol (and high in polyunsaturated fats), which the green tea group was not. Therefore, lipid profile could have improved in the control group, making even a lack of change from baseline in the green tea group appear to be adverse.

## 7. Green Tea and Cholesterol Lowering Mechanisms

### *7.1 Suppression of Biosynthesis*

Recognizing the pathways by which green tea may interfere with lipid regulation is a matter of active research. Researchers have employed various methods to test the

hypothesis that catechins may inhibit or suppress enzymes in lipogenic pathways[71–74]. Though exact mechanisms underlying these interactions are not fully understood, most of what is known has been contributed by animal and *in vitro* experiments.

Green tea extract (GTE) administered daily to obese mice was found to suppress hepatic malic enzyme activity by 67.3% ($P<0.001$), as well as significantly reduce acyl CoA: cholesterol acyltransferase activity ($P<0.01$), indicating hypolipidemic effects on fatty acid synthesis (by reducing substrate) and cholesterol regulation in green tea treated groups. There was no change in HMG-CoA reductase activity between groups[71]. Contrary to this, Cuccioloni *et al.* showed green tea to be a potent inhibitor of HMG-CoA reductase *in vitro* through competitive binding of the active site on the enzyme, as well as a potential for it to act synergistically with statin drugs to increase efficacy of treatment[72]. Additionally, increased presence and activity of cellular LDL receptor has been suggested. A study by Bursill *et al.* reported a dose-dependent increase in LDL receptor activity *in vivo*, using a rat model. In rats supplemented with 2% GTE in rat chow daily, LDL receptor protein transcription was found to be significantly higher ($P<0.05$) than in control animals, indicating increased uptake of LDL from plasma in treated rats, exerting a hypocholesterolemic effect[75].

Inhibition of biosynthesis does not only refer to the enzymes involved in lipid synthesis, it may pertain to its carriers as well. Apolipoprotein B-100 (apoB) is one of the main structural proteins on VLDL-C and LDL-C molecules, and it can be used as a biomarker of coronary artery disease risk[73]. In an *in vitro* experiment by Yee *et al.*, the effects of EGCG on inhibition of apoB secretion in a human hepatoma cell line, HepG2,

was tested. 25-hydroxycholesterol was added to test medium to simulate a lipid-rich environment, causing a two-fold stimulation in apoB secretion as compared to control cells. The addition of EGCG to this test medium reduced the effect of the 25-hydroxycholesterol by about 95%, indicating a protective effect of the catechins against cholesterol-stimulated apoB (VLDL-C, LDL-C) secretion in the *in vitro* setting[73]. Though the direct extrapolation of findings from *in vitro* and animal studies to clinical human use is complicated, they do provide a basis for the mechanistic understanding of how catechins may interact with lipid metabolism in favor of improved homeostasis.

### *7.2 Interference with Lipid Absorption*

Several *in vitro* studies have provided data pointing toward the possible mechanism of green tea inhibition of lipid absorption. A 2006 study by Shishikura *et al.* tested the emulsification properties of a lipid mixture (olive oil, phosphatidylcholine, and bile salts) exposed to various green tea catechins. The amount of catechins used in this experiment were considered achievable by typical daily consumption. Lipid droplet size increased with the addition of the catechins, thus decreasing the surface area exposed to the hydrolyzing effects of the lipases[76]. Lipolysis of core triglycerides in emulsion droplets is essential for their absorption, since they cannot be absorbed as whole triglycerides. A distinguishing characteristic of EGCG is the presence of multiple hydroxyl groups on its ring structure. In the Shishikura study, EGCG was one of the main components found on the lipid-water interface of the emulsion droplet, demonstrating its role as an inhibitor of intestinal lipolysis. The investigators

hypothesized these hydroxyl groups allow for EGCG-mediated hydrogen bonding between neighboring emulsion droplets, causing coalescence and an increase in droplet size[76]. A 2003 study by Raederstorff *et al.* also found EGCG to modify micelle formation and solubilization, contributing to reduced intestinal absorption of cholesterol. In rats given a lipid emulsion test meal supplemented with 0.1-0.5 g/kg EGCG, intestinal cholesterol absorption was reduced by ~7% - 21%, compared to controls[77].

Wang, Noh, and Koo demonstrated *in vitro* the ability of green tea catechins to inhibit pancreatic phospholipase $A_2$ ($PLA_2$), an enzyme necessary for the hydrolysis of phospholipids on the surface of emulsion droplets. Without the action of $PLA_2$ on lipid droplets, the lipid core cannot be hydrolyzed, preventing formation of mixed micelles and hindering the uptake of the lipids. EGCG exerted the greatest inhibitory effect on $PLA_2$[78]. Further compounding these effects, a study with intestinal cells suggested a minimum amount of triglyceride must first be hydrolyzed from micelles in order to stimulate cellular uptake of other hydrophobic compounds, such as cholesterol and fat-soluble vitamins[79]. Animal studies showing an increase in fecal lipid excretion in mice given an EGCG extract also support this theory[43]. This may explain interesting findings of another study by Bursill *et al.*, who propose a potentially greater cholesterol-lowering ability of tea catechins occurs when consumed in the same medium as the cholesterol (ie: diet)[80]. The physiologic barriers complicating the absorption of green tea catechins in the intestine further support the likelihood of decreased intestinal absorption being a primary mechanism for their lipid-lowering effects[7].

## 8. Conclusion

Interest in the health-promoting properties of green tea has continued to fuel literature published in this area. Existing clinical trials investigating the effects of green tea catechins on lipid profile have provided intriguing evidence for the hypolipidemic properties of these compounds in humans, particularly in the ability of green tea and GTE to significantly reduce LDL-C. As shown in section 5 however, these trials have been relatively small ($n$ = 9 to 111), short in duration (3 to 24 weeks), and produced mixed or contradictory results relative to other studies. A few also co-administered green tea treatment with a diet intervention, making it hard to distinguish the impact of the diet versus that of the catechins on lipid profile. Many of these reports also include in their conclusion a call for larger, randomized, placebo-controlled intervention trials to support the suggestive results of smaller trials, in order to better define any cause-effect relationship present. Postmenopausal women in particular are a population at increased risk of various conditions including CVD, and to our knowledge, only one other small and short-duration study[65] has investigated the effects of green tea on lipid profile in this population. Three recent meta-analyses summarizing trials of green tea and serum lipids have also been conducted[3,68,81]. Though both meta-analyses from 2011 reported significant overall reductions in TC and LDL-C with green tea consumption, one reported no statistically significant heterogeneity between studies[68], and the other did report significant heterogeneity between studies which impacted results[3]. This is despite about half the studies in each review being the same as those analyzed in the other review. Overall, the results of epidemiological studies and randomized clinical trials showing

beneficial effects of green tea on lipid and disease biomarkers is promising, and warrant further study in this potentially high-impact area.

# Chapter Two:

# Effects of Green Tea Catechin Extract on Serum Lipids in Postmenopausal Women: A Randomized, Placebo-Controlled Clinical Trial

## 1. Background

Green tea and epigallocatechin gallate (EGCG) have been shown in various human and animal trials to exert hypocholesterolemic effects *in vivo*, particularly on total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C). Recent meta-analyses of green tea and lipid clinical trials (2-24 weeks long; $n$=10-270; men and women, all ages, healthy, obese, metabolic syndrome, dyslipidemic, hypertensive, diabetic) have shown a significant average reduction in both TC (-5.90 mg/dL) and LDL-C (-4.95 mg/dL) in participants consuming green tea extract (GTE) or green tea beverage (250-2500 mg daily), compared to placebo[3,68,81]. EGCG is of great interest as it is believed to be the main bioactive catechin in green tea responsible for its hypolipidemic properties, exerting this effect through a number of proposed mechanisms (explained previously: Chapter 1, section 7). The present trial was conducted to test the reproducibility of these results in a group of postmenopausal women, a population of interest due to increased risk and incidence of dyslipidemia and related complications. With menopause there are established adverse shifts in lipid metabolism, including an increase in triglycerides (TGs), TC, LDL-C, body mass index (BMI), central adiposity, increased waist to hip ratio, and a slight reduction in high-density lipoprotein cholesterol (HDL-C), after controlling for age[82–84].

This trial was a sub-study of a larger clinical trial to evaluate the effects of consumption of green tea extract on breast cancer biomarkers in postmenopausal women at high risk of breast cancer due to dense breasts. We hypothesized that after one year, participants consuming green tea extract would exhibit significantly reduced levels of

total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and increased levels of high-density lipoprotein cholesterol (HDL-C). Further sub-group analyses were conducted with the intention of highlighting certain populations which may respond more strongly to, or derive a greater benefit from, green tea consumption. These sub-groups included women with baseline hypercholesterolemia (TC >240 mg/dL), women using statins during the study, and categorizing by baseline BMI to determine any potential influence of these factors on response to intervention.

To our knowledge there have been no large-scale, long-duration (> 6 months) clinical trials to evaluate the impact of green tea catechins on lipid profile in postmenopausal women. Significant findings in this trial may provide an additional option of care to practitioners in lipid risk management, using green tea extract as an adjunct or supplementary therapy. Moreover, the catechin dose used in this study is in general widely attainable, equating to approximately 8-10 cups of green tea per day[65].

## 2. Methods

### *2.1 Study Population*

The current trial is a sub-study of the Minnesota Green Tea Study (MGTS, not yet published), a double-blind, randomized clinical trial. The purpose of the MGTS was to evaluate the effects of consumption of GTE on a number of breast cancer biomarkers, including mammographic density, reproductive hormones, IGF axis proteins, and markers of oxidative stress, in women at high risk of breast cancer due to having dense breasts. From 2009-2013, 1,084 women were randomized into the study, and a total of

945 completed the study (12.8% withdrawal, $n$=139). The MGTS was approved by the University of Minnesota Institutional Review Board. The parent study (MGTS) design follows an intent-to-treat model (ITT), and 59 women enrolled in the study chose to stop taking supplement but remain in the study. They were designated ITT and followed through completion as normal. For purposes of this thesis a compliance model that did not include these 59 participants was used. Thus 886 women were included in the final data analysis (**Table 1**). Study participants were followed for one year while they consumed either a placebo capsule or a green tea extract (GTE) capsule containing an average of 320 mg total catechins (approximately 65% EGCG, 15% ECG, 10% EGC, 10% EC). Participants were asked to take two capsules twice per day, totaling ~1200 mg catechins (800 mg EGCG) per day in the treatment group.

Mammographic density, as a primary endpoint of the MGTS, was a parameter for recruitment eligibility. Potential participants diagnosed with heterogeneously or extremely dense breasts and at increased risk for breast cancer were sent letters of invitation to the parent study. After an initial phone screening, eligible subjects went through an orientation session on the University of Minnesota St. Paul Campus (UMNSP). All participants signed an informed consent before going forward with the study, and were provided with a copy for their records. Consented participants attended a clinical screening for eligibility at either the Delaware Clinical Research Unit (prior to July 2010) or the Human Nutrition Research Clinic (HNRC, after July 2010) at UMNSP. The clinical screening included measurement of anthropometrics and a small non-fasting blood draw for genotype and hepatitis B or C exposure. Exclusionary criteria included:

current smoker; use of hormone modification therapy (within the past 6 months); weight fluctuation of >10 pounds within the past year; experiencing a menstrual period within the last 12 months; diabetic; positive for hepatitis B or C; taking >10 prescription medications; currently taking methotrexate or Enbrel; elevated liver enzymes >1.5 upper limit of normal; consuming >7 alcoholic drinks per week; history of any cancer within the last 5 years; any history of breast cancer, ovarian cancer, or proliferative breast disease; BMI <18.5 or >40 $kg/m^2$; and not willing to consume <1 cup of green tea per week.

If eligibility was confirmed at their clinical screening, women were enrolled in the study. Randomization was completed by Investigational Drug Services (IDS) at the University of Minnesota Medical Center, Fairview, and the randomization code was kept confidential from all participants and investigators during the study and analysis. All supplements were dispensed by the IDS pharmacy, and both participants and investigators were blind to individual participant assignment throughout the entirety of the study.

### *2.2 Study Design*

Nutrient and calorie intake was determined for each participant by completion of a Diet History Questionnaire (DHQ; National Cancer Institute) at their baseline and endpoint visits. Height was measured at screening, baseline (month 0) and endpoint (month 12) visits. Weight and vitals were collected at every clinic visit, and included systolic and diastolic blood pressure, heart rate, respirations, and temperature. After

randomization, participants attended their baseline visit at the HNRC at UMNSP for fasting blood draws, urine collection, measurement of vitals, and to receive study supplement. Baseline, month 6, and month 12 were the largest and most-involved visits, which included a larger fasting blood draw for biochemical assays, 24-hour urine collection, and completion of study questionnaires. A comprehensive health history questionnaire (HHQ) was completed at the baseline visit, as well as a Menopause-Specific Quality of Life (MENQOL) questionnaire and DHQ. MENQOL was completed again at both months 6 and 12, and DHQ was repeated at month 12. A small volume of blood was drawn monthly through month 6, for monitoring of liver function enzymes. After month 6, participants came in every three months for the remainder of the study. Spot urine collections were also collected at months 3 and 9, for compliance analysis. A sample participant time line is shown in **Figure 3** (Note: months 7, 8, 10, and 11, grayed out in the figure, are depicted in the timeline to maintain month sequence, however no regularly scheduled study visits occurred during those months).

Serum collected at the HNRC for analysis was separated from whole blood by centrifugation at 1,500 *g* for 25 minutes, then separated into 1.5 mL aliquots and stored at -80 degrees Celsius until analysis. Frozen samples were sent in batches to Quest Diagnostic laboratories for measurement of a standard lipid panel. Batches were prepared by arranging participants in to GTE-placebo pairs, and all samples from each participant and pair in a batch were analyzed together in the same run. Total cholesterol, HDL-C, and triglycerides were measured in serum using the Beckman Olympus AU5400 chemistry analyzer. LDL-C was calculated using the Friedewald method[85].

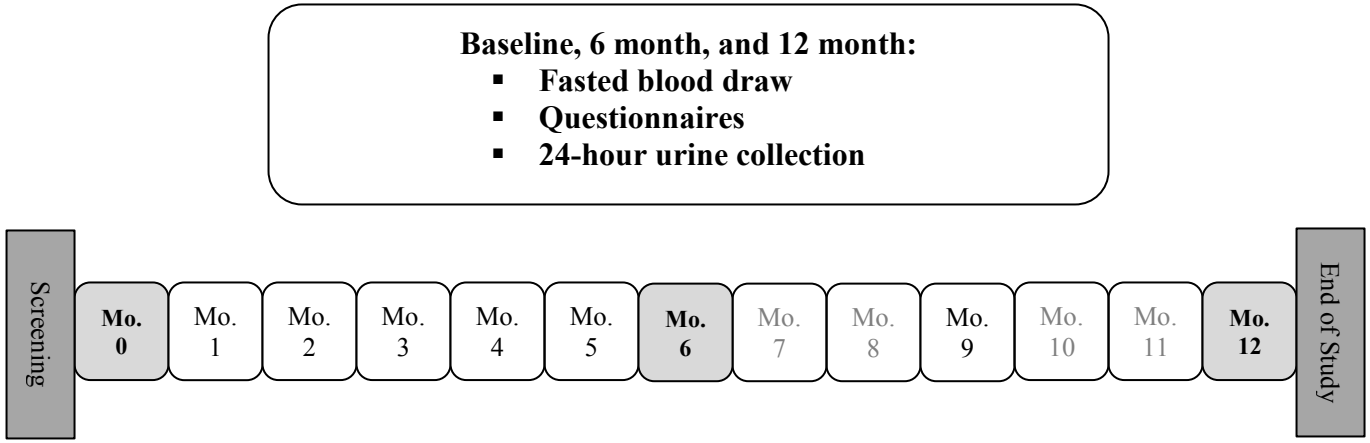


**Figure 3.** Sample Participant Timeline

*2.3 Statistical Analyses*

Data in tables are presented as lipid concentrations in mg/dL, unless otherwise noted (ie: Nutrient Intake table). Statistical analyses were performed by the study biostatistician; details of statistical method can be found below each table. Successful randomization showed an even distribution between treatment groups for the following factors: dietary (soluble dietary fiber, saturated fat, dietary cholesterol, mono- and polyunsaturated fats, alcohol, caffeine, total kcal, and macronutrients), self-reported activity level, and statin use. In all tables (except Nutrient Intake, **Table 2**), data are presented as the least squares mean and 95% confidence interval with adjustment for age and BMI.

Per protocol, all reported adverse events in our study population were coded and monitored. The two highest categories of participant adverse events were colds/infections/infestations (*n*=286), and gastrointestinal distress or disorders (*n*=260). Serious adverse events were rare (SAE, 2.21% of enrolled participants). Events in this category included those possibly attributable to supplementation as well as those most likely unrelated (ie: a planned surgery). Any participant experiencing an SAE was treated by an IRB-approved protocol, including being taken off supplement and monitored as necessary.

*2.4 Study Supplement*

The study supplement, Green Tea Extract Catechin Complex (Corban Complex GTB; abbreviated as "GTE" for use in this thesis), was manufactured and provided by

Corban Laboratories in Plymouth, MN. GTE was made from green tea leaves grown by Youshan Tea Farm in China, and supplied by Taiyo Green Power Co. Ltd. in China. The decaffeinated catechin complex was prepared by a two-step ethanol extraction of dried green tea leaves, with the first extraction producing a crude extract to be filtered and concentrated. The polyphenols were separated in the second (70%) ethanol extraction, concentrated, and recovered by a vacuum system. This process was repeated 1-3 times before the concentrated extract was spray dried into a powder, sifted, and ready for use. Corban provided eight batches of green tea supplement over the course of the trial. Catechin composition of each batch was analyzed by two separate laboratories (Corban company and C.S. Yang laboratory) to ensure consistency. A table of catechin content by batch can be found in **Appendix: 1**. Placebo capsules were identical in appearance to active GTE capsules, and contained placebo (50% maltodextrin, 49.5% cellulose, 0.5% magnesium stearate). All capsules were stored and dispensed by IDS based on individual patient schedule, and were stored in a temperature-controlled locked cabinet until given to participants by professional clinic staff during appropriate visits. Participants were provided a three-month supply of supplement at corresponding visits: baseline, month 3, month 6, and month 9 (135 capsules of green tea or placebo provided for each month). Participants were instructed to take two pills with breakfast and two pills with dinner, each day.

Each bottle contained approximately 15 extra capsules to help cover lost or damaged capsules, to prevent participant deviation from supplement schedule. Participants also received pill diaries to record when they took their supplements, to

facilitate tracking and compliance. Unused pills were returned to the HNRC at participant's visits, and study staff counted the remaining capsules to calculate compliance. Compliance based on this method was excellent (mean: 96.5%).

## 3. Results

There were no statistically significant differences between treatment and placebo groups for any baseline characteristics. The GTE group did have a borderline higher baseline BMI (25.8 kg/m$^2$ versus 25.1 kg/m$^2$, *P*=0.072; **Table 1**). The majority of the study population were Caucasian (~97%), held a high school or college degree (~93.5%), and reported regular engagement in exercise (~81%). Though 61% of women in each treatment group were self-reported tea drinkers at baseline, all were asked to reduce their intake of green tea to <1 cup per week upon enrollment.

There were no differences in average nutrient intake between treatment groups at baseline (month 0) or end of study (month 12), nor an overall treatment by time interaction (**Table 2**). A time effect was observed, however, with both groups exhibiting a post-intervention decrease in all nutrient categories except MUFA, EPA, DHA, cholesterol, and alcohol (alcohol was borderline significantly reduced, *P*=.056). The average reduction in daily calories after one year was -5.4%.

Serum levels of TC, HDL-C, LDL-C, and non-HDLC showed no difference between groups at baseline (**Table 3**). TG concentration averaged 10 mg/dL lower in the GTE group as compared to placebo group (*P*=.0007), and Chol:HDL ratio was also lower in the GTE group ( *P*=.023). After 6 months on treatment, there were significant

reductions in the GTE group compared to placebo for total cholesterol (TC, *P*<.0001), HDL-C (*P*=.0016), LDL-C (*P*<.0001), and non-HDLC (*P*=.0004). This significance remained after 12 months on treatment for TC, LDL-C, and non-HDLC, ending with overall average reductions of -1.79% for TC (*P*<.0001), -4.15% for LDL-C (*P*=.0001), and -2.13% for non-HDLC (*P*<.0001). The Chol:HDL ratio remained relatively stable over the course of intervention in both groups. TG increased in the GTE group (+3.5 mg/dL) and decreased in the placebo group (-1.7 mg/dL, *P*=.023) after 12 months on treatment. Due to the lower baseline TGs observed in the GTE group however (GTE: 91.8 mg/dL vs. Placebo: 101.9 mg/dL at Mo.0, *P*=.0007), TG levels at endpoint were quite similar in both groups, and did not differ significantly (*P*=.2897). No treatment by time effects were observed for any lipid parameter (all *P*>.203).

Further analysis tested the significance of the change in lipid biomarkers. Difference in overall change (reduction) remained significant for treatment effect in TC (*P*<.0001), LDL-C (*P*<.0001), and non-HDLC (*P*<.0001). The change of HDL-C seen in the GTE group at month 6 (-1.2 mg/dL, *P*=.0016) was corrected by the month 12 visit (-0.2 mg/dL, *P*=.084). However, the overall reduction of HDL-C in the GTE group, and overall increase in the placebo group, resulted in a significant treatment effect on HDL-C in a direction opposing the hypothesis (*P* overall for change from baseline = .004).

Subgroup analyses were conducted to investigate six factors of interest relating to the overall impact of catechin supplementation. The factors examined were baseline dyslipidemia (elevated TC or LDL-C and reduced HDL-C), baseline BMI, *COMT* genotype, and statin use. Findings from previous trials on green tea effects on cholesterol

concentrations suggest that catechins may exert a stronger cholesterol-lowering effect in people who are hypercholesterolemic at baseline[59,64]. However, the addition of a dietary or exercise intervention in these trials makes interpretation of the results difficult. Our study design should allow for a clearer analysis of effects of GTE supplementation. To measure treatment effect by baseline cholesterol, all participants were stratified into one of the following three clinical sub-categories, as set by the National Cholesterol Education Program of the National Institutes of Health[86]: <200 mg/dL ("Desirable"), 200-239 ("Borderline High"), ≥ 240 mg/dL ("High") . Participant allocation and results are shown in **Table 4**.

Significant reductions in TC, LDL-C, and non-HDLC were observed in the two lowest BMI categories (≤ 24.9, and 25-29.9 kg/m$^2$, **Table 5**). A decrease in HDL-C was observed in the lowest BMI category, measuring -1.6 mg/dL at midpoint and rising to -0.4 mg/dL by endpoint ($P$=.03). In contrast, a small increase in HDL-C was seen in the highest BMI category at month 12, though this elevation proved insignificant (+1.1 mg/dL, $P$=.338). An overall interaction was found between treatment group, BMI, and TG ($P$=.017), particularly relating to the moderate increase in TGs seen in the lowest (≤ 24.9 kg/m$^2$, $P$=.029) and highest (≥30 kg/m$^2$, $P$=.006) BMI categories (no change in TG in the 25-29.9 kg/m$^2$ group). Baseline BMI did not appear to effect Chol:HDL ratio (**Table 5**).

Statin use was not restricted during the trial, and approximately 20% of study participants reported using statins during their enrollment (GTE: 21.2%, Placebo: 19.9%). The inclusion of these participants provided a unique opportunity to explore any potential

effects of simultaneous use of statins and GTE. A general decrease in the impact of GTE was observed in those who concomittantly took statins, versus those taking the GTE alone (**Table 6**). Within statin users, GTE versus placebo group comparisons showed a significant reduction of LDL-C in the GTE group at 6 months (-5.2 mg/dL, *P*=.011). This change was comparable to a similar reduction seen at 6-months in the GTE group of non-users ("non-users", -6.0 mg/dL, *P*=.0004). This significant reduction remained by month 12 in non-users (-5.9 mg/dL, *P*=<.0001), but waned in the statin users (-1.6 mg/dL at month 12, *P*=.133). Significant reductions in LDL-C were seen in both statin users and non-users taking GTE (*P*=.026 and *P*<.0001, respectively), however the overall treatment effect was lessened in statin users. Reduction of TC was borderline significant after 6 months in statin users taking GTE (-4.0 mg/dL, *P*=.051), however TC rose again by one year to near-baseline levels (-0.2 mg/dL, *P*=.344, between group comparison at month 12). Curiously, TGs were the only other lipid to show a change in statin users, as well as the only lipid parameter to show an overall interaction between statin use and GTE supplementation. By month 6, statin users taking GTE showed an average increase in TGs of +7.9 mg/dL (*P*=.021) when compared to those not taking GTE. This trend continued to an average increase of +10.0 mg/dL by month 12 (*P*=.0145). The difference between treatment groups in statin users was significant (*P*=.005), as was the overall association between TG, effect of GTE, and statin use (*P*=.014). Aside from TGs, no additional overall interactions between treatment group, statin use, and lipid parameters, were seen (**Table 6**).

We were also interested to see if *COMT* genotype influenced a participant's

response to GTE supplementation. All 886 participants were stratified in to their respective genotype category, and a genotype-lipid-treatment group interaction was analyzed (mixed model). No significant interactions were found between *COMT* genotype and treatment effect (p values: TC=0.960, LDL-C=0.793, HDL-C=0.445, TG=0.943, Chol:HDLr=0.222, Non-HDLC=0.773). Similar to the stratification for baseline total cholesterol sub-analysis (**Table 4**), baseline HDL-C and LDL-C were examined and analyzed. No significant category-lipid-treatment group interactions were found for either of these (data not shown).

## 4. Summary of Major Findings

A. Total cholesterol (*P*<.0001), LDL-C (*P*<.0001), and non-HDLC (*P*<.0001) were significantly reduced in the GTE group after one year on treatment compared to placebo. Average reductions in percent mg/dL were -1.79% (TC), -4.15% (LDL-C) and -2.13% (non-HDLC).

B. HDL-C decreased significantly in the GTE group by month 6 (*P*=.0016), and though it increased and was comparable to levels in the placebo group by month 12 (*P*=.0836), the overall treatment effect for change from baseline was significant in an adverse direction (*P*=.0038).

C. Average triglyceride (TG) concentration was 10 mg/dL lower in the GTE group than placebo group at baseline. TGs increased in the GTE group and decreased in the placebo group over the course of the intervention, resulting in an overall significant treatment effect (*P*=.013) between groups despite average endpoint values being similar (GTE: 99.0 mg/dL vs. Placebo: 96.8 mg/dL, *P*=.2897).

D. A significant overall interaction was seen between baseline cholesterol level and treatment effect for TC (*P*=0.028, **Table 4**). A borderline significant interaction was also seen for non-HDLC (*P*=0.065).

## 5. Discussion

Previous clinical trials investigating the effect of green tea catechins on serum lipids have called for the need for larger, longer-duration clinical trials to verify or refute earlier findings[32,57,59,65]. The results of our study provide evidence to support the ability of green tea catechins to reduce total cholesterol (-4.6 mg/dL, *P*=<.0001), LDL-C (-5.0 mg/dL, *P*=<.0001), and non-HDLC (-4.4 mg/dL, *P*=<.0001) in postmenopausal women. Circulating estradiol has been positively associated with HDL-C, and negatively associated with TC and LDL-C[87]. Thus, these are significant findings as menopause is independently associated with adverse changes in lipid profile[82,83,88]. A borderline significant decrease in the Chol:HDL ratio was seen in the GTE group after one year on treatment, versus no change in the placebo group. This is of interest to note, as chol:HDL ratio can be used clinically as an indicator for cardiovascular risk as well[86]. The final concentrations of HDL-C in the treatment group were not significantly different than the placebo group. However due to the opposing trend seen at the month 6 time point (GTE: -1.2 mg/dL, Placebo: +0.6 mg/dL, *P*=.0016), the overall treatment effect was deemed significant (change in HDL-C from baseline: *P*=.004). Our study demonstrated supplementation with GTE for one year resulted in significant overall reductions in TC, LDL-C, and non-HDLC.

In the first sub-analysis (**Table 4**), overall treatment effect by baseline TC category was examined. The largest (magnitude) improvement in any lipid parameter in our study was the change in TC concentration seen after one year on GTE in women with a baseline cholesterol level of >240 mg/dL (considered "high" by NCEP standards).

Though reductions in TC, LDL-C, and non-HDL-C were also seen in the placebo group in this category, the overall change in in the >240 group (-23.0 mg/dL, *P*=.0012) is still striking and shown to be significant. TC was significantly reduced in the 200-240 group (-7.4 mg/dL overall, *P*=.0001) and the <200 group (+4.2 mg/dL, *P*=.065) as well. Likewise, LDL-C was significantly reduced in the >240 mg/dL baseline group (-20.7 mg/dL, *P*=.007) and 200-240 mg/dL group (-6.6 mg/dL *P*<.0001), and LDL-C increased slightly in the <200 mg/dL group (+1.7 mg/dL, *P*=.024). A thorough review by M.C. Houston on non-pharmacologic therapies for dyslipidemia suggests that similar to green tea catechins, a number of compounds appear to reduce cholesterol or triglycerides more effectively in hyperlipidemic participants, such as dietary/soluble fiber[89] and omega-3 fish oils[90]. A similar association has been reported for statins as well[91].

BMI and lipids have been shown to be positively correlated as well, particularly related to TC and LDL-C[82]. Given the suggested increase in efficacy of catechins in hypercholesterolemic groups, we felt it was plausible to explore this effect by BMI. Changes in lipid profile for the lowest BMI category ($\leq$24.9 kg/m$^2$) tended to follow the trend of the other two categories, though change in levels were often less pronounced. Women in the highest BMI category ($\geq$30 kg/m$^2$) exhibited larger magnitudes of change, in both GTE and placebo groups, so no significant interactions were shown for this category except a rise in TGs in the GTE group after one year on treatment (+6.0 mg/dL, *P*=.0064). The only lipid parameter to show an overall interaction between treatment group and statin use were triglycerides (TG). After one year on GTE treatment, TGs rose +10.0 mg/dL in statin users (*P*=0145), though only +1.8 mg/dL in non-users (*P*=.244).

The average TG level at baseline was 101.9 mg/dL in the placebo group, and 91.8 mg/dL in the GTE group (*.P*=0007), thus the +10.0 mg/dL increase in TG seen with GTE+statin supplementation brought both groups to a similar endpoint concentration by month 12. This is surprising because combination therapy is sometimes used to help maximize a patient's response to or efficacy of a treatment. For example, nicotinic acid may be prescribed to increase HDL-C in a patient who is also taking a statin to decrease LDL-C. Additional combinations may include a concomitant use of plant sterol/stanols, soluble fiber, statin, fish oil, or bile acid sequestrants, all of which have been independently associated, to varying degrees, with lipid profile improvement[89]. Adverse diet-drug interactions are also known to exist however, such as the inhibitory effect of grapefruit juice on statin metabolism[92]. Though slight increases in TG have been shown in some previous green tea and lipid clinical trials, the greater potency of TG-reduction by statins would be expected to override that. Somewhat complicating this however, is paper by Stein *et al.* which showed statins effectively reduced triglyceride levels, though only in patients who were hyperlipidemic at baseline[91]. It is possible this is why no triglyceride-lowering effect was seen in the GTE, since baseline levels were already low.

This was not a mechanistic study, and the exact nature of this interaction is not fully understood. It has been suggested the degradation of green tea catechins in the high pH of the small intestine decreases their overall absorption and bioavailability[10,93]. An *in vitro* study by Green and colleagues tested the stabilizing effects of various additives, including grapefruit juice, to green tea in a simulated small intestine environment. The addition of a grapefruit juice+catechin mixture to the simulated intestine increased the

recovery (post-digestion presence) of full catechins from <20% to 61% [93]. Assuming these results translate to greater *in vivo* bioavailability would be inappropriate, however it highlights the need to be open to unexpected interactions, including any potential diet-catechin-drug interactions.

Participants were also separated into groups based on their *COMT* genotype (A/A: low-activity, A/G: intermediate-activity, G/G: high-activity), an enzyme involved in catechin metabolism. Previous studies have suggested carriers of the low-activity *COMT* allele may derive more benefit from consuming green tea, due to the decreased enzyme activity and thus slower excretion of catechins from the body[94,95]. However, if reduced intestinal absorption of lipids is indeed the primary mechanism by which green tea catechins exert their hypolipidemic effects, it is understandable that the *COMT* enzyme would have little effect on this process, as the action would take place before the catechins would be metabolized.

Strengths of the current study include having a large study population ($n$=886), high compliance to treatment (mean: 96.5%), and being an extended intervention trial (12 months) with a well-executed, rigorous study design (randomized, double-blind, placebo-controlled). The treatment being tested was a concentrated catechin extract of Chinese green tea leaves, equating to approximately 8-10 cups of brewed green tea per day. Though some constituents of the whole leaf are lost in a catechin extraction (ie: vitamins, amino acids, fiber, minerals), this method provides more control over treatment amount and helps to reduce the variability in daily catechin intake that may happen if participants were asked to brew their own tea. Additionally, some studies have demonstrated

enhanced catechin absorption and bioactivity with green tea extract capsules as compared to similar amounts provided in brewed green tea[96]. Our use of an encapsulated green tea extract in this trial also helped encourage compliance, and allowed for successful blinding of treatments.

Limitations of this study include a lack of diversity in race/ethnicity, and the self-reported data on medication use and health history, physical activity habits, and dietary intake of nutrients. Although participants were asked to maintain stable body weight, consume four study capsules per day, and to inform study staff of any changes in medication, the integrity of this data is reliant on their compliance to our requests. Participants were expected to come to blood-draws in a fasted state, and unless gross lipemia was seen during processing of their blood work, it was believed the participant was fasting. Non-compliance with fasting requirements for blood draws was not believed to be an issue during sample collection. Participants often self-reported if they had consumed anything prior to their blood draw (ie: coffee), and if they had, they were asked to re-schedule and return fasting at a later date. Though fasting is more important for triglyeride and LDL-C measurement (which can be underestimated in a non-fasting blood sample), TC, HDL-C, Non-HDL, and Chol:HDL ratio are essentially unaffected by fasting status[97]. Even so, the large sample size of our population is likely to neutralize an overall impact of any occasional non-fasting lipid panel. Our biochemical assays were also based on a single sample collection for each time point, though the use of an automated chemistry analyzer for the lipid panels helped to maintain consistency of testing protocol and accuracy.

As shown in **Table 1**, the majority of our study population was Caucasian. This is possibly a consequence of the centers used for recruitment for the parent study, primarily located in the University and suburban neighborhoods of Minneapolis and St. Paul. Worth noting, a smaller ($n$=103) study from 2012 of very similar design to the current trial, reported similar trends in lipid change in a majority Hispanic population (53% of participants)[65]. These limitations may somewhat reduce our ability to extrapolate these results to a general population, however we believe the underlying trend in the results is promising.

In conclusion, consuming 1200 mg GTE (800 mg as EGCG) for one year significantly reduced total cholesterol (-4.6 mg/dL, $P$=<.0001), LDL-cholesterol (-5.0 mg/dL, $P$<.0001), and non-HDL cholesterol (-4.4 mg/dL, $P$<.0001) in postmenopausal women. Triglycerides rose in women taking both GTE and statins, as well as overall with GTE supplementation. In the overall analysis by treatment effect, TGs were raised an average of +3.5 mg/dL ($P$=.0133) after one year, though the underlying mechanism responsible for this increase has yet to be elucidated. A small but significant decrease in HDL-C was observed in the GTE supplement group after six months (-1.2 mg/dL, $P$=.0016), returning to near normal after one year though remaining statistically significant (-0.2 mg/dL, $P$=.0038). A generally stronger lipid-modifying effect was seen at the 6 month time point, with mild to moderate attenuation in some parameters by month 12. Exploratory subgroup analyses showed GTE had the greatest and most favorable impact on serum lipids in women with a baseline total cholesterol >240 mg/dL.

This study was a well-designed, placebo-controlled clinical intervention demonstrating a strong association between consumption of a green tea catechin extract and reduction of total, LDL-, and non-HDL cholesterol in postmenopausal women. Research into dosing guidelines, possible drug interactions, and reproducibility in hypercholesterolemic patients, would be beneficial, to further the potential therapeutic use of these findings.

## 6. Tables

**Table 1. Baseline demographic and lifestyle variables by treatment group.**

| Variable | GTE: (*n*=429) | Placebo: (*n*=457) | *P* value[1] |
|---|---|---|---|
| **Age (SD), years** | 59.9 (5.0) | 59.6 (5.0) | 0.362 |
| **BMI (SD), kg/m$^2$** | 25.8 (7.8) | 25.1 (3.6) | 0.072 |
| **Race** | | | |
| **White, *n* (%)** | 418 (97.4) | 441 (96.5) | 0.481 |
| **Education** | | | |
| **College or High, *n* (%)** | 400 (93.9) | 423 (93.4) | 0.753 |
| **Ever smoking** | | | |
| **Yes, *n* (%)** | 139 (32.4) | 142 (31.1) | 0.739 |
| **Alcohol drinking** | | | |
| **Yes, *n* (%)** | 340 (79.3) | 380 (83.2) | 0.268 |
| **Tea drinking** | | | |
| **Yes, *n* (%)** | 263 (61.5) | 283 (62.2) | 0.228 |
| **Regular exercise** | | | |
| **Yes, *n* (%)** | 347 (80.9) | 372 (81.4) | 0.957 |
| **Taking aspirin** | | | |
| **Yes, *n* (%)** | 25 (5.8) | 31 (6.8) | 0.559 |
| **Taking statins** | | | |
| **Yes, *n* (%)** | 91 (21.2) | 91 (19.9) | 0.632 |
| **Total energy intake (SD), kcal** | 1435 (545) | 1443 (530) | 0.809 |

[1]From t-test for continuous variables and $\chi^2$ test for categorical variables.

**Table 2. Nutrient Intake at Month 0 (baseline) & Month 12 (endpoint) by Treatment Group.[1]**

| Nutrient, Time Point | GTE: *n*=428 Mean (SD) | Placebo: *n*=455 Mean (SD) | *P* values | | |
|---|---|---|---|---|---|
| | | | Visit[2] | Trt[3] | Time[3] |
| **Total Energy (kcal)** | | | | 0.9519 | **<.0001** |
| Month 0 | 1436 (1384.9-1486.0) | 1441 (1391.8-1490.0) | 0.8825 | | |
| Month 12 | 1361 (1314-1408.3) | 1360 (1314.5-1405.0) | 0.9770 | | |
| **Carbohydrate (g)** | | | | 0.9031 | **<.0001** |
| Month 0 | 179.6 (172.9-186.2) | 181.4 (175-187.9) | 0.6901 | | |
| Month 12 | 168.4 (162.2-174.5) | 167.6 (161.6-173.5) | 0.8534 | | |
| **Protein (g)** | | | | 0.4132 | **<.0001** |
| Month 0 | 58.7 (56.5-60.8) | 57.4 (55.3-59.5) | 0.4278 | | |
| Month 12 | 55.7 (53.6-57.7) | 54.6 (52.6-56.6) | 0.4781 | | |
| **Total Fat (g)** | | | | 0.7182 | **0.0272** |
| Month 0 | 52.9 (50.5-55.3) | 53.7 (51.4-56) | 0.6368 | | |
| Month 12 | 51.7 (49.5-53.9) | 52.0 (49.8-54.2) | 0.8456 | | |
| **Saturated Fat (g)** | | | | 0.9356 | **0.0269** |
| Month 0 | 16.2 (15.5-16.9) | 16.4 (15.7-17.1) | 0.7328 | | |
| Month 12 | 15.9 (15.2-16.6) | 15.8 (15.2-16.5) | 0.8393 | | |
| **MUFA (g)** | | | | 0.6081 | 0.1240 |
| Month 0 | 20.5 (19.5-21.5) | 20.8 (19.9-21.8) | 0.6751 | | |
| Month 12 | 20.1 (19.2-21) | 20.4 (19.5-21.3) | 0.5894 | | |
| **PUFA (g)** | | | | 0.6249 | **0.0162** |
| Month 0 | 12.2 (11.6-12.8) | 12.5 (11.9-13.1) | 0.4712 | | |
| Month 12 | 11.9 (11.3-12.5) | 11.9 (11.4-12.5) | 0.8787 | | |
| **Omega-3 FAs (g)** | | | | | |
| **EPA (20:5)** | | | | 0.6171 | 0.4182 |
| Month 0 | 0.0 (0-0) | 0.0 (0-0) | 0.6981 | | |
| Month 12 | 0.0 (0-0) | 0.0 (0-0) | 0.6328 | | |

| | | | | | |
|---|---|---|---|---|---|
| **DHA (22:6)** | | | | 0.6009 | 0.1410 |
| Month 0 | 0.0 (0-0.1) | 0.0 (0-0.1) | 0.6055 | | |
| Month 12 | 0.0 (0-0.1) | 0.0 (0-0) | 0.7070 | | |
| **ALA (18:3)** | | | | 0.9434 | **<.0001** |
| Month 0 | 1.0 (1-1.1) | 1.0 (1-1.1) | 0.6594 | | |
| Month 12 | 1.0 (0.9-1) | 1.0 (0.9-1) | 0.7409 | | |
| **Trans-FA (g)** | | | | 0.5005 | **0.0041** |
| Month 0 | 2.7 (2.6-2.8) | 2.7 (2.5-2.8) | 0.7925 | | |
| Month 12 | 2.6 (2.5-2.7) | 2.5 (2.4-2.7) | 0.3204 | | |
| **Cholesterol (mg)** | | | | 0.6275 | 0.1077 |
| Month 0 | 145.5 (138.6-152.3) | 142.1 (135.5-148.8) | 0.4986 | | |
| Month 12 | 141.3 (134.5-148) | 140.2 (133.6-146.8) | 0.8321 | | |
| **Total Fiber (g)** | | | | 0.7524 | **<.0001** |
| Month 0 | 17.6 (16.8-18.3) | 17.6 (16.9-18.4) | 0.9442 | | |
| Month 12 | 16.7 (16-17.4) | 16.4 (15.7-17.1) | 0.4948 | | |
| **Soluble Fiber (g)** | | | | 0.8359 | **<.0001** |
| Month 0 | 5.8 (5.5-6.1) | 5.8 (5.6-6.1) | 0.8542 | | |
| Month 12 | 5.5 (5.3-5.8) | 5.4 (5.2-5.7) | 0.5449 | | |
| **Caffeine (mg)** | | | | 0.2569 | **0.0046** |
| Month 0 | 358.3 (327.9-388.7) | 379.0 (349.6-408.5) | 0.3371 | | |
| Month 12 | 337.3 (308.8-365.9) | 361.1 (333.4-388.8) | 0.2416 | | |
| **Alcohol (g)** | | | | 0.9785 | 0.0564 |
| Month 0 | 7.0 (5.8-8.2) | 6.5 (5.4-7.7) | 0.5927 | | |
| Month 12 | 5.8 (5-6.6) | 6.2 (5.5-7) | 0.4296 | | |

[1] Data is presented in mean (95% CI); adjusted for Age and BMI.
[2] Test for difference between treatment and placebo group by time point using ANCOVA (GLM).
[3] Test for overall difference between treatment groups, and change over time, using mixed methods for repeated measures data.
*Abbreviations:* MUFA (monounsaturated fatty acid), PUFA (polyunsaturated fatty acid), FA (fatty acid), EPA (eicosapentaenoic acid), DHA (docosahexaenoic acid), ALA (α-linolenic acid), Total Fiber = Total Dietary Fiber.

**Table 3. Mean level and change of blood lipids by treatment group and time point.**

| Measure (mg/dL)[1] | GTE: *n*=429 | Placebo: *n*=455 | *P* [3] | Overall Trt[4] | Overall Time[4] |
|---|---|---|---|---|---|
| **TC** | | | | | |
| Value at Mo.0 | 206.5 (203.6-209.5) | 208.8 (205.9-211.7) | 0.2760 | | |
| Value at Mo.6[2] | 200.8 (199-202.7) | 207.7 (205.9-209.5) | <.0001 | | |
| Change from 0 | -6.6 (-8.6-4.7) | -0.3 (-2.2-1.6) | <.0001 | | |
| Value at Mo.12[2] | 202.8 (200.9-204.8) | 209.5 (207.6-211.4) | <.0001 | | |
| Change from 0 | -4.6 (-6.7-2.5) | 1.6 (-0.5-3.6) | <.0001 | | |
| *P* (actual value) | | | | **<.0001** | **0.0068** |
| *P* (change) | | | | **<.0001** | **0.0068** |
| **HDL** | | | | | |
| Value at Mo.0 | 70.2 (68.5-71.9) | 68.9 (67.3-70.5) | 0.2778 | | |
| Value at Mo.6[2] | 68.4 (67.7-69.2) | 70.0 (69.3-70.7) | 0.0028 | | |
| Change from 0 | -1.2 (-2-0.4) | 0.6 (-0.2-1.3) | 0.0016 | | |
| Value at Mo.12[2] | 69.4 (68.6-70.1) | 70.2 (69.4-70.9) | 0.1306 | | |
| Change from 0 | -0.2 (-1-0.6) | 0.8 (0-1.5) | 0.0836 | | |
| *P* (actual value) | | | | **0.0071** | **0.0416** |
| *P* (change) | | | | **0.0038** | **0.0405** |
| **TG** | | | | | |
| Value at Mo.0 | 91.8 (87.7-96) | 101.9 (97.9-105.9) | 0.0007 | | |
| Value at Mo.6[2] | 98.2 (95.5-101) | 96.4 (93.7-99.1) | 0.3628 | | |
| Change from 0 | 2.0 (-0.9-4.8) | -1.4 (-4.1-1.4) | 0.0987 | | |
| Value at Mo.12[2] | 99.0 (96.1-102) | 96.8 (93.9-99.7) | 0.2897 | | |
| Change from 0 | 3.5 (0.3-6.7) | -1.7 (-4.8-1.4) | 0.0232 | | |
| *P* (actual value) | | | | 0.1920 | 0.6438 |
| *P* (change) | | | | **0.0133** | 0.6454 |

| | | | | | |
|---|---|---|---|---|---|
| **LDL** | | **(Placebo: *n*=454)*** | | | |
| Value at Mo.0 | 118.0 (115.3-120.6) | 119.5 (116.9-122.1) | 0.4204 | | |
| Value at Mo.6[2] | 112.3 (109.6-114.9) | 118.9 (116.4-121.5) | 0.0004 | | |
| Change from 0 | -5.8 (-7.5-4.1) | -0.5 (-2.1-1.2) | <.0001 | | |
| Value at Mo.12[2] | 113.1 (110.5-115.7) | 120.4 (117.9-123) | 0.0001 | | |
| Change from 0 | -5.0 (-6.8-3.1) | 1.1 (-0.7-2.9) | <.0001 | | |
| *P* (actual value) | | | | **<.0001** | 0.0610 |
| *P* (change) | | | | **<.0001** | 0.0571 |
| **Chol:HDL ratio** | | | | | |
| Value at Mo.0 | 3.1 (3-3.2) | 3.2 (3.2-3.3) | 0.0231 | | |
| Value at Mo.6[2] | 3.1 (3.1-3.1) | 3.1 (3.1-3.2) | 0.0577 | | |
| Change from 0 | -0.1 (-0.1-0) | 0.0 (-0.1-0) | 0.2576 | | |
| Value at Mo.12[2] | 3.1 (3-3.1) | 3.2 (3.1-3.2) | 0.0210 | | |
| Change from 0 | -0.1 (-0.1-0) | 0.0 (-0.1-0) | 0.2117 | | |
| *P* (actual value) | | | | **0.0137** | 0.9099 |
| *P* (change) | | | | 0.1696 | 0.9046 |
| **Non-HDLC** | | | | | |
| Value at Mo.0 | 136.3 (133.4-139.2) | 139.9 (137.1-142.7) | 0.0837 | | |
| Value at Mo.6[2] | 132.4 (130.6-134.1) | 137.7 (136-139.3) | <.0001 | | |
| Change from 0 | -5.4 (-7.3--3.6) | -0.9 (-2.6-0.9) | 0.0004 | | |
| Value at Mo.12[2] | 133.4 (131.5-135.3) | 139.4 (137.5-141.2) | <.0001 | | |
| Change from 0 | -4.4 (-6.4-2.4) | 0.8 (-1.2-2.7) | 0.0003 | | |
| *P* (actual value) | | | | **<.0001** | **0.0446** |
| *P* (change) | | | | **<.0001** | **0.0453** |

[1] Data is presented in mean (95% CI); adjusted for Age and BMI.
[2] Adjusted for Age, BMI, and baseline lipid value, for that parameter (ie: HDL measured value at Month 6 and Month 12 adjusted for baseline HDL).
[3] Test for treatment effect between GTE and placebo groups at time point using ANCOVA (GLM).

[4] Test for overall treatment, time, and treatment by time, effects between GTE and placebo groups using mixed methods for repeated measures.
* LDL values could not be calculated for one participant due to triglyceride levels >400 mg/dL.

**Table 4. Treatment Effect: by Baseline Total Cholesterol (TC) Category**

| Measure & Group (*n*)[1] | Time point | | Overall *P* values: | | | |
|---|---|---|---|---|---|---|
| | change Mo.0 to Mo.6 (mg/dL) | change Mo.0 to Mo.12 (mg/dL) | Trt[3] | Time[3] | Trt* Time[3] | Interaction[4] |
| **TC** | | | | | | **0.028** |
| **<200 mg/dL** | | | 0.0652 | **0.0038** | 0.3683 | |
| GTE (181) | 0.2 (-2.6-3) | 4.2 (1.2-7.2) | | | | |
| Placebo (184) | 4.5 (1.8-7.3) | 6.5 (3.5-9.4) | | | | |
| *P*[2] | 0.0314 | 0.2792 | | | | |
| **200-240 mg/dL** | | | **<.0001** | 0.1296 | 0.8220 | |
| GTE (191) | -9.3 (-12.1-6.5) | -7.4 (-10.2-4.6) | | | | |
| Placebo (204) | -1.5 (-4.2-1.2) | -0.1 (-2.8-2.6) | | | | |
| *P*[2] | <.0001 | 0.0003 | | | | |
| **>240 mg/dL** | | | **0.0012** | 0.9402 | 0.2354 | |
| GTE (57) | -19.4 (-25.4-13.3) | -23.0 (-29.6-16.4) | | | | |
| Placebo (67) | -10.2 (-15.8-4.6) | -7.0 (-13.1-1) | | | | |
| *P*[2] | 0.0338 | 0.0007 | | | | |
| **HDL** | | | | | | 0.535 |
| **<200 mg/dL** | | | 0.3085 | 0.3043 | **0.0303** | |
| GTE | 0.0 (-1.2-1.2) | 1.4 (0.2-2.7) | | | | |
| Placebo | 1.7 (0.5-2.9) | 1.2 (0-2.4) | | | | |
| *P*[2] | 0.0464 | 0.8035 | | | | |
| **200-240 mg/dL** | | | **0.0106** | 0.1111 | 0.9522 | |
| GTE | -2.1 (-3.3-0.9) | -1.4 (-2.6-0.3) | | | | |
| Placebo | -0.3 (-1.5-0.9) | 0.4 (-0.7-1.5) | | | | |
| *P*[2] | 0.0421 | 0.0215 | | | | |
| **>240 mg/dL** | | | 0.0663 | 0.4565 | 0.9521 | |
| GTE | -2.1 (-3.8-0.4) | -1.3 (-3.7-1.1) | | | | |
| Placebo | 0.1 (-1.5-1.7) | 0.7 (-1.5-2.9) | | | | |
| *P*[2] | 0.0715 | 0.2404 | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| **TG** | | | | | | 0.139 |
| **<200 mg/dL** | | | **0.0281** | 0.2661 | 0.5012 | |
| GTE | 4.9 (0.7-9.1) | 5.2 (1-9.4) | | | | |
| Placebo | -2.1 (-6.3-2) | 1.0 (-3.1-5.1) | | | | |
| *P*[2] | 0.0204 | 0.1615 | | | | |
| **200-240 mg/dL** | | | **0.0411** | 0.7652 | 0.6148 | |
| GTE | 3.0 (-1.2-7.2) | 3.2 (-1.2-7.7) | | | | |
| Placebo | -1.4 (-5.5-2.7) | -2.7 (-7-1.6) | | | | |
| *P*[2] | 0.1469 | 0.0615 | | | | |
| **>240 mg/dL** | | | 0.6416 | 0.8275 | 0.1973 | |
| GTE | -9.3 (-17.7-0.9) | -2.5 (-15.9-11) | | | | |
| Placebo | -0.4 (-8.2-7.4) | -5.0 (-17.4-7.4) | | | | |
| *P*[2] | 0.1334 | 0.7864 | | | | |
| **LDL** | | | | | | 0.142 |
| **<200 mg/dL** | | | **0.0245** | **0.0138** | 0.8183 | |
| GTE | -0.7 (-3.2-1.7) | 1.7 (-1-4.4) | | | | |
| Placebo | 3.3 (0.9-5.7) | 5.1 (2.4-7.8) | | | | |
| *P*[2] | 0.0235 | 0.0847 | | | | |
| **200-240 mg/dL** | | | **<.0001** | 0.2576 | 0.9127 | |
| GTE | -7.8 (-10.2-5.4) | -6.6 (-9-4.2) | | | | |
| Placebo | -0.9 (-3.2-1.4) | 0.0 (-2.3-2.4) | | | | |
| *P*[2] | <.0001 | 0.0002 | | | | |
| **>240 mg/dL** | | | **0.0066** | 0.5015 | 0.1163 | |
| GTE (57) | -15.4 (-20.9-10) | -20.7 (-26.9-14.6) | | | | |
| Placebo (66*) | -9.7 (-14.8-4.6) | -7.2 (-12.9-1.5) | | | | |
| *P*[2] | 0.1400 | 0.0021 | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Chol:HDLratio** | | | | | | 0.754 |
| **<200 mg/dL** | | | 0.2685 | 0.2370 | 0.1814 | |
| GTE | 0.0 (-0.1-0.1) | 0.0 (-0.1-0.1) | | | | |
| Placebo | 0.0 (-0.1-0.1) | 0.1 (0-0.1) | | | | |
| *P*[2] | 0.6853 | 0.1121 | | | | |
| **200-240 mg/dL** | | | 0.5576 | 0.5961 | 0.3640 | |
| GTE | -0.1 (-0.1-0) | -0.1 (-0.1-0) | | | | |
| Placebo | 0.0 (-0.1-0) | -0.1 (-0.1-0) | | | | |
| *P*[2] | 0.3047 | 0.9681 | | | | |
| **>240 mg/dL** | | | 0.4588 | 0.4793 | 0.8300 | |
| GTE | -0.2 (-0.3-0.1) | -0.3 (-0.5-0.1) | | | | |
| Placebo | -0.1 (-0.3-0) | -0.2 (-0.3-0) | | | | |
| *P*[2] | 0.5940 | 0.4106 | | | | |
| **Non-HDLC** | | | | | | 0.065 |
| **<200 mg/dL** | | | 0.1420 | **0.0074** | 0.9843 | |
| GTE | 0.2 (-2.4-2.9) | 2.7 (-0.2-5.6) | | | | |
| Placebo | 2.8 (0.2-5.5) | 5.3 (2.4-8.1) | | | | |
| *P*[2] | 0.1720 | 0.2154 | | | | |
| **200-240 mg/dL** | | | **0.0003** | 0.3422 | 0.7839 | |
| GTE | -7.2 (-9.7-4.7) | -5.9 (-8.6-3.2) | | | | |
| Placebo | -1.2 (-3.6-1.3) | -0.5 (-3.1-2.1) | | | | |
| *P*[2] | 0.0009 | 0.0048 | | | | |
| **>240 mg/dL** | | | **0.0072** | 0.7229 | 0.2365 | |
| GTE | -17.3 (-23-11.6) | -21.7 (-28.7-14.8) | | | | |
| Placebo | -10.3 (-15.6-5.1) | -7.7 (-14.1-1.3) | | | | |
| *P*[2] | 0.0836 | 0.0047 | | | | |

[1] Data is presented in mean (95% CI); adjusted for Age and BMI.
[2] Test for treatment effect between GTE and placebo groups at time point using ANCOVA (GLM).
[3] Test for overall treatment, time, and treatment by time, effects between GTE and placebo groups using mixed methods for repeated measures.

[4] Test for overall interaction between treatment group and stratified variables using mixed methods for repeated measures.
* LDL values could not be calculated for one participant due to triglyceride levels >400 mg/dL.

**Table 5. Treatment Effect: by Baseline BMI Category**

| Measure (kg/m$^2$) & Group (*n*)[1] | Time point | | Overall $P$ values: | | | |
|---|---|---|---|---|---|---|
| | change Mo.0 to Mo.6 (mg/dL) | change Mo.0 to Mo.12 (mg/dL) | Trt[3] | Time[3] | Trt* Time[3] | Interaction[4] |
| **TC** | | | | | | 0.556 |
| **≤ 24.9** | | | **0.0011** | **0.0254** | 0.9635 | |
| GTE (213) | -6.4 (-9.1-3.8) | -4.2 (-7.2-1.3) | | | | |
| Placebo (238) | -0.9 (-3.4-1.6) | 1.4 (-1.4-4.1) | | | | |
| *P** | 0.0030 | 0.0071 | | | | |
| **25-29.9** | | | **0.0002** | 0.1393 | 0.4584 | |
| GTE (158) | -6.2 (-9.7-2.8) | -5.4 (-8.7-2) | | | | |
| Placebo (175) | 0.9 (-2.4-4.1) | 3.5 (0.3-6.7) | | | | |
| *P** | 0.0033 | 0.0002 | | | | |
| **≥ 30** | | | 0.3628 | 0.7764 | 0.0786 | |
| GTE (58) | -9.1 (-14.5-3.8) | -4.2 (-10.2-1.8) | | | | |
| Placebo (42) | -1.5 (-7.7-4.8) | -5.0 (-12-2.1) | | | | |
| *P** | 0.0716 | 0.8741 | | | | |
| **HDL** | | | | | | 0.980 |
| **≤ 24.9** | | | **0.0299** | **0.0139** | 0.6003 | |
| GTE | -1.6 (-2.8-0.4) | -0.4 (-1.5-0.7) | | | | |
| Placebo | 0.2 (-1-1.3) | 0.9 (-0.1-2) | | | | |
| *P** | 0.0376 | 0.0994 | | | | |
| **25-29.9** | | | 0.0644 | 0.9208 | 0.5952 | |
| GTE | -0.7 (-1.9-0.5) | -0.5 (-1.7-0.7) | | | | |
| Placebo | 0.8 (-0.3-2) | 0.5 (-0.6-1.7) | | | | |
| *P** | 0.0665 | 0.2414 | | | | |
| **≥ 30** | | | 0.3375 | 0.3527 | 0.1235 | |
| GTE | -1.2 (-2.7-0.4) | 1.1 (-1.5-3.7) | | | | |
| Placebo | 1.6 (-0.2-3.5) | 1.1 (-2-4.1) | | | | |
| *P** | 0.0260 | 0.9874 | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| **TG** | | | | | | **0.017** |
| **≤ 24.9** | | | **0.0287** | 0.2530 | 0.8133 | |
| GTE | 3.2 (-0.2-6.6) | 4.3 (0.6-8.1) | | | | |
| Placebo | -1.8 (-5.1-1.4) | 0.0 (-3.5-3.6) | | | | |
| *P** | 0.0368 | 0.1020 | | | | |
| **25-29.9** | | | 0.9899 | 0.8699 | 0.4201 | |
| GTE | -1.0 (-6.2-4.2) | 1.4 (-4.4-7.1) | | | | |
| Placebo | 0.9 (-4-5.9) | -0.7 (-6.1-4.8) | | | | |
| *P** | 0.5941 | 0.6182 | | | | |
| **≥ 30** | | | **0.0064** | 0.4755 | 0.4115 | |
| GTE | 5.4 (-3.5-14.2) | 6.0 (-5.4-17.4) | | | | |
| Placebo | -7.8 (-18.2-2.7) | -15.7 (-29.1--2.3) | | | | |
| *P** | 0.0633 | 0.0179 | | | | |
| **LDL** | | | | | | 0.768 |
| **≤ 24.9** | | | **0.0007** | 0.2923 | 0.8511 | |
| GTE | -5.4 (-7.6-3.2) | -4.7 (-7.3-2.1) | | | | |
| Placebo | -0.6 (-2.7-1.5) | 0.4 (-2-2.9) | | | | |
| *P** | 0.0022 | 0.0046 | | | | |
| **25-29.9** | | | **0.0004** | 0.1215 | 0.1700 | |
| GTE | -5.3 (-8.3-2.3) | -5.2 (-8.2-2.1) | | | | |
| Placebo (174) | 0.2 (-2.7-3) | 3.0 (0.1-5.9) | | | | |
| *P** | 0.0105 | 0.0002 | | | | |
| **≥ 30** | | | 0.1482 | 0.6988 | 0.2995 | |
| GTE | -9.0 (-14.2-3.9) | -5.7 (-11.3--0.1) | | | | |
| Placebo | -1.6 (-7.6-4.5) | -2.9 (-9.5-3.6) | | | | |
| *P** | 0.0694 | 0.5340 | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Chol:HDLratio** | | | | | | 0.416 |
| **≤ 24.9** | | | 0.2186 | 0.8287 | 0.5616 | |
| GTE | 0.0 (-0.1-0) | -0.1 (-0.1-0) | | | | |
| Placebo | 0.0 (-0.1-0) | 0.0 (-0.1-0) | | | | |
| *P** | 0.4128 | 0.1966 | | | | |
| **25-29.9** | | | 0.2067 | 0.6616 | 0.8829 | |
| GTE | -0.1 (-0.2-0) | -0.1 (-0.1-0) | | | | |
| Placebo | 0.0 (-0.1-0.1) | 0.0 (-0.1-0.1) | | | | |
| *P** | 0.2926 | 0.2637 | | | | |
| **≥ 30** | | | 0.4597 | 0.4667 | 0.5834 | |
| GTE | -0.1 (-0.3-0) | -0.1 (-0.3-0.1) | | | | |
| Placebo | -0.2 (-0.3-0) | -0.3 (-0.5-0) | | | | |
| *P** | 0.6835 | 0.4232 | | | | |
| **Non-HDLC** | | | | | | 0.517 |
| **≤ 24.9** | | | **0.0106** | 0.1830 | 0.7876 | |
| GTE | -4.8 (-7.2-2.4) | -3.9 (-6.7-1.1) | | | | |
| Placebo | -1.0 (-3.3-1.2) | 0.4 (-2.2-3.1) | | | | |
| *P** | 0.0241 | 0.0289 | | | | |
| **25-29.9** | | | **0.0011** | 0.0955 | 0.2883 | |
| GTE | -5.5 (-8.8-2.3) | -4.9 (-8.2-1.6) | | | | |
| Placebo | 0.0 (-3-3.1) | 2.9 (-0.2-6) | | | | |
| *P** | 0.0148 | 0.0008 | | | | |
| **≥ 30** | | | 0.5934 | 0.9439 | 0.2365 | |
| GTE | -8.0 (-13.2-2.7) | -5.3 (-11.5-0.9) | | | | |
| Placebo | -3.1 (-9.3-3.1) | -6.0 (-13.3-1.2) | | | | |
| *P** | 0.2434 | 0.8834 | | | | |

[1] Data is presented in mean (95% CI); adjusted for Age.
* Test for treatment effect between GTE and placebo groups at time point using ANCOVA (GLM).

[3] Test for overall treatment, time, and treatment by time, effects between GTE and placebo groups using mixed methods for repeated measures.
[4] Test for overall interaction between treatment group and stratified variables using mixed methods for repeated measures.

**Table 6. Treatment Effect: by Statin Users and Non-Users**

| Measure (mg/dL)[1] | Statin Users GTE (*n*=91) Placebo (*n*=91) | | | | | Non-Users of Statins GTE (*n*=338) Placebo (*n*=364) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | change Mo.0 to Mo.6 | change Mo.0 to Mo.12 | ***p* values** Trt[3] | Time[3] | Trt* Time[3] | change Mo.0 to Mo.6 | change Mo.0 to Mo.12 | ***p* values** Trt[4] | Time[4] | Trt* Time[4] | Inter.[5] |
| **TC** | | | 0.1000 | 0.2964 | 0.3955 | | | **<.0001** | **0.0111** | 0.6948 | 0.865 |
| GTE | -4.0 (-9.3-1.2) | -0.2 (-5.9-5.5) | | | | -7.3 (-9.4-5.3) | -5.8 (-7.9-3.7) | | | | |
| Placebo | 3.4 (-1.8-8.7) | 3.7 (-2-9.5) | | | | -1.3 (-3.2-0.7) | 1.0 (-1-3.1) | | | | |
| *p*[2] | 0.0510 | 0.3444 | | | | <.0001 | <.0001 | | | | |
| **HDL** | | | 0.3155 | 0.4441 | 0.6302 | | | **0.0056** | **0.0056** | 0.2216 | 0.688 |
| GTE | -0.4 (-2.1-1.4) | -0.5 (-2.1-1) | | | | -1.4 (-2.3-0.6) | -0.1 (-1-0.8) | | | | |
| Placebo | 1.0 (-0.7-2.7) | 0.1 (-1.5-1.7) | | | | 0.5 (-0.4-1.3) | 1.0 (0.1-1.8) | | | | |
| *p*[2] | 0.2882 | 0.5849 | | | | 0.0023 | 0.0915 | | | | |
| **TG** | | | **0.0047** | 0.5614 | 0.8735 | | | 0.2435 | 0.8199 | 0.4535 | **0.014** |
| GTE | 7.9 (0.9-14.8) | 10.0 (2.8-17.1) | | | | 0.4 (-2.7-3.4) | 1.8 (-1.8-5.3) | | | | |
| Placebo | -3.9(-10.9-3.1) | -2.8 (-9.9-4.4) | | | | -0.7 (-3.7-2.2) | -1.4 (-4.9-2) | | | | |
| *p*[2] | 0.0208 | 0.0145 | | | | 0.6080 | 0.2101 | | | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **LDL** | | | **0.0257** | **0.1637** | **0.4303** | | | **<.0001** | **0.1797** | **0.2538** | **0.441** |
| GTE | **-5.2 (-9.8-0.7)** | **-1.6 (-6.9-3.7)** | | | | **-6.0 (-7.8-4.2)** | **-5.9 (-7.7-4)** | | | | |
| Placebo | **3.3 (-1.3-7.8)** | **4.2 (-1.1-9.5)** | | | | **-1.4 (-3.2-0.3)** | **0.3 (-1.5-2.1)** | | | | |
| $p^2$ | **0.0108** | **0.1331** | | | | **0.0004** | **<.0001** | | | | |
| **C:HDLr** | | | 0.6500 | 0.3835 | 0.9465 | | | 0.1698 | 0.4945 | 0.7858 | 0.913 |
| GTE | 0.0 (-0.1-0.1) | 0.0 (-0.1-0.2) | | | | -0.1 (-0.1-0) | -0.1 (-0.1-0) | | | | |
| Placebo | 0.0 (-0.1-0.1) | 0.1 (-0.1-0.2) | | | | 0.0 (-0.1-0) | -0.1 (-0.1-0) | | | | |
| $p^2$ | 0.6821 | 0.7016 | | | | 0.2781 | 0.2045 | | | | |
| **NonHDLC** | | | 0.1721 | 0.1533 | 0.4594 | | | **<.0001** | 0.1533 | 0.3303 | 0.964 |
| GTE | -3.7 (-8.6-1.3) | 0.3 (-5.5-6.2) | | | | -5.9 (-7.8-4) | -5.7 (-7.7-3.6) | | | | |
| Placebo | 2.5 (-2.5-7.4) | 3.6 (-2.2-9.5) | | | | -1.7 (-3.5-0.1) | 0.1 (-1.9-2) | | | | |
| $p^2$ | 0.0868 | 0.4355 | | | | 0.0019 | <.0001 | | | | |

[1] Data is presented in mean (95% CI); adjusted for Age and BMI.
[2] Test for treatment effect between GTE and placebo groups at time point using ANCOVA (GLM).
[3] Test for overall treatment, time, and treatment by time, effects between GTE and placebo groups (statin users) using mixed methods for repeated measures.
[4] Test for overall treatment, time, and treatment by time, effects between GTE and placebo groups (non statin-users) using mixed methods for repeated measures.
[5] Test for overall interaction between treatment effect and statin use using mixed model for repeated measures. (inter.=interaction)

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# Supplementary Material

**Appendix 1:** Catechin Content of Study Capsules

**Catechin Content of Capsules (mean value in mg, by batch)**

| | EGC (mg) | EC (mg) | EGCG (mg) | ECG (mg) | Total (mg) |
|---|---|---|---|---|---|
| **Batch 1** | 25 | 25 | 198 | 42 | 297 |
| **Batch 2** | <1 | 21 | 211 | 63 | 307 |
| **Batch 3** | 1 | 24 | 210 | 73 | 323 |
| **Batch 4** | 1 | 22 | 205 | 68 | 310 |
| **Batch 5** | 1.8 | 21 | 209 | 64 | 309 |
| **Batch 6** | 64 | 33.5 | 237.5 | 33 | 384 |
| **Batch 7** | 56 | 29 | 207 | 28 | 334 |
| **Batch 8** | 63 | 37 | 204 | 32 | 359 |
| **Mean** | 26.6 | 26.6 | 210.2 | 50.4 | 327.9 |

**Appendix 2:** Copy of Study Consent Form

IRB Approved
Version: 11/18/2011 (latest version)

**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 your 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.