Effects of Green Tea Extract on Biomarkers of Breast Cancer Risk Including
Reproductive Hormones and IGF axis Proteins

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Dedication

This dissertation is dedicated to my parents, my late generous father, Mohammad Bagher, and my beautiful mother, Maliheh, for their unconditional love and endless encouragement.

And to **Omar Khayyam**, Persian poet, philosopher, mathematician, and astronomer.

"We are no other than a moving row
Of Magic Shadow-shapes that come and go
Round with the Sun-illumined Lantern held
In Midnight by the Master of the Show."

Abstract

Objective: To investigate the effects of daily intake of a highly concentrated green tea extract (GTE) for one year on circulating sex hormones and insulin-like growth factor (IGF) proteins as well as urinary estrogens and estrogen metabolites in postmenopausal women with different catechol-O-methyltransferase (*COMT*) genotypes.

Method: The Minnesota Green Tea Trial (MGTT) is a randomized, double-blind, placebo-controlled, phase II clinical trial. Healthy postmenopausal women with heterogeneously or extremely dense breast tissue (age = 59.78 ± 5.02 years; body mass index= 25.70 ± 8.21 kg/m^2) were randomly assigned to the GTE group (n=538) and were given 4 capsules a day, each containing 200 mg epigallocatechin gallate (EGCG) and the others (n=537) to the placebo group. Participants were 93% non-Hispanic white and non-current hormone users. Twenty-four hour urine samples were collected at month 0 and at the end of the study, and fasting blood samples were drawn at months 0, 6 and 12. Circulating and urinary estrogens, as well as urinary estrogen metabolites were quantified by the liquid chromatography-tandem mass spectrometry method. Blood IGF axis proteins were analyzed by ELISA.

Results: GTE supplementation was associated with reduced urinary estriol levels (P= 0.02) and higher urinary 2-hydroxyestrone (P= 0.02) compared to the placebo. There was also less of a reduction in the urinary levels of 16α-hydroxyestrone in the GTE versus placebo group. Intake of the GTE resulted in significant increase of circulating estradiol and testosterone and their corresponding free and bioavailable fractions, whereas these measures were reduced in the placebo group. Additionally, *COMT* genotype did not modify the GTE effect on either circulating sex hormones and IGF proteins or urinary estrogens.

Conclusion: Daily intake of high-dose of green tea extract for 12 month exerts modest effects on urinary excretion of estrogen metabolites, yet these effects are not modified by the *COMT* polymorphisms. Potential breast cancer protective effects of GTE are not mediated by alterations in circulating sex hormones or IGF axis proteins.

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List of Abbreviations

%MD, percent mammographic density

2-MeOE$_1$, 2-methoxyestrone

2-MeOE$_2$, 2-methoxyestradiol

2-OHE$_1$, 2-hydroxyestrone

2-OHE$_2$, 2-hydroxyestradiol

4′-MeEGC, methylated-epigallocatechin

4-MeOE$_1$, 4-methoxyestrone

4-MeOE$_2$, 4-methoxyestradiol

4-OHE$_1$, 4-hydroxyestrone

4-OHE$_2$, 4-hydroxyestradiol

16α-OHE$_1$, 16α-hydroxyestrone

ACE, Angiotensin converting enzyme

ALT, alanine aminotransferase

ANOVA, analysis of variance

anti-HCV, antibodies to hepatitis C virus

AUC, area under the curve

BI-RADS, breast imaging and reporting data system

BMI, body mass index

CG, catechin gallate

CI, confidence interval

Cmax, peak plasma concentrations

COMT, catechol-O-methyltransferase

CV, coefficient of variation

CPT, cell preparation tubes

DGT, decaffeinated green tea

DHA, docosahexaenoic acid

DHEA, dehydroepiandrosterone

DHEAS, dehydroepiandrosterone sulfate

DHQ1, diet history questionnaire I

DSMB, data and safety monitoring board

E$_1$, estrone

E$_2$, estradiol

E$_3$, estriol

EC, epicatechin

ECG, epicatechin gallate

EDTA, ethylenediaminetetraacetic acid

EGC, epigallocatechin

EGCG, epigallocatechin gallate

EPA, eicosapentaenoic acid

EPIC, European prospective investigation into cancer and nutrition

FDA, food and drug administration

GC, gallocatechin

GCG, gallocatechin gallate

GC-MS, gas chromatography-mass spectrometry

GTE, green tea extract

HBsAg, hepatitis B surface antigen

HHQ, health history questionnaire

HPLC, high-performance liquid chromatography

HRT, hormone replacement therapy

IDS, investigational drug services

IGF, insulin-like growth factor

IGFBP-3, IGF binding protein 3

IRB, institutional review board

IRS1, insulin-receptor substrate 1

ITT, intention-to-treat

LC/MS/MS, liquid chromatography/tandem mass spectrometry

M4, 5-(3′, 4′, 5′-trihydroxy-phenyl)-γ-valerolactone

M6, 5-(3′, 4′-dihydroxy-phenyl)-γ-valerolactone

MAPK, mitogen-activated protein kinase

MENQOL, menopause-specific quality of life questionnaire

MET, metabolic equivalent

MGTT, Minnesota green tea trial

MTHFR, methylenetetrahydrofolate reductase

NCI, national cancer institute

NIH, national institutes of health

NHS, nurses' health study

OR, odds ratio

PAPS, 3′-phosphoadenosine-5′-phophosulfate

PI3K, phosphatidylinositol 3-kinase

PPE, polyphenone E

PTEN, phosphatase and tensin homologue

SAM, *S*-adenosyl-L-methionine

SERMS, selective estrogen receptor modulators

SHBG, sex hormone binding globulin

S6K, S6 kinase

SNP, single nucleotide polymorphism

SULT, sulfotransferase

$t_{1/2}$, elimination half-life

Tmax, time to reach Cmax

TOR, target of rapamycin

TYMS, thymidylate synthase

UGT, uridine diphosphate-glucuronosyltransferases

UMMC, university of Minnesota medical center

WHR, waist-to-hip ratio

Chapter 1: Background

Hypothesis and Study Aims

Breast cancer is the most common and second deadliest cancer of women living in the United States[1]. Many risk factors for breast cancer have been identified. Some of these factors are modifiable, such as change in dietary habits. However, the role and mechanisms of diet as a modifiable factor in prevention or treatment of breast cancer remains either controversial or unclear. One dietary factor which has received great attention among researchers within the last two decades is tea. Proposed health benefits of tea, particularly green tea, varies from affecting cardiovascular disease [2, 3] to bone metabolism [4] and cancer risks [5]. There is convincing evidence from animal model and in vitro studies that green tea has protective effects against cancer although epidemiological studies do not strongly support these results and are still inconsistent. The chemoprotective effect of green tea is primarily attributed to the bioactive polyphenol compound known as catechins, wherein (-)-epigallocatechin-3-gallate (EGCG) is the most potent and of special interest [6]. Some of the purported mechanisms by which green tea intake is believed to influence breast cancer risk in human include changes in circulating sex hormone or urinary estrogen metabolites levels, and insulin-like growth factor (IGF) system.

The purpose of this research study was to test, as part of the NIH-funded grant "Green Tea and Reduction of Breast Cancer Risk study", the hypothesis that green tea extract supplementation decreases breast cancer risk by affecting recognized breast cancer biomarkers such as endogenous sex hormones and estrogen metabolites as well as IGF axis proteins. We hypothesized that daily consumption of green tea extract containing 800 mg epigallocatechin gallate (EGCG) for one year will alter the levels of aforementioned biomarkers in a breast cancer preventive direction. Furthermore, we took advantage of a nutrigenetic approach in which we hypothesized that protective effects of green tea intake will be more remarkable in women who possess low activity catechol-O-

methyl transferase (COMT) genotype than participants who have high activity *COMT* genotype.

The specific objectives of this research study were to investigate the effect of daily consumption of green tea extract (GTE) containing 800mg epigallocatechin gallate (EGCG) for one year on:

- Circulating levels of estrone (E_1), estradiol (E_2), androstenedione, testosterone, and sex hormone binding globulin (SHBG)
- Urinary estrogens including estrone (E_1), estradiol (E_2), estriol (E_3), and their metabolites such as estrone and estradiol 2-hydroxy, 4-hydroxy, 2-methoxy, and 4-methoxy metabolites as well as $16-\alpha$ hydroxyestrone
- Circulating levels of insulin-like growth factor 1 (IGF-1) and IGF binding protein 3 (IGFBP-3)

Other biomarkers evaluated in the study, but not part of this dissertation, included mammographic density, plasma concentrations of F-2 isoprostanes as well as urinary green tea polyphenols and their metabolites.

Introduction: Tea Types, Chemical Composition, Metabolism and Consumption

After water, tea is the most commonly consumed beverage in the world. Depending on the preparation method, tea is produced from the plant leaf of *camellia sinensis* in three basic forms. Black tea requires leaves that are dried and crushed after harvesting, and oxidation is promoted. In this process, naturally occurring polyphenols, such as catechins, in the tea leaves will leave and convert to other polyphenols (primarily theaflavins and the thearubigins). Black tea accounts for 78% of tea consumption in the world where the United States, the United Kingdom and Europe are among the usual consumers. On the other hand, green tea constitutes 20% of the world tea production and is most popular in Japan and parts of China. Green tea is manufactured by steaming and heating the fresh leaves of tea immediately following harvesting. This process avoids

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oxidation of green leaf polyphenols and results in higher levels of catechins in comparison with black tea. Finally, Oolong tea is a partially oxidized product of the tea leaf which accounts for the remaining 2% of the tea consumption [7].

Tea composition varies not only by type of the tea but also by climate, season, horticultural practices, the age of the leaf, quantity of tea leaves, and brewing time [8]. Green tea contains primarily catechins, flavonols, lignans, and phenolic acids. Catechins, the most interesting group of green tea components, constitute 30-40% of the dry tea leaf weight and are monomeric flavonoids that in turn belong to a larger class i.e. polyphenols [9-11]. The major catechins found in green tea, as shown below in

Figure 1, are epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG) and epicatechin (EC), wherein EGCG is the most abundant and active catechin that may account for 50% to 80% of the total catechins found in green tea. Other catechins found in lower levels in green tea include gallocatechin gallate (GCG), gallocatechin (GC), catechin gallate (CG), and catechin and are also shown in

Figure 1. In comparison, black tea is largely comprised of complex polymeric polyphenols such as theaflavins and thearubigins, and has smaller amounts of catechins equal to one-third of the weight of green tea. In contrast, black tea usually contains more caffeine than green tea (3-10% vs. 3-6%, respectively, in a typical cup of tea) [12, 13].

Metabolism of catechins is accomplished through three different metabolic pathways of methylation, glucuronidation and sulfation, in which COMT, uridine diphosphate-glucuronosyltransferases (UGT) and sulfotransferases (SULT) are the responsible enzymes, respectively[14]. Major biotransformation pathways of green tea EGCG and EGC have been shown in Figure 2.
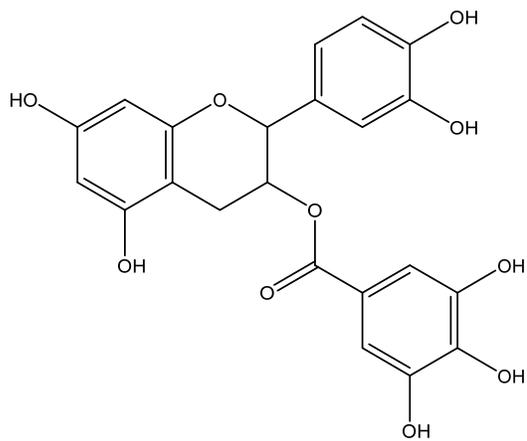
(-)-Catechin Gallate (CG)
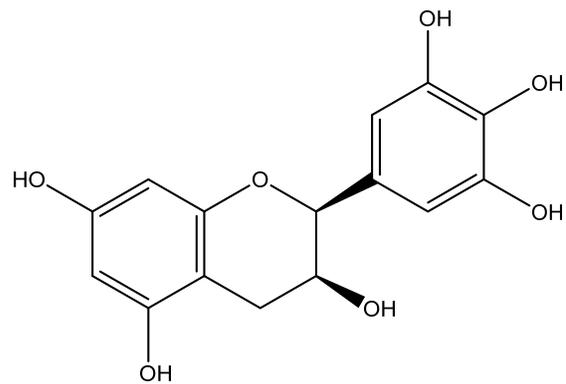
(-)-Gallocatechin (GC)

(-)-Gallocatechin Gallate (GCG)

(-)-Epicatechin (EC)

(-)-Epicatechin Gallate (ECG)

(-)-Epigallocatechin (EGC)
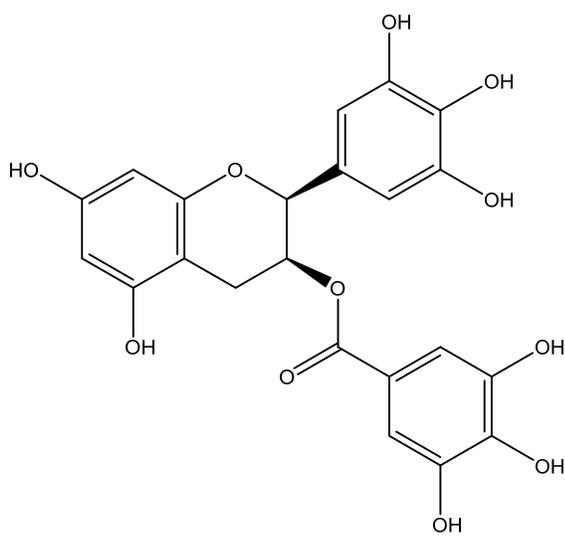
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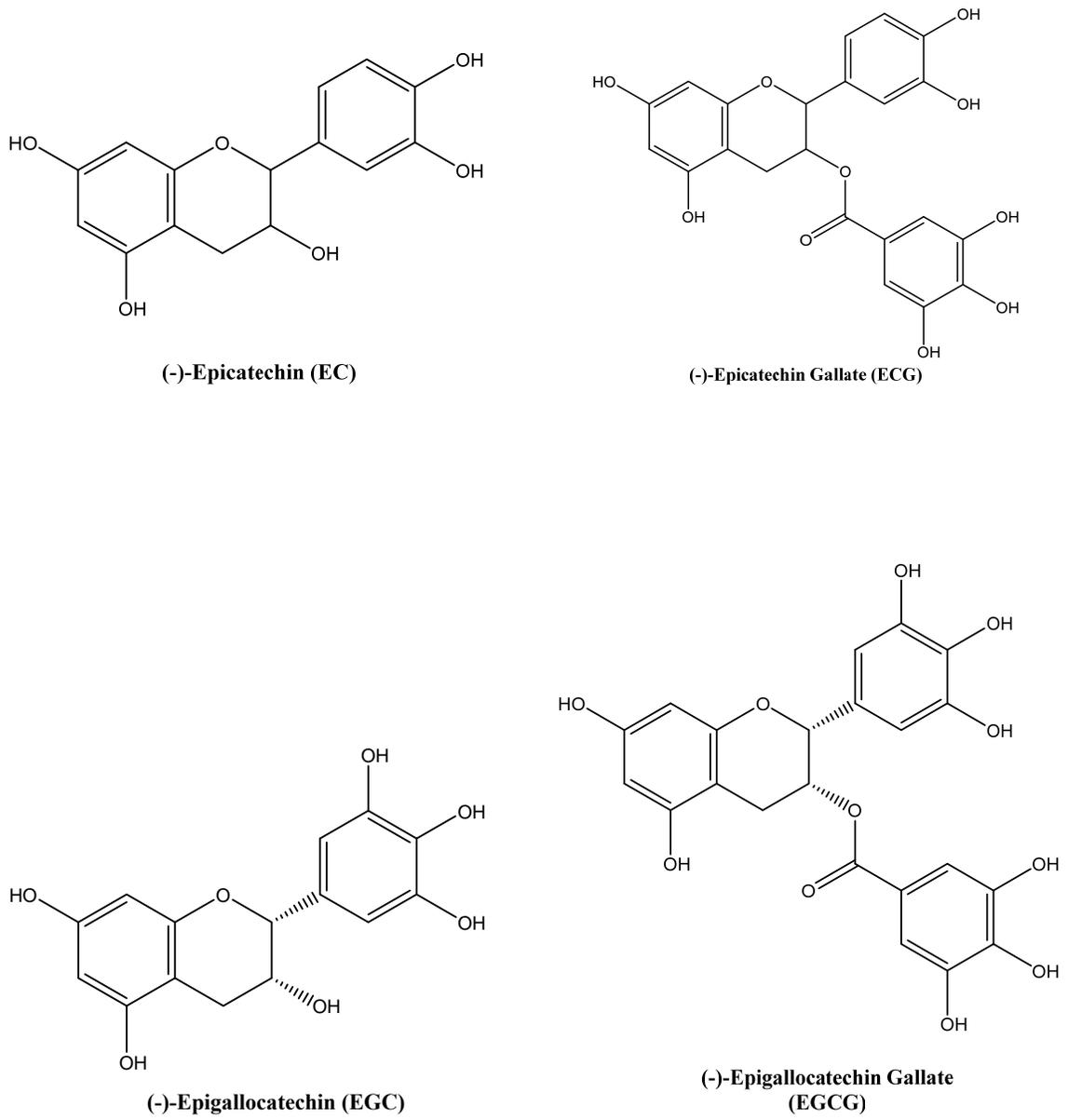
Figure 1. Different types of catechins in green tea.

Glucuronidation by UGT is an important pathway in phase II metabolism. UGT facilitates binding of glucuronic acid to different entities with hydroxyl or amine groups. Vaidyanathan and Walle [15] have shown that EC was not glucuronidated by the human liver and small intestinal microsomes or even by recombinant UGT1A7, but was readily glucuronidated by rat liver microsomes. Contrary to EC, both EGC and EGCG are glucuronidated in humans, mice, and rats, and to date at least 4 EGCG glucuronides and 2 EGC glucuronides have been identified [16]. It has been reported that the glucuronidation rate of EGCG is much faster than EGC and mice have more identical glucuronidation patterns to human than rats [16]. In the sulfation conjugation pathway, the sulfate group from a donor molecule such as 3′-phosphoadenosine-5′-phophosulfate (PAPS) is bound to substances like alcohol or amine [17]. EC, EGC, and EGCG are sulfated in human liver cytosol and intestine, yet their chemical structures as well as their active sites for sulfation need to be elucidated.

Methylation conjugation by the action of COMT enzyme is one of the most prominent reactions of phase II metabolism of xenobiotics. Of importance is the *O*-methylation pathway of catechins because individual genetic variability in COMT enzyme has been shown to influence the rate of catechin excretion from the body. Cytosolic COMT enzyme is widely present in all human tissues with the most activity observed in the liver, kidney, and gastrointestinal tract [18]. With the aid of a potential methyl donor such as *S*-adenosyl-L-methionine (SAM), COMT enzyme acts upon catecholic compounds such as xenobiotic catechols, catecholamine neurotransmitters, catechol estrogens, and green tea catechins, and usually yields less toxic and active products.

COMT gene is polymorphic; a single G to A transition at codon 108/158 of *COMT* (SNP rs4680) results in a valine to methionine substitution in the cytosolic or membrane-bound form of this enzyme, which shows a 3- to 4-fold decrease in enzymatic activity. The reason for decreased enzymatic activity of variant isoform has been attributed to its thermolability [19]. Individuals with heterozygous genotype (H/L) also

show intermediate levels of COMT activity [20, 21]. Wu *et al*[22] have previously shown that breast cancer protective effect of green tea intake is significantly more prominent in women with low-activity *COMT* than those with high-activity *COMT* genotype. More recently, Inoue-Choi *et al* [23] have also reported that men with low-activity *COMT* genotype (LL) in the Shanghai Cohort Study exhibited statistically significantly lower urinary levels of tea polyphenol metabolites than participants with high-activity *COMT* genotype. These results suggest individuals with low-activity *COMT* genotype metabolize tea catechins slower and retain these bioactive components longer in their bodies which consequently may contribute to greater benefits from green tea intake.

Methylation enzymology studies of EGCG and EGC by cytosolic COMT have determined that EGCG is methylated to 4″-O-methyl-EGCG and then to 4′, 4″-di-O-methyl-EGCG [18]. Similarly, EGC is methylated to 4′-O-methyl-EGC. Cytosolic COMT enzyme has lower affinity for EGC than EGCG (i.e. higher K_m for methylation of EGC than EGCG). Among different methylated catechin metabolites, 4′-*O*-methyl EGC and 4′, 4″-di-*O*-methyl-EGCG are present in higher levels in human urine [24, 25]. Interestingly, it has been shown that EGCG and its metabolites inhibit the activity of COMT; hence, these constituents have the potential to reduce the rate of *O*-methylation of endogenous catechol estrogens or catechol drugs such as L-DOPA. Reduction of methylation activity of COMT results in improved efficacy of L-DOPA and treatment of Parkinson's disease [26, 27]. In fact, there are currently studies indicating neuroregenerative and neuroprotective effects of tea polyphenols [28-30], which may be partly due to inhibition of COMT enzyme activity by EGCG.

In addition to phase II enzymatic metabolism, catechins such as EGC, EC, and ECG undergo further degradation and anaerobic fermentation by intestinal microflora in the colon, and produce ring fission metabolites including 5-(3′, 4′, 5′-trihydroxyphenyl)-γ-valerolactone (M4), 5-(3′, 4′-dihydroxyphenyl)-γ-valerolactone (M6), and 5-(3′, 5′-dihydroxyphenyl)-γ-valerolactone (M6′) [31]. These metabolites are detected in human urine and plasma close to 13 hours following intake of 20 mg/kg body weight of a

decaffeinated green tea [31]. Detected concentrations are usually much higher in urine than plasma. M4 and M6′ are derived from EGC, and EC is the substrate for M6. These ring fission products can be sulfated or glucuronidated, or even be more degraded to phenolic compounds. Among catechins, urinary EGC, and its methylated or ring-fission products are considered as good exposure markers of green tea consumption. Since EC is found abundantly in various food sources (e.g., dark chocolate, wine, or fruits) [32, 33], its urinary level does not accurately reflect tea intake.
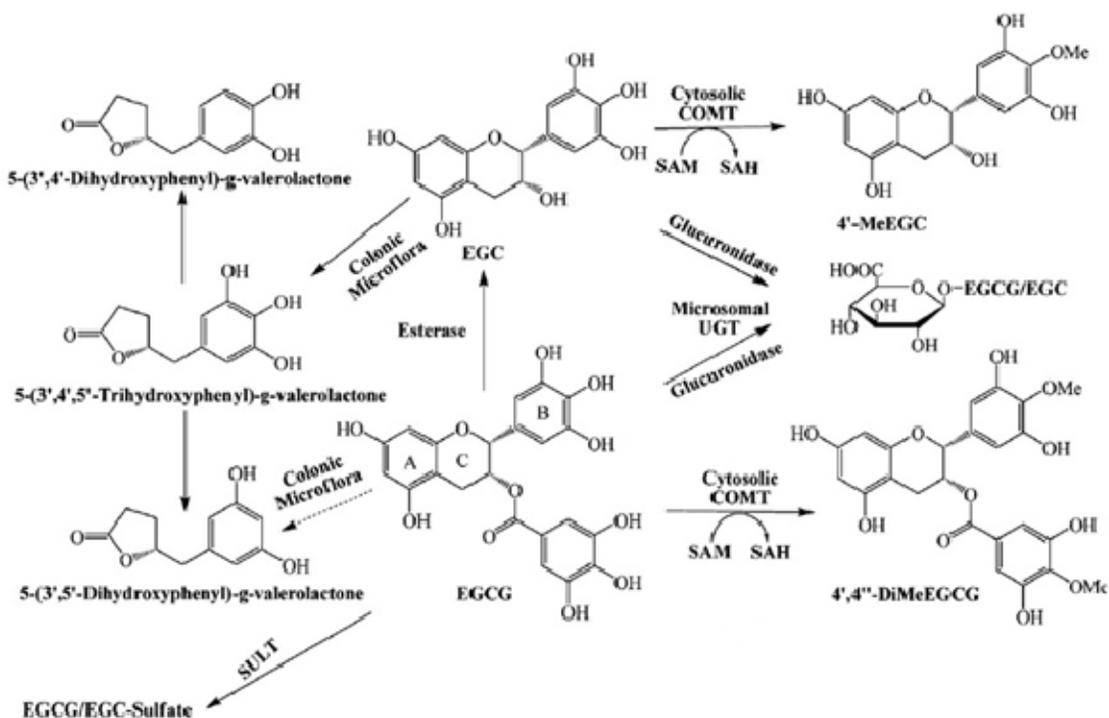
Figure 2. Primary biotransformation pathways of EGCG and EGC [7].

Green tea catechins possess both antioxidant and pro-oxidant activities *in vitro* and *in vivo* [34]. Currently, there is stronger evidence for antioxidant cancer preventive effect of catechins than their pro-oxidant activities. Green tea catechins (particularly EGCG) exerts their antioxidant effects directly by trapping reactive oxygen species

(ROS) such as superoxide, hydroxyl or peroxyl radicals, or chelating transition metals [35], or indirectly by upregulating phase II antioxidant enzymes [36]. Chemopreventive effect of green tea catechins with regard to its pro-oxidant role has been attributed to generating genotoxic substances such as hydrogen peroxide and superoxide radicals. It is thought that formation of these elements induces apoptosis and inhibits cancer cell growth [37, 38]. And it has been suggested that green tea catechins act as antioxidant or pro-oxidant depending on the their concentrations in the sense that in levels above 10 μM they demonstrate pro-oxidant activities [39].

Pharmacokinetic and Bioavailabilities of Green Tea Catechins

As reviewed in this work, and described in further details by Lambert and Yang [40], *in vitro* and animal studies have consistently shown that tea constituents possess antiangiogenic and antiproliferative properties, and inhibit carcinogenesis at multiple different sites. However, in extrapolating these results to humans, the poor bioavailability of tea catechins should be taken into account. It is important to note that tea catechin concentrations used *in vitro* and in cell culture studies are above physiological levels usually found in human plasma. The non-conjugated plasma concentrations of tea catechins in humans or animals fall under sub-μM or nM ranges with the peak plasma level of EGCG close to 1 μM/L [41]. However, required EGCG levels in the majority of *in vitro* studies to show effective response are about 10-100 times of the plasma and tissue concentrations that can be reached *in vivo*. This issue raises the key question of how the results of *in vitro* studies are applicable to the proposed cancer preventive mechanisms *in vivo* [42]. Figure 3 illustrates potential targets of EGCG and corresponding required concentrations by which it can exert its anticarcinogenic activity.

Pharmacokinetic and bioavailability of tea catechins have been investigated in several animal [6, 43-45] and human studies [41, 46-50]. Chen *et al* [43] in a study of rats showed that administration of 200 mg/kg of decaffeinated green tea (DGT) via intragastric route (i.e. orally) resulted in plasma bioavailabilities or absorptions of 0.1%,

14.0%, and 31.0% for EGCG, EGC, and EC, respectively. Plasma elimination half-lives of the foregoing polyphenols were also reported as 165, 66, and 67 minutes, respectively. The DGT contents included 73, 68, and 27 mg/g of EGCG, EGC, and EC, respectively. When pure EGCG solution was administered orally at a dose of 75 mg/kg, the fraction of absorbed EGCG was 1.6%. The difference in EGCG bioavailability between the DGT and pure EGCG could be due to other contents in the DGT; differences in disposition kinetics of EGCG; or the fact that total catechins (sum of conjugated and free catechins) can be influenced by pre-systemic first-pass metabolism by intragastric administration. Plasma bioavailability of EGCG following oral administration of EGCG at a dose of 163.8 μmol/kg (~75 mg/kg) has been reported to be higher in mice than rats (26.5 % vs. 1.65 %)[44]. Other studies in rats have also shown that either intravenous or intrahepatic administration of EGCG exhibits similar plasma EGCG bioavailabilities [45], or intraperitoneal administration of EGCG in doses close to 75 mg/kg leads to higher plasma bioavailabilities of EGCG compared to intragastric route [6]. These findings are consistent with the notion that loss through pre-systemic first-pass metabolism within the GI tract is one of the major factors responsible for the low bioavailabilities of green tea catechins.

In one of the earliest human pharmacokinetic studies of green tea catechins, Yang *et al* [46] reported peak plasma concentrations (C_{max}) of EGCG, EGC, and EC as 0.71, 1.8, and 0.65 μM, respectively, following ingestion of 1.5 g of a DGT. Using higher doses of the DGT up to 3.0 g increased C_{max} of the catechins notably, yet 4.5 g of the DGT did not markedly alter the C_{max}. Formulation used in this study included 73 mg EGCG, 68 mg EGC, 22 mg ECG, and 25 mg EC, and it took 1.4-2.4 h for the aforementioned catechins to reach their C_{max} (T_{max}). Also, elimination half-lives ($t_{1/2}$) of catechins were 2.5 to 5.7 h.
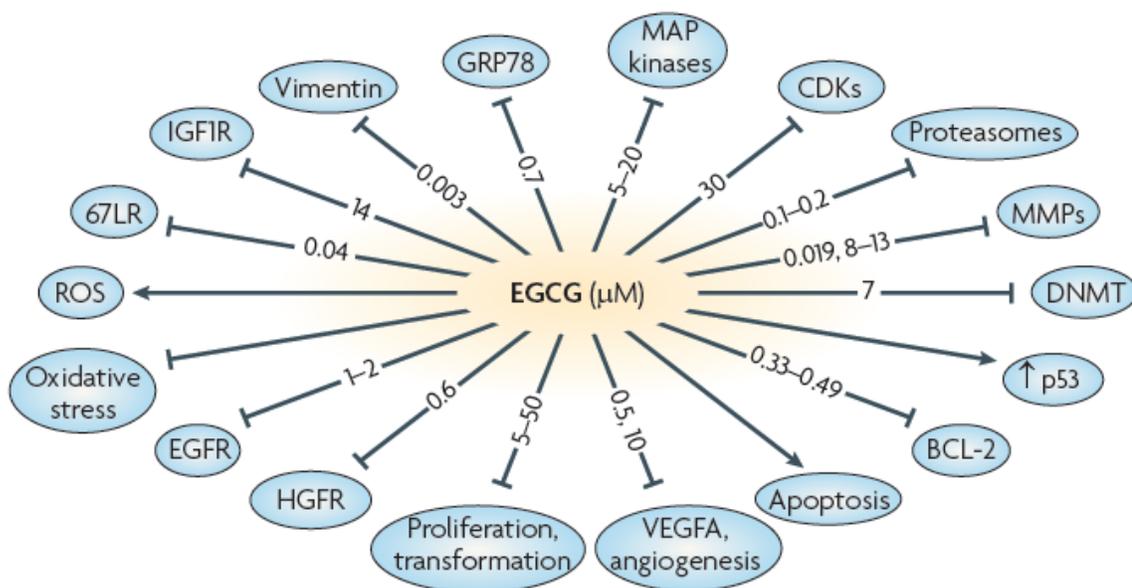
Figure 3. Potential anticarcinogenic targets of EGCG [12].

Noted values are effective levels in IC_{50}, K_i (inhibition constant) or K_d (dissociation constant), and are from *in vitro* studies in µM unit. *Abbreviations:* 67LR, 67 kDa laminin receptor; CDK, cyclin-dependent kinase; DNMT, DNA methyltransferase; EGFR, epidermal growth factor receptor; GRP78, glucose-regulated protein 78kDa; HGFR, hepatocyte growth factor receptor; IGF1R, insulin-like growth factor 1 receptor; MMP, matrix metalloproteinase; ROS, reactive oxygen species; VEGFA, vascular endothelial growth factor A.

In another study [41] with healthy human subjects, after intake of a single oral dose of pure EGCG or Polyphenon E (PPE) in amounts equal to 200, 400, 600, and 800 mg of EGCG, C_{max} of EGCG in corresponding doses were comparable between two catechin formulations. The average C_{max} of free EGCG increased in a dose-dependent manner in which administration of 200, 400, 600, and 800 mg of pure EGCG resulted in the average C_{max} of 0.16, 0.24, 0.37, 0.96 µM free EGCG, respectively. Accordingly, the average C_{max} of free EGCG were 0.16, 0.27, 0.36, 0.82 µM following intake of PPE in doses equal to 200, 400, 600, and 800 mg of EGCG, respectively. The authors also reported that EGCG was mostly present in plasma in free form as treatment with deconjugating enzymes did not significantly change the plasma EGCG concentrations.

EGCG was not detected in urine. In marked contrast to EGCG, the EGC and EC were mainly detected in plasma and urine in the conjugated forms of glucuronide or sulfate after PPE administration. EGC and EC were hardly detectable following pure EGCG administration.

Lee *et al* [47] have demonstrated similar patterns of findings and reported that 77% of EGCG was present in the free form in plasma, whereas only 31% of EGC and 21% of EC were in the free form by one hour following consumption of green tea. The tea regiments used in this study were a single oral dose of either 2 mg/kg body weight of pure EGCG, or 20 mg tea solids/kg green tea or DGT that is equivalent to 195 mg EGCG, 154 mg EGC, and 45 mg EC. In addition, authors also noted that the T_{max} for EGCG was 1.6 h, and the EGCG $t_{1/2}$ was 3.7 h after a single oral intake of EGCG.

The longest pharmacokinetic study [48] to date showed that after consumption of 800 mg of EGCG once daily for four weeks, area under the plasma EGCG concentration-time curve increased significantly by 60% in healthy men and women. However, this result was not observed following daily intake of 400 mg of EGCG twice per day. Furthermore, area under the curve (AUC) of EGC or EC did not change in response to either 800 mg of EGCG one daily or 400 mg of EGCG twice daily during 4 weeks. In a follow-up study by the same investigators [49], pharmacokinetics of green tea catechins in a single dose of PPE with EGCG contents of 400, 800, and 1200 mg were compared between the fed and fasting status. Similar to previous studies, EGCG and EGC were hardly detectable in the urine, and were predominantly found in plasma in the free form. Fasting condition led to higher AUC and C_{max}, and lower T_{max} compared with the fed state. In general, total concentrations of gallated catechins including EGCG and ECG were 3-5 fold higher in the fasting versus the fed state. The maximum plasma level of EGCG was achieved by administration of the highest dose of PPE (i.e. 1200 mg EGCG) in the fasting condition, which was equal to 7.4 μM and highest level of EGCG observed *in vivo* studies so far.

Current human studies suggest that green tea catechins are absorbed very quickly with C_{max} of 1-3 h. Also, they have very short half-lives of 2-4 h, and their transition time

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from small colon to large intestine is very fast. Intestinal microbial degradation pathway products such as M4 and M6 have also been found in human urine and plasma [31], implying parent catechins are also degraded and excreted in the urine in these forms. Generally, nongallated catechins are better absorbed in the small intestine than the gallated catechins, which might be due to their lower molecular weight and smaller number of phenolic groups. Although dosing has some effects on systemic bioavailability, parent catechins are not accumulated in the blood. Mechanisms involved in changes of catechins exposure after administration of green tea are not well understood and remains to be elucidated. However, as suggested by Chow and Hakim [51], alterations in non-enzymatic degradation or microflora metabolism, extensive first-pass metabolism, interindividual variability in gastric stability, or even food interaction with different dosing states are among potential reasons. Active efflux of catechins by multidrug resistance-associated proteins from enterocytes into the intestinal lumen or from the liver to the bile has been suggested as another potential contributing factor to the low bioavailability of catechins [52]. Further research is required to explore different approaches to enhance bioavailability of green tea polyphenols.

Tea catechin tissue distribution has been examined in a very limited number of studies in laboratory animals and humans. In rats, EGCG amount was detected in the descending order in the small intestine mucosa, colon mucosa, liver and plasma following single oral dose of EGCG at 500 mg/kg body weight [53]. Kim *et al* have also demonstrated that mice receiving a 0.6% green tea polyphenol for 12 days had higher levels of EGCG than EGC and EC in the lung, but lower amounts of EGCG than EGC in the liver [54]. Major green tea catechins have also been found in prostate tissues of human subjects after drinking 5 cups of green tea for five days prior to prostatectomy. In another study by the same investigators [55], 4″-O-methyl-EGCG was detected in levels close to the EGCG. The possibility of EGCG methylation in prostate tissue has also been suggested by the same group.

Tea and Breast Cancer Risk

There has been a substantial body of evidence from multiple experimental studies suggestive of anti-cancer properties of green tea extracts or tea catechins in different cancer sites. In vitro and animal model studies have consistently shown that green tea can inhibit development of tumors at different stages of carcinogenesis including initiation, promotion and progression [56-58]. Of particular interest is the effect of green tea extract on mammary cancer cells. Sartippour *et al* [59] have reported that green tea extract or EGCG (40mg/L) significantly and dose-dependently decreases the levels of vascular endothelial growth factor (VEGF) in MDA-MB231 human breast cancer cells. These findings have been recently confirmed by Leong *et al* [60] in C3(1)/SV40 mouse model by showing that administration of 0.5% Polyphenon E (PPE), a highly concentrated EGCG green tea extract, slows tumor proliferation through inhibition of VEGF expression in mammary cells. The chemoprotective effect of green tea including reducing mammary tumor incidence, size, volume and weight as well as increasing latency and survival times has been reproduced in several other animal models as well [61-64].

In contrast, epidemiological studies on the association of green tea intake and breast cancer risk look inconsistent. As lately reviewed by Wu and Butler [65], three case-control studies [66-68] conducted in the U.S. and China so far have shown significant reduced risk of breast cancer with regular green tea intake compared with non-green tea drinkers (combined odds ratio (OR)= 0.70; 95% confidence interval (CI), 0.61-0.79). These studies had adjusted for a wide range of potential confounding factors such as reproductive status, soy intake, and black tea. On the contrary, green tea intake has not been inversely associated with breast cancer risk in a recent hospital-based case-control study among Japanese women newly diagnosed with breast cancer [69]. Findings from this study were not changed following considering potential effect modifiers such as menopausal status, single nucleotide polymorphisms (SNP) in *CTP19A1* (rs10046) and *COMT* (rs4680) genes, or dietary intake of folate or isoflavone. On the other hand, data from prospective cohort studies are not in line with the majority of case-control studies and are conflicting. Findings from five cohort or nested case-control studies [70-74] show

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no association between green tea intake and breast cancer risk (combined OR= 1.06; 95% CI = 0.93-1.20) [65]. Reasons for the observed inconsistency between the cohort and case-control studies are unclear at this time, however, crude assessment of green tea intake, or recall bias among breast cancer cases in case-control studies may have contributed to these inconclusive results. An important factor that might influence the association between green tea and breast cancer risk is genetic variability in metabolism of green tea. For example, genetic polymorphism in the enzymes such as COMT can modify the exposure levels to tea catechins which in turn may affect breast cancer risk assessment [22].

Angiotensin converting enzyme (ACE) catalyzes the conversion of angiotensin I to angiotensin II. Epidemiological studies have indicated that angiotensin II is implicated in the development of human breast cancer by which risk of breast cancer is lower among women with the low-activity genotype of the *ACE* gene versus women with the high-activity genotype [75, 76]. Evidence from *in vitro* study [77] also shows that green tea polyphenols attenuate angiotensin II-induced generation of ROS. Yuan *et* al [78] have reported that in the Singapore Chinese Health Study, reduced breast cancer risk with higher intake of green tea was only seen in women who possessed the high-activity ACE genotype (OR= 0.33; 95% CI, 0.13- 0.82). To date, very few epidemiological studies have investigated the effect modification of *COMT* or *ACE* genotypes on the association between green tea intake and risk of breast cancer.

Current data do not support an association between black tea intake and breast cancer risk. In a meta-analysis of 5 cohort and 8 case-control studies, Sun *et al* [79] found a moderate positive association between black tea intake and breast cancer risk in the cohort studies (OR= 1.15; 95% CI, 1.02-1.31), but no association in the case-control studies. In addition, data from cross-sectional studies support this notion further by higher concentrations of estrogen and prolactin related with black tea intake, which subsequently may increase the risk of hormone-dependent breast cancer [80, 81].

Numerous mechanisms have been suggested for the observed association between green tea and cancer risk including anti-oxidant [82], hormonal mediated pathways [6,

83], anti-angiogenic [84], change in breast density [85], anti-inflammatory [86] as well as anti-telomerase activity [87]. This doctoral dissertation aimed to study the effects of green tea extract intake on risk of breast cancer via hormonal mediated pathways including modulation of circulating estrogens and IGF axis proteins by considering the genetic variation role in the metabolism of tea catechins. To the best of my knowledge, to date only two human intervention studies [88, 89] have investigated the role of green tea catechins intake on biomarkers of breast cancer risk.

Circulating Endogenous Estrogens, Urinary Estrogen Metabolites and Breast Cancer Risk

The association of circulating endogenous estrogens and urinary estrogens and estrogen metabolites (referred collectively as EM throughout this dissertation) and breast cancer risk have been extensively reviewed in manuscript 1.

Effects of Green Tea on Endogenous Sex Hormones and Estrogen Metabolism

Life time exposure to estrogens is a well-recognized risk factor for breast cancer development. One of the proposed mechanisms by which green tea may inhibit breast carcinogenesis is thought to be through reducing circulating sex hormone concentrations and urinary estrogen metabolite levels. Green tea might exert its sex hormone-related activities through inhibition of aromatase (via disrupting estrogen synthesis) or by blockading estrogen receptor from estrogen binding. It is true that SHBG is not a sex hormone but the term of "sex hormone" is henceforth considered for it for the ease of expression.

Aromatase (CYP19) is the key enzyme that catalyzes the conversion of androgens to estrogens (testosterone and androstenedione to estradiol and estrone, respectively) [90]. Several *in vitro* and *in vivo* studies [91-93] have shown that estrogen reduction

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activity of green tea, in particular EGCG constituent, may result from its modulatory effect on aromatase enzyme. Furthermore, Goodin *et al* [94] have demonstrated that green tea catechins, especially EGCG, compete with 17-β-estradiol for binding to estrogen receptor alpha (ER-α) and estrogen receptor beta (ER-β). Along the same lines, it has been shown that green tea reduces the ER-α level in tumors both *in vitro* and *in vivo* [95]. Similarly, Farabegoli *et al* [96] have recently reported that EGCG treatment downregulates ER-α pathway in MCF-7 breast carcinoma cell line.

   To date, very few epidemiological studies have investigated the role of green tea intake on circulating sex hormones or urinary estrogens and estrogen metabolite levels in women. In a cross-sectional study of 50 premenopausal Japanese women aged 21-42 years, Nagata *et al* [97] reported that green tea intake was significantly inversely correlated with follicular, but not luteal serum estradiol level, even after adjusting for potential confounders such as age, body mass index (BMI), menstrual cycle length, and fat and fiber intake (Spearman correlation coefficient (r)= –0.32, *P* <0.05). Green tea was also positively correlated with serum SHBG concentrations in the luteal phase of the menstrual cycle while additional adjustment for fat and fiber intakes was done (r= 0.31, *P*= 0.05). Black tea was not correlated with neither estradiol nor SHBG in this study. In another cross-sectional study, Wu *et al* [80] investigated the relationship between tea intake and plasma levels of estrone, estradiol and androstenedione in 130 healthy postmenopausal participants of the Singapore Chinese Health Study. Findings from this study showed that regular (daily or weekly) green tea drinkers had 13% lower levels of plasma estrone in comparison to non- or irregular tea drinkers (*P*= 0.03) controlling for age, BMI, soy intake, etc. Interestingly, regular black tea drinkers had 19% higher levels of estrone compared to non- or irregular tea drinkers. A similar trend of changes between tea intake and plasma levels of estradiol and androstenedione was seen, however, none of these differences reached statistical significance (*P*= 0.08 and *P*= 0.14, respectively). Moreover, reported tea-sex steroid hormone associations were not influenced by COMT genotype.

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More recently two human intervention studies have investigated the effects of green extract high in EGCG on circulating levels of sex hormones. Green tea supplement used in these two trials was Polyphenon E (PPE), which is a hard gelatin decaffeinated green tea extract capsule containing 200 mg EGCG, 37 mg EGC, 31 mg EC, and other green tea polyphenols. Daily consumption of PPE with doses up to 1600 mg EGCG for 6 months was unrelated to serum levels of sex hormones including estradiol, testosterone, and SHBG compared to placebo group. When comparisons from baseline to month 6 were conducted within PPE group, both estradiol and SHBG concentrations were significantly decreased ($P= 0.05$ and 0.03, respectively). Participants in this randomized controlled trial were 40 women aged 21-65 years with a history of hormone receptor-negative breast cancer and did not have any evidence of disease at the time they started the study. Although COMT genotyping was conducted in these subjects, sex hormone analysis was not stratified by COMT genotype status, probably due to very small sample size [89]. In another randomized placebo-controlled intervention [88], 103 healthy postmenopausal women were randomized into three different groups of 400-mg EGCG as PPE (n= 37), 800-mg EGCG as PPE (n= 34), and placebo (n= 32) and consumed trial capsules daily for two months. Participants were overweight and mostly Hispanic, non-current menopausal hormone therapy users (i.e. within last 6 months), and not a regular green tea drinker (less than one cup of green tea per week). Following supplementation with high doses of EGCG equal to 400mg to 800 g, serum levels of estrone, estradiol, testosterone, androstenedione as well as SHBG did not change significantly between two different PPE and placebo groups.

The effects of green tea catechins on estrogen metabolism has much less been studied. Michnovicz and Bradlow [98] studied the effect of adding 3% green tea polyphenols extract to the diets of B ALB/c mice on hepatic estradiol 2-hydroxylation. This diet was capable of increasing the activity of liver 2-hydroxylase (P450 enzyme) which yields 2-hydroxylated estrogens. These findings are important because the ratio of $2\text{-OHE}_1/16\alpha\text{-OHE}_1$ has been used as a surrogate biomarker of breast cancer risk. Also, it

has been shown that catechin and (-)-epicatechin can reduce COMT activity in human mammary tissues [99]. Since COMT enzyme is both involved in catechin and estrogen metabolism, it is likely that *COMT* genotype activity influences these pathways along with subsequent oxidative DNA damage.

In the only epidemiological study of green tea intake and urinary estrogens[100], the association of green tea consumption with urinary estrogen profiles was investigated in 119 premenopausal women in luteal phase and 72 postmenopausal women. In this cross-sectional study of healthy Japanese American women, daily drinking of green tea versus less than once per week was associated with lower luteal total estrogen metabolites (P_{trend}= 0.01) and 16-hydroxylation pathway (P_{trend}= 0.001) in premenopausal women. Additionally, daily intake of green tea in postmenopausal women was related to lower concentrations of estrone and estradiol (P_{trend}= 0.01 and 0.09, respectively) when compared to drinking less than once weekly. Although no significant association was found for any estrogens or estrogen metabolites ratios in premenopausal women, daily green tea intake was inversely linked to the ratio of parent estrogens to estrogen metabolites (P_{trend}= 0.04), and positively associated with ratio of 4-pathway methylated catechols to 4-pathway catechols in postmenopausal subjects. The ratio of 2-hydroxylation pathway to 16-hydroxylation pathway was not associated with green tea intake in neither premenopausal nor postmenopausal women, and all results were adjusted for age and study center.

To the best of my knowledge, no human intervention study has investigated the effects of either green tea catechins intake or COMT genotype on urinary estrogen metabolites levels. Taken together, overall existing data from observational (cross-sectional) or *in vitro* studies support the proposed mechanism that green tea intake may exert its beneficial effect against breast cancer by lowering circulating estrogen levels.

Circulating Insulin-like Growth Factors and Breast Cancer Risk

The insulin-like growth factor (IGF) system is a family of peptides including two ligands (IGF1 and IGF2), two cell surface receptors (IGF-1R and IGF-2R), and at least six high affinity binding proteins (IGFBP-1 to IGFBP-6). IGFBP-3 is the most abundant IGFBP and predominant binding protein of IGF1 and IGF2 [101]. Additionally, and as shown in **Figure 4**, intracellular downstream signaling of the IGF pathway is consisted of several proteins including but not limited to insulin-receptor substrate 1 (IRS1); phosphatidylinositol 3-kinase (PI3K); Akt; target of rapamycin (TOR); S6 kinase (S6K); Raf, and mitogen-activated protein kinase (MAPK). IGF1 is mostly synthesized by the liver and to a lesser extent in extrahepatic organs through autocrine and paracrine mechanisms by interactions between stromal and epithelial cells. IGF1is mainly regulated by growth hormone (GH) and to a smaller extent by nutritional factors. Circulating concentrations of IGF1 are correlated with energy and dietary intake of protein, and IGF-1 levels may be decreased by the effect of malnutrition on GH [102, 103]. Production of IGF2 is similar to IGF1 but its regulation is not strongly controlled by GH.

IGF-1R is a tyrosine kinase cell-surface receptor and has binding affinity for both IGF1 and IGF2. Activation of IGF-1R stimulates higher cancer cell proliferation and metastasis rate. These effects have been attributed to either autocrine secretion of IGF-I/II by the cancer cells or increase in blood concentrations of IGF-1 in the host [105]. IGF-1R signals can be improperly amplified by pathology in downstream molecules such as loss of function of phosphatase and tensin homologue (PTEN), which is a tumor suppressor gene. Protein encoded by the PTEN gene is an adverse key regulator of PI3K signaling and weakens signaling deriving from IGF-1R.

IGF-2R has only affinity for binding to IGF2 and does not have tyrosine kinase domain. As a result, IGF-2R is not involved in downstream signaling pathway, and even shows properties of a tumor suppressor gene in the sense that it attenuates proliferation by decreasing IGF2 levels and subsequent inactivation of IGF-1R [104].
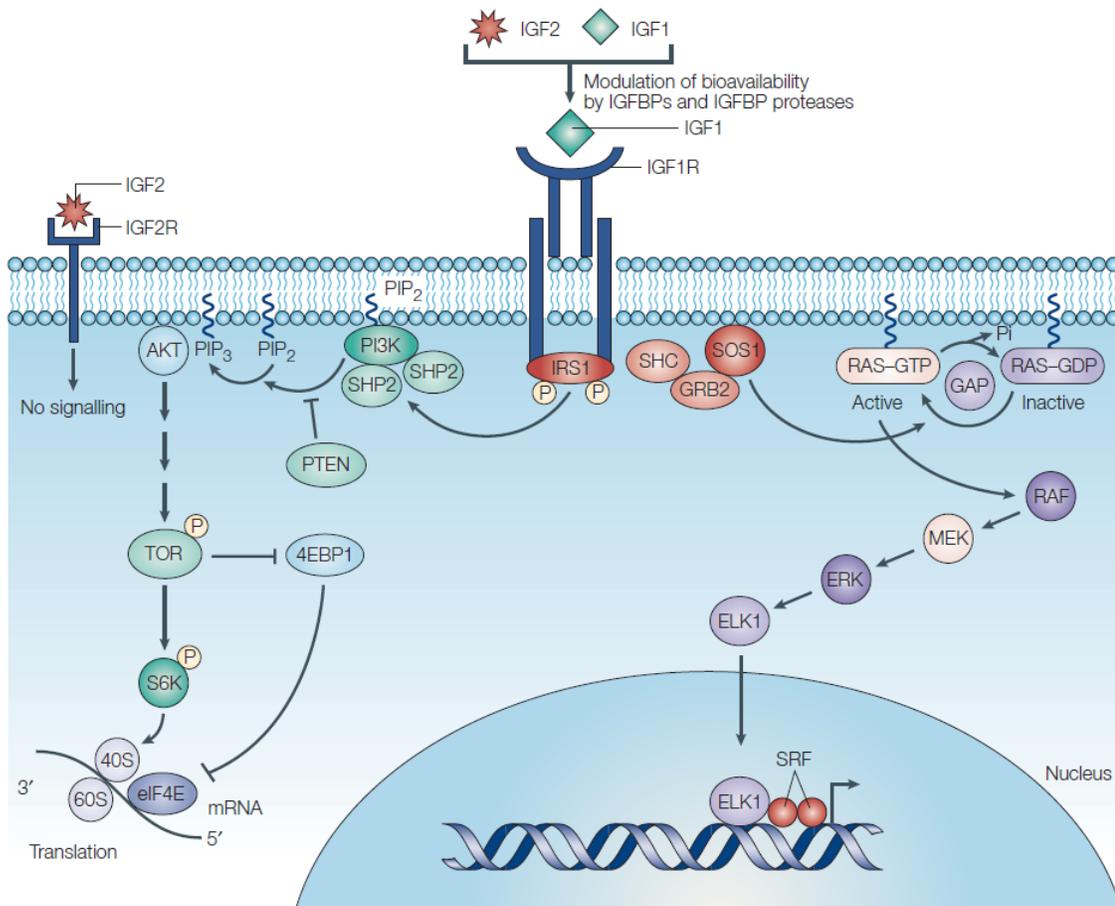
Figure 4. Insulin-like growth factor system and its downstream signaling pathways [104].

Circulating levels of IGF1, IGF2 and IGFBPs vary noticeably among normal individuals. This high between-person variation is thought to be due to genetic and environmental factors [106]. Bioavailability of IGF proteins is determined by IGFBPs levels. For instance, IGFBP-3 prolongs the half-life of IGF-1 in blood but decreases its bioavailability. The role of IGFBPs in growth regulation is complicated and poorly understood. IGFBPs can compete with IGF-1R for free ligands such as IGF-1 and 2 and therefore it diminishes IGFs signaling. On the other hand, it has been suggested that IGFBPs can trigger IGFs signaling possibly by increasing the half-lives of IGFs in circulation. Along the same lines, preclinical studies in MDA-MB-231BP-2 breast cancer

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cells have shown that overexpression of IGFBP-2 is associated with breast cancer [107], and loss of function of PTEN gene [108]. Other growth regulatory actions that may modulate cell cycle and apoptosis, such as influencing Wnt signaling pathway [109] or interaction with TGF-β signaling [110], have also been attributed to the IGFBPs. These biological activities of IGFBPs are independent of its IGFs binding properties.

IGF pathway is involved in mammalian growth, development and metabolism, and plays an important role in cellular processes like proliferation, survival, and differentiation. IGF1, the main and most-studied ligand of IGF pathway, is believed to promote carcinogenesis by stimulating mitosis, increasing DNA synthesis and inhibiting apoptosis [111]. In vitro studies suggest that IGF-1 and estradiol act through a cross-talk pathway by which mammary epithelial proliferation is promoted, and consequently, the risk of breast cancer is increased [112]. This mechanism works through estrogens (estradiol) stimulating the expression of IGF-1 and IGF-1R, and as a result of that, circulating concentrations of IGFBPs, in particular IGFBP-3, are reduced. Successively, binding of IGF-I to its receptor starts an intracellular signal transduction pathway which activates transcription factors such as the estrogen receptor. This in turn enhances estrogenic activity by converting estrone sulfate to more active compound, i.e. estrone [113, 114]. In addition to synergistic relationship with estrogens, high levels of IGF-1 are associated with increased mammographic density, which is an established independent predictor of breast cancer risk [115, 116]. High percent breast density has also been associated with common genetic variations in the IGF pathway such as rs1520220, rs6220, and rs2946834 SNPs [117, 118]. These findings support the notion that the relation between breast density and breast cancer risk may be mediated at least partly thorough the IGF axis-related pathway(s).

Although findings from the majority of individual epidemiological studies [119-122] are inconsistent, a recent large meta-analysis of 17 prospective studies have shown that high serum concentrations of IGF-1 and IGFBP-3 are significantly positively

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associated with postmenopausal breast cancer risk (OR= 1.33; 95% CI, 1.14– 1.55, $P<0\cdot0002$ for IGF-1; and OR= 1.23; 95% CI, 1.04– 1.45, $P<0\cdot001$ for IGFBP-3) [123]. Neither IGF-1 nor IGFBP-3 was related to breast cancer risk in premenopausal women in this meta-analysis. Ahlgren *et al* [124] have previously reported that high birth weight and growth during childhood and adolescence, most specifically during puberty, modify breast cancer risk later in life in Danish women population. Given IGF-1 exert its peak growth activity over the first 1-2 decades of life, it is intriguing to speculate that IGF1 levels in early life exerts much stronger effect on breast cancer risk later in life. Therefore, designing large prospective studies to evaluate the effects of early exposure of IGF axis proteins on breast cancer risk is warranted. In addition, efforts are due to standardize measurement methods and sample storage conditions, and identify potential factors that may either modify the effect (e.g., other hormonal mediators) or act as confounding variable in studying the relationship between IGFs and breast cancer risk.

In confirming the proposed cross-talk between IGF pathway and estrogens, data from the European Prospective Investigation into Cancer and nutrition (EPIC), a multi-center prospective cohort study, showed that IGF-I was positively associated with postmenopausal estradiol, estrone and androstenedione, while inversely correlated with SHBG levels [125]. In accordance with these findings, Pollak *et al* have demonstrated that administration of Tamoxifen, a common anti-estrogen therapy for hormone receptor-positive breast cancers, in patient with breast cancer resulted in significant reduction in serum IGF-1 levels [126]. Biological mechanisms underlying the association between IGF1levels and cancer risk are mainly thought to involve activation of survival pathways and making apoptosis of damaged cells less effective. In this manner, stepwise accumulation of genetically damaged cells and cell proliferation occur at a faster pace and concurrently the risk of errors in cell division is escalated [104].

Interestingly, low serum levels of IGF1 and high circulating concentrations of IGFBP-3 have been related to adverse cardiac outcomes such as ischemic heart disease [127]. Similarly, low circulating concentrations of IGF1 has been negatively associated

with the congestive heart failure risk in elderlies participating in the Framingham Heart Study [128].

Current strategies in treatment of cancer with focus on IGF pathway mostly revolves around targeting IGF-1R. There are several different approaches in attaining this goal including inhibitors of ligand binding; anti-receptor antibodies; inhibitors of IGF-1R expression; inhibitors of kinase activity; Akt inhibitors; and TOR inhibitors. Results from some of phase II clinical trials of IGF-1R specific antibodies showed major responses; however, phase III studies did not prove to be safe and efficacious. Pharmacology research is presently more directed toward using predictive biomarkers in identifying responder population and combination therapy with agents originally designed for treatment of diabetes such as metformin [129].

Effects of Green Tea on Circulating Insulin-like Growth Factors

Given the role of IGF axis in carcinogenesis, it has been suggested that blocking or lowering circulating concentration of IGF-1R and IGF-1 can result in reduced risk of cancer. Animal models have also demonstrated that lacking the IGF-1 gene or inhibition of IGF-IR function can in turn delay the onset of breast cancer or disturb breast cancer growth [130, 131]. Other than drug therapeutic approach, it has been suggested that dietary components in green tea can also modify the circulating level of IGF-1, IGF receptors and its binding proteins. Results from *in vitro* and *in vivo* studies have shown that EGCG can bind to active IGF-1R and selectively inhibits its kinase activity in a dose-dependent manner with the half maximal inhibitory concentration (IC50) equal to 14 μmol/L [132]. Zhou *et al* [133] reported that a combination of soy phytochemical and green tea reduce serum levels of IGF-1 in male and female FVB/N mice. In another study by Zhou [134], it was shown that green tea infusion at 1.5 g tea leaf/100 mL water reduced serum IGF-1 levels by 19% in female severe combined immune deficient mice. Similarly, Adhami *et al* [135] reported that combination of EGCG (10-40 μmol/L) and a

Cyclooxygenase-2 (COX-2) inhibitor reduced the levels of IGF-1 *in vitro* (human prostate cancer cells) and *in vivo* (athymic nude mice). Lately, it has been reported that physiological levels of EGCG inhibit the growth of ER-α -positive MCF7 cells, which in turn leads to down-regulation of the ERα, reduction in IGFBP-2 concentrations and higher levels of the tumor suppressor genes such as p53/p21. On the other hand, ERα - positive cell line T47D, EGCG induced an increase in expression of ERα protein levels and as a result of that cells responded more efficiently to Tamoxifen. In addition, EGCG triggered cell death in ERα-negative cell line of MDA-MB-231. Contrary to cancer cell lines, cell growth or death in normal breast epithelial cells were not altered in response to physiological levels of EGCG in amounts close to 1μM [136].

Epidemiological studies on green tea and circulating IGF levels are inconsistent and limited. Maruyama *et al* [137] found significant positive association of IGF-1 levels with higher intake of green tea in both Japanese men and women (P_{trend}= 0.01 and 0.04, respectively). IGFBP-3 levels were unrelated to the green tea intake. These results should be interpreted cautiously since the investigators had only adjusted for age. Circulating levels of IGF-1 and IGFBP-3 were not correlated with green tea intake in a cross-sectional study of healthy Asian-American women in Los Angeles County where non-green tea drinkers were compared to those drinking green tea more than 1 cup per day [65].

To my knowledge, only two clinical trials have so far examined the effects of green tea catechins intake on the circulating levels of IGF-1 or IGFBP-3. These two studies were described earlier in the "Effects of Green Tea on Endogenous Sex Hormones and Estrogen Metabolism" section, page 16. Briefly, in the study conducted by Crew *et al* [89], 34 women with a history of hormone receptor negative breast cancer received PPE in doses varying from 800 mg to 1600 mg EGCG daily for two months. Findings from this trial did not show any significant differences in IGF-1 and IGFBP-3 concentrations, and IGF-1: IGFBP-3 ratio between the PPE and placebo groups. However, women in the PPE group experienced favorable yet statistically insignificant

changes in IGFBP-3 levels and IGF-1: IGFBP-3 ratio. Given very small sample size of this trial, these results should be considered preliminary and interpreted with caution. Although *COMT* genotype activity was determined for each participant, results were not presented based on COMT genotype, likely because of small sample size. In another human intervention study [88] in which 103 healthy postmenopausal women were randomized into either placebo (n= 32), 400 mg EGCG as PPE (n= 37), or 800 mg EGCG as PPE (n= 34) group, daily green tea extract supplementation for two months did not result in significant changes in IGF-1 and IGFBP-3 concentrations between experiment and placebo groups. Nonetheless, a borderline statistically significant decrease in IGFBP-3 levels in both groups of 400 mg and 800 mg EGCG as PPE was observed (*P* for within-group comparison= 0.068 and 0.053, respectively). Data regarding IGF-1: IGFBP-3 ratio, or results based on *COMT* genotype status was not reported in this study.

Manuscript 1: Estrogen Metabolism and Breast Cancer

This work has been published in Cancer Lett. 2015 Jan 28;356 (2 Pt A):231-43. The association of androgens with breast cancer risk in postmenopausal women has also been described at the end of this manuscript to accomplish the objective of this dissertation.

SUMMARY

There is currently accumulating evidence that endogenous estrogens play a critical role in the development of breast cancer. Estrogens and their metabolites have been studied in both pre- and postmenopausal women with more consistent results shown in the latter population, in part because of large hormonal variations during the menstrual cycle and far fewer studies having been performed in premenopausal women. In this review we describe in detail estrogen metabolism and associated genetic variations, and

provide a critical review of the current literature regarding the role of estrogens and their metabolites in breast cancer risk.

INTRODUCTION

Breast cancer remains an overwhelming health burden, with an estimated 232,670 new breast cancer cases and 40,000 deaths among women living in the U.S in 2014 [138]. Age is the strongest risk factor for breast cancer. Unlike many cancers that increase beginning at the end of the fifth decade of life, breast cancer begins to rise in the third decade of life, most likely due to the effects of ovarian hormones on breast tissue [139-141]. More than 2/3 of all new cases occur after the age of 55 and women older than 65 have a relative risk greater than 4.0 when compared with those younger than 65.

To date, many additional risk factors for breast cancer have been identified. Some risk factors are non-modifiable, such as age, BRCA1 and BRCA2 gene mutations, family history, reproductive history, and high-dose radiation to the chest. Others are potentially modifiable, such as high endogenous estrogens, hormone therapy, obesity (for postmenopausal breast cancer) and alcohol consumption [139, 140]. There is some controversy regarding whether or not the risk factor of high mammographic density is modifiable [142-146].

Since a number of these known risk factors are related to endogenous estrogen levels, the effect of estrogens on breast carcinogenesis has drawn a great deal of attention in the last two decades, with evidence suggesting that estrogens play a causal role in the etiology of breast cancer [147]. In this review, we will discuss the metabolism of estrogens and will present a detailed analysis of published data evaluating the role of circulating and urinary estrogens and their metabolites in human breast cancer.

ESTROGEN METABOLISM

All steroid hormones originate from C27 cholesterol (Figure 5). The main source of cholesterol required for the synthesis of steroid hormones (steroidogenesis) is LDL-

cholesterol [148]. Cholesterol is metabolized down a number of enzymatic pathways and is converted to the 21-, 19-, and 18-carbon steroid hormones, respectively.

The first step in ovarian steroidogenesis is the movement of cholesterol into the mitochondrion. This step is regulated by the steroidogenic acute regulatory protein (*StAR*) encoded by the *STAR* gene [149] The next step involves the conversion of cholesterol to pregnenolone, catalyzed by the mitochondrial side-chain cleavage enzyme complex. Pregnenolone acts as a precursor for all steroid hormones. It is metabolized by different enzymes, and under the action of 17-hyroxylase/17, 20-lyase enzyme, a product of the CYP17A1 gene is converted to progesterone or androstenedione. Androstenedione, in turn, is further metabolized to other androgens or estrogens.

Estrogens are among very few aromatic molecules in humans. They are all C18 steroids and consist of one benzene ring, a phenolic hydroxyl group at C3, and a hydroxyl group (17β-estradiol) or a ketone group (estrone) at C17 (Figure 5). The main estrogens circulating in the human body are estradiol and estrone, as well as 16-hydroxyestradiol (estriol). Although estriol is usually the major estrogen in pregnant women [150], and is the most abundant estrogen in the urine of all women, estradiol is the most biologically active estrogen, primarily secreted by ovarian granulosa cells located next to theca cells and regulated by follicle-stimulating hormone (FSH). Estrone is reversibly converted to estradiol through the action of 17β-hydroxysteroid dehydrogenase enzyme [151]. Androstenedione, the most important product of the theca cells during the follicular phase of the menstrual cycle, is not biologically active; however, it acts as a precursor for both estrone and testosterone in the ovaries and peripheral tissues [90]. Testosterone, in turn, is converted to estradiol by the action of aromatase enzyme in the peripheral tissues (Figure 5).

In premenopausal women, estradiol synthesized in the ovaries is the most important estrogen, while in postmenopausal women, estrone synthesized in peripheral tissues is predominant. Aromatase (CYP19), encoded by the *CYP19A1* gene, is the rate-limiting enzyme in catalyzing the conversion of androgens to estrogens [152, 153]. Given

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the importance of this enzyme, blocking aromatase activity is an important pharmacological tool used for the treatment of estrogen-dependent diseases such as breast cancer, endometriosis, and endometrial cancer.

Estradiol and estrone are metabolized by three competitive pathways involving irreversible hydroxylations catalyzed by the NADPH-dependent cytochrome P450 (CYP) enzymes including CYP1A1, CYP1B1, and CYP1A2 (Figure 6). Estrone and estradiol are hydroxylated at positions C2, C4 and C16 and are converted to catechol estrogens (2-hydroxyestrone, 4-hydroxyestrone, 2-hydroxyestradiol, and 4-hydroxyestradiol), and 16α-hydroxyestrone. Estriol is produced by the hydroxylation of estradiol or 16α-hydroxyestrone. Catechol estrogens are further metabolized (methylated) to methoxyestrogens (2-methoxyestrone, 4-methoxyestrone, 2-methoxyestradiol and 4-methoxyestradiol) by the catechol-O-methyltransferase (COMT) enzyme (Figure 6 and Figure 7).

In addition to methylation, parent estrogens and catechol estrogens are also conjugated with glucuronic acid and sulfate by hepatic phase II enzymes including UDP-glucuronosyltransferases and sulfotransferases, respectively. Conjugation is considered a detoxification reaction by which hormones either become water soluble and are excreted in the urine or feces, or turn into a more lipophilic moiety with elevated half-lives (Figure 6) [154-156].

2-hydroxylation pathway. Quantitatively, the 2-hydroxylation pathway is the major metabolic pathway compared to the 4- and16-hydroxylation pathways. The cytochrome P-450 enzymes, including CYP1A1 and CYP1B1, are major phase I enzymes mainly expressed in breast and liver tissues [157]. These enzymes, along with CYP1A2, catalyze the C2 hydroxylation of parent estrogens to their respective catechol estrogens [158]. Two-hydroxylated estrogens possess low binding affinity for the estrogen receptor (ER) [159, 160]. These metabolites demonstrate reduced hormonal potency when compared with estradiol, and both non-estrogenic and anti-estrogenic activities have been attributed to them. There is some evidence from cell culture studies

in ER+ human MCF-7 breast cancer cells suggesting that 2-hydroxyestrone and 2-hydroxyestradiol inhibit cell growth and proliferation [161, 162]. In addition, 2-hydroxy metabolites have been associated with normal cell differentiation and apoptosis [163, 164]. Taken together, these findings have led some researchers to classify 2-hydroxyestrone as a "good estrogen" [165]. The lack of tumorigenic activity of 2-hydroxy metabolites has been attributed to a few mechanisms including a high rate of clearance, more rapid rate of *O*-methylation by the COMT enzyme, lower hormonal potency in estrogen target tissues, and methylated products of 2-hydroxyestradiol such as 2-methoxyestradiol, which suppress tumor cell proliferation and angiogenesis [159]. At the same time, it has been shown that 2-hydroxyestrogens can damage DNA and generate free radicals as they go through redox cycling or when COMT is inhibited [166, 167]. It is important to note that high inter-individual variability in 2-hydroxylation has been shown in human liver samples, possibly explaining the high variability of metabolite levels in individuals [168].

Methoxyestrogens, including 2-methoxyestradiol, have been shown to inhibit carcinogenesis by suppressing cell proliferation and estrogen oxidation due to effects on microtubule stabilization [169-171]. Lottering *et al*. [172] investigated the effects of 17-β estradiol and its metabolites on cell cycle in MCF-7 cells and reported that 2-methoxyestradiol acts as a cytostatin and inhibits mitosis.

4-hydroxylation pathway. CYP3A4/3A5 has been shown to be the primary enzyme in the 4 hydroxylation of estradiol in human liver microsomes [168]. 4-hydroxylated catechol estrogens possess carcinogenic potential due to their ability to cause DNA damage by forming depurinating adducts, which in turn, generate mutations with subsequent oxidative damage and initiation of breast cancer [173]. In microsomal preparations of human mammary fibroadenoma and adenocarcinoma, formation of 4-hydroxyestradiol was four times higher than 2-hydroxyestradiol formation, indicating that the ratio of 4-/2- hydroxyestradiol may be used as a biomarker for detection of malignant breast tumors [174]. In addition, it has been shown that the ratios of quinone-estrogen

DNA adducts to their parent or conjugated catechol estrogens were significantly higher in women with breast cancer or at high risk of breast cancer compared with control women [175]. On the other hand, it has been suggested that the 4-methoxyestrogens prevent oxidative metabolism of estradiol [171] and oxidative DNA damage [167]. These findings are in agreement with a more recent study in which inhibition of COMT enzyme activity was associated with higher levels of depurinating 4-hydroxyestrone (estradiol)-1-N3Adenine and 4-hydroxyestrone (estradiol)-1-N7Guanine adducts in MCF-10F cells [176].

16-hydroxylation pathway. 16α-hydroxyestrone is the most important metabolite of the 16-hydroxylation pathway. 16α-hydroxyestrone is a potential tumor initiator, which promotes unscheduled DNA synthesis and anchorage independent growth in mouse mammary epithelial cells [177-179]. Animal studies have shown that urinary concentrations of 16α-hydroxyestrone are associated with increased proliferation of mammary cells [178, 179], Ras oncogene expression [180], and mammary tumor incidence [181]. Osborne *et al*. investigated the extent of estradiol 16α-hydroxylation in relation to the risk of developing breast cancer in human breast tissue. They reported that 16α-hydroxyestrone levels were eight-fold higher in cancerous mammary terminal duct lobular units than nearby mammary fat tissue, suggesting that 16α-hydroxyestrone production may play an important role in breast cancer induction [182].

There are currently substantial data suggesting a link between concentrations of individual metabolites or the ratio of specific metabolites and breast cancer risk in humans; this will be discussed in further detail later in this review.

ROLE of GENETIC VARIATION in ESTROGEN METABOLISM

It has been postulated that genetic polymorphisms in genes encoding enzymes involved in estrogen metabolism pathways and the genes encoding the ERs are associated with breast cancer risk. Polymorphic variations in genes encoding COMT, CYP1A1,

CYP1B1, estrogen receptor alpha (ERα), estrogen receptor beta (ERβ), CYP17A1, and CYP19A1 have received extensive attention within the last decade.

COMT is a phase II enzyme that inactivates catechol estrogens by conjugating them into nongenotoxic methoxyestrogens [27]. COMT also prevents biotransformation of catechol estrogens to quinone-DNA adducts and development of reactive oxygen species (ROS) capable of damaging cellular macromolecules such as DNA, lipids, and proteins [175, 183, 184]. *COMT*, located on chromosome 22q11[185], is polymorphic; a single G to A transition at codon 158 of *COMT* (single nucleotide polymorphism (SNP) rs4680) results in a 3- to 4-fold decrease in enzymatic activity (GG vs. AA genotype). Individuals with heterozygous genotype (A/G) show intermediate levels of COMT activity [20, 21].

Given the role of COMT in the conversion of catechol estrogens to methoxyestrogens, genetic variations in this enzyme may influence the risk of breast cancer as a result of significant changes in catechol estrogen and methoxyestrogen levels [186]. It has been hypothesized that women possessing the low activity *COMT* genotype (AA or Met/Met) might be at greater risk of breast cancer due to higher concentrations of catechol estrogen intermediates [187-189]. Unexpectedly, results from the most recent meta-analysis [190] of 56 studies including 34,358 breast cancer cases and 45,429 controls show no evidence of significant associations between the *COMT* Val158Met polymorphism and breast cancer risk in any genetic model (comparing recessive and dominant models with each other). Although these findings did not change in subgroup analyses by ethnicity, source of controls, or menopausal status, they must be interpreted with caution because of the large heterogeneity between studies and lack of data for adjustment of other covariates such as age, body mass index (BMI), lifestyle, and environmental factors.

In addition to the hydroxylation of parent estrogens to catechol estrogens, CYP along with peroxidase enzymes catalyze the oxidation of catechol estrogens to estrogen semiquinones and quinones, which are carcinogenic metabolites of estrogens (Figure 7)

[191]. It has been hypothesized that polymorphic variations in CYP1A1 and CYP1B1genes are linked with increased risk of breast cancer. In a case-control study, Taioli *et al*. [192] demonstrated that African-American breast cancer cases with the Msp1 homozygous variant polymorphism in CYP1A1 had an odds ratio (OR) of 8.4 (95% CI, 1.7- 41.7) compared with controls. This association was not observed in Caucasian women. In contrast, Miyoshi [193] reported an inverse association between the Msp1 (6235(T/C)) and breast cancer risk in Japanese women (OR= 0.60; 95% CI, 0.41-0.88). Taken together, these findings are mixed and more research is needed to clarify this discrepancy.

SNPs in CYP1B1genes have also been investigated in relation to breast cancer risk [194-197]. In a population-based case-control study conducted by Reding *et al.*[198] of 891 breast cancer cases and 878 controls, women homozygous with the T allele in CYP1B1*2 (Ser^{119}; rs1056827) were compared with women homozygous with the G allele. Those homozygous for the CYP1B1*2 Ser allele (T/T) had a 1.69 times higher risk of breast cancer (95% CI, 1.17-2.46); however, results from this study were not in agreement with an earlier meta-analysis [199] that showed no overall associations of breast cancer risk with CYP1B1 polymorphisms. This inconsistency may be due to selection bias or the result of chance in Redwing's study, or limitations of meta-analysis, such as variability in populations and publication bias.

In a meta-analysis of more than 10,000 mostly Caucasian breast cancer cases, multiple potentially functional SNPs in ERα including rs2234693, rs9340799, rs1801132, rs3798577, and rs2228480 have been studied. The only SNP that showed borderline significant association with reduced risk of breast cancer was rs2234693 (CC genotype vs. TT; OR=0.92; 95 % CI, 0.86- 0.99; *P* =0.08 for heterogeneity test) [200]. Polymorphisms in the ERβ gene and breast cancer risk have also been studied in a recent systematic review [201] that reported that rs2987983, and rs4986938 SNPS are significantly associated with overall breast cancer risk

Most recently, Chattopadhyay *et al.* [202] reported significant associations between SNPs in ERα (rs2234693), ERβ (rs2987983), CYP17A1 (rs743572) and CYP19A1 (rs700519) and breast cancer risk in a case control study performed in North India. The study included 360 cases with corresponding controls matched on age, sex, ethnicity, and geographical location. For the ERα genetic polymorphism, the CC genotype was the reference genotype for all comparisons. The CT and CT+TT genotypes were positively associated with postmenopausal status (P=0.018 and P=0.017; respectively) and histological grade I and II cases (P=0.022 and P=0.008; respectively), and negatively associated with advanced clinical stage (III+IV) (P=0.008 and P=0.021; respectively). However, in each case the significance was lost after conducting Bonferroni corrections for multiple comparisons. For the other SNPs, a number of associations remained statistically significant after performing Bonferroni corrections and adjusting for age. For ERβ, the TC+CC genotypes were inversely correlated with premenopausal status when compared with the TT genotype (OR=0.31; 95 % CI, 0.15-0.62; *P* value=0.001). For CYP17A1, the TC+CC genotypes were positively associated with ER- status compared with TT genotype (OR=2.77; 95 % CI, 1.52- 5.04; *P* value =0.001). Finally, a SNP in CYP19A1 or the aromatase gene (rs700519) was linked with an increased risk among postmenopausal women (CT+TT vs. CC; OR=2.72; 95 % CI, 1.47- 5.10; *P* value=0.001).

Studies assessing different SNPs in the CYP19A1 and CYP17A1 genes in relation to breast cancer have yielded inconclusive results. Talbott *et al*. [203] have demonstrated that polymorphic variations in the CYP 19 gene in rs1008805 (A/G) SNP with at least one G allele, but not rs730154 (C/T) SNP, is linked with higher risk of premenopausal breast cancer (OR = 1.72; 95% CI, 1.20- 2.49). There was no association between postmenopausal breast cancer and rs1008805 SNP. Yet Reding and other investigators [204] from the Women's Contraceptive And Reproductive Experiences (CARE) study observed no substantial association with breast cancer risk for neither SNPs in *CYP19A1*, nor for *CYP17A1*, ERα, *COMT*, *CYP1A1*, or *CYP1B1* genes in more than 1,600 White

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and Black cases. Similar results have been noted from the Shanghai Breast Cancer Study for 19 SNPs in CYP19A1gene [205]. Polymorphic variations in the CYP17A1 gene in relation to breast cancer have been also considerably evaluated, but findings are mixed and no firm conclusion can be drawn at this time [206-209].

Further studies with more homogenous populations and larger sample sizes are required to substantiate the role of ERs and estrogen-metabolizing gene polymorphisms in breast cancer risk pathogenesis.

ESTROGENS and BREAST CANCER RISK

There is increasing evidence from epidemiological, animal, and *in vitro* studies that endogenous estrogens are involved in breast carcinogenesis [210]. Evidence suggesting a hormonal role in breast cancer development began with an early observation that bilateral oophorectomy significantly reduces breast-cancer risk, and that risk reduction is greater if the ovaries are removed earlier in life [211]. In addition, some of the well-established risk factors for breast cancer, including early onset of menarche (<12 years), late menopause (>55 years), nulliparity or having child late in life, are related to lifetime exposure of breast tissue to sex hormones. Given that approximately 2/3 of breast tumors are ER positive (ER+) [212] and responsive to circulating estrogens, and that almost all ER negative (ER-) cases are resistant to endocrine therapy, it is important to elucidate the specific mechanisms by which estrogens are related to elevated breast cancer risk. We will discuss the association of estrogens with breast cancer risk in the following sections separately based on menopausal status. Assessing the role of androgens and progesterone in breast cancer is beyond the scope of this review and will not be discussed here.

A. Postmenopausal Women

A. 1. Circulating primary hormones. In postmenopausal women, increased circulating concentrations of estradiol, estrone, estrone-sulfate, and androstenedione have been associated with higher breast cancer risk, whereas higher levels of sex hormone binding globulin (SHBG) have been associated with lower risk [213-220]. Key *et al.*

[221], in a pooled analysis of 9 prospective studies of 663 women who developed breast cancer and were not on any exogenous sex hormones, showed that risk of breast cancer significantly increases with higher levels of total estradiol, free estradiol, estrone, estrone-sulfate, androstenedione, dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), and testosterone. The relative risk (RR) and 95% CI for the highest quintile versus the lowest quintile of estradiol levels was 2.0 and 1.47–2.71, respectively (**Table 1**). Since this analysis was published, a few more prospective and case-control studies have been reported that have found similar results [222-226]. It should be noted that the majority of populations studied were general populations with average breast cancer risk who were not taking any exogenous sex hormones. However, when breast cancer risk category (low versus high based on Gail or Rosner and Colditz risk model scores) [227, 228] was taken into consideration, no difference in the association between sex hormones and breast cancer was observed in low versus high breast cancer risk subjects [225, 229]. These findings should be evaluated in further studies.

Surprisingly, the results from the Women's Health Initiative (WHI) are in disagreement with those from observational studies. Manson and colleagues [230] have recently published updated data from the WHI with 13 years of cumulative follow-up. Briefly, WHI randomized 27,347 postmenopausal women 50-79 years to take either conjugated equine estrogens alone (CEE) for 7.2 years or CEE plus medroxyprogesterone acetate (MPA) for 5.6 years. Compared with the placebo group, the CEE group showed a 21% reduced risk of invasive breast cancer (95% CI, 0.65- 0.97) while the CEE+MPA group showed a 28% increased risk (95% CI, 1.11- 1.48) **(Table 1).** As suggested by Chlebowski and others [231, 232] the discrepancy between observational studies and the WHI may be due to methodological issues. For example, in non-research setting, women using hormones usually have more screening mammograms than non-hormone users, and consequently breast cancer is detected earlier; however, in the WHI study, all participants were required to receive screening mammograms at baseline and annually. In addition, there were a relatively small number of breast cancer cases in the estrogen –alone group (n=168 for the intervention group and n=216 for the control group).

There are a number of limitations of observational studies, including the collection of a single blood sample. It has been suggested that one blood sample with a long period of follow up time, which is characteristic of epidemiological studies, may not be a good predictor of breast cancer risk. However, Zhang *et al*. in a nested case–control analysis within the Nurses' Health Study (NHS), showed that one single measurement of blood reproductive hormones is sufficient to predict ER+/PR+ breast cancer in postmenopausal women 16-20 years following blood draw. In addition, when two blood measurements, collected 10 years apart, were compared, the intra-class correlation coefficients were found to be 0.69 (95 % CI, 0.61- 0.75) for estradiol, and 0.74 (95 % CI, 0.67- 0.80) for SHBG, indicating that hormone levels are well correlated over long period of time [233].

Due to scarce available data, it is unclear if the relationship between circulating sex hormones and breast cancer differs according to receptor status. In a recent short review by Key, results from four studies were compared, and it was reported that estradiol was directly linked with ER+ breast cancer in postmenopausal women. However, since the number of ER- breast cancer cases was very small, no firm conclusion could be established [234].

A number of known breast cancer risk factors have been proposed to influence risk via effects on estrogens. Obesity, defined as BMI> 30 kg/m^2, raises the risk of postmenopausal breast cancer, and this has been attributed to the higher circulating levels of estrogens synthesized in the adipose tissue of obese women. At the same time, an inverse association between obesity and SHBG blood levels has been reported, which in turn, contributes to higher concentrations of free estradiol (bioavailable fraction) in the circulation.

The magnitude of the associations of estrogens with a number of breast cancer risk factors including obesity, reproductive, demographic, and life style factors has been investigated by the Endogenous Hormones and Breast Cancer Collaborative Group in several studies. In a pooled analysis of eight prospective studies in postmenopausal

women, adjusting data for free estradiol concentrations attenuated breast cancer risk by 17% for each 5 kg/m^2 increase in BMI, resulting in a loss of statistical significance for the association between BMI and breast cancer risk [235]. In another cross-sectional analysis of 13 prospective studies by the same group, estrogen and androgen levels were positively associated with obesity, smoking (15+ cigarettes daily) and alcohol consumption (20+ g alcohol daily), and inversely linked with age. By contrast, SHBG concentrations were greater in older women and lower in obese women and those consuming alcohol [236].

Mammographic density, a known risk factor for breast cancer development, is a measure of the amount of fibroglandular tissue that appears on a mammogram [237]. It has been hypothesized that sex steroid effects on breast cancer are mediated through mammographic density [238, 239]; however, available data do not consistently support the hormonal basis for mammographic density mostly because of the confounding influence of BMI [240-242].

It has long been of concern that circulating estrogen may not be an appropriate surrogate for breast tissue levels. Some studies have shown that estrogen concentrations in normal or breast tumor tissue are greater than in the circulation [243, 244]. It has been suggested that inhibition of local estrogen aromatization in tumor tissue may be an appropriate breast cancer prevention strategy. This notion has recently been rejected by Lønning *et al.* [245] who proposed a model in which plasma-to-tissue equilibration explains the high estrogen levels in breast tissue. According to this model, malignant breast tumors are constantly exposed to circulating estrogens through ER binding or active uptake of estrogens. Therefore, they propose that systemic suppression of estrogen production may be superior to targeting local aromatase enzyme in hormone responsive breast cancer. Given the strong positive association observed between blood sex hormones and breast cancer in postmenopausal women, circulating estrogens seem to be an appropriate marker of tissue exposure, as suggested by Hankinson and Eliassen [246].

A. 2. Urinary and circulating estrogen metabolites. The role of estrogen metabolites in breast cancer has been the subject of discussion for the last three decades; however, compared to circulating primary estrogens, few studies have investigated the association between breast cancer and individual estrogen metabolites, their pathways or ratios. Among catechol estrogens, 16α-hydroxyestrone and 4-hydroxy metabolites are relatively more estrogenic and have genotoxic potential while 2-hydroxy metabolites are considered to have little estrogenic activity or antiestrogenic properties [165].

Findings from small observational studies [161, 247, 248] have led to the hypothesis that a lower ratio of urinary 2-hydroxyestrone to 16α-hydroxyestrone (2/16-hydroxyestrone) is a breast cancer risk factor. The most recent combined analysis [249] reviewed the results of 5 trials comprised of 385 invasive breast cancer cases and 723 controls. All metabolites were analyzed utilizing an enzyme linked immunosorbent assay (ELISA), and odds ratios adjusted for known breast cancer risk factors were calculated. Results comparing the women in the lowest tertiles with those in the highest tertiles did not reveal any significant association for either the 2/16-hydroxyestrone ratio (OR=1.02; 95% CI, 0.71-1.48) (**Table 2**) or any individual metabolites (OR for 2-hydroxyestrone =0.93; 95% CI, 0.67-1.30, and OR for 16α-hydroxyestrone =1.01; 95% CI, 0.73-1.41). Additionally, when data were stratified by ER status, no significant difference in the relative risk was found. The pattern of results was similar in a systematic review by Obi *et al.* [250] in which 6 prospective and 3 retrospective studies including 1189 breast cancer cases and 1888 matched controls were reviewed. Women in the top category of either urinary or circulating 2/16-hydroxyestrone did not differ in risk of breast cancer compared to those in the bottom category (**Table 2**). Risk associations were not changed when ER subtype was also taken into account.

Recently, Fuhrman *et al.* [251] examined the associations of circulating levels of 15 estrogens and estrogen metabolites individually and grouped by pathway, as well as metabolic pathway ratios, with breast cancer risk in a prospective case-control study nested within the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. Participants were 277 invasive breast cancer cases and 423 matched controls, all

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postmenopausal women not using exogenous hormones at the time of the blood draw. In contrast to previous studies, an assay with high sensitivity and specificity, liquid chromatography–tandem mass spectrometry (LC-MS/MS), was utilized to measure circulating levels of hormones and their metabolites. The study demonstrated positive associations between breast cancer risk and unconjugated estradiol levels (HR = 2.07; 95% CI, 1.22- 3.51; P_{trend}=0.01) and the ratio of 4-hydroxylation pathway catechol estrogens to 4-hydroxylation pathway methylated catechol estrogens (HR = 1.76; 95% CI, 1.06- 2.93; P_{trend}=0.02) comparing the highest to the lowest quintile. Interestingly, the ratio of 2-hydroxylation pathway to parent estrogens was found to be inversely associated with breast cancer risk (HR $_{Q5\ vs.\ Q1}$ = 0.54; 95% CI, 0.32- 0.90; P_{trend}=0.003) (**Table 2**).

Since the publication of the Fuhrman *et al.* results, another two studies have been conducted by the same lab and core investigators with inconsistent findings. Dallal *et al.* [252] reproduced similar results in a prospective case-cohort study within the Breast and Bone Follow-up to the Fracture Intervention Trial (B~FIT), of 407 cases and 496 controls. In this study, high circulating levels of estradiol were associated with elevated breast cancer risk (HR$_{top\ vs.\ bottom\ quintile}$=1.86; 95% CI, 1.19-2.90; P_{trend}=0.04). Additionally, increased ratios of the 2-hydroxylation pathway to parent estrogens, and 2:16-hydroxylation pathways were associated with lower risk (HR=0.69; 95% CI, 0.46-1.05; P_{trend}=0.01; and, HR=0.60; 95% CI, 0.40- 0.90; P_{trend}=0.002, respectively) (**Table 2**). Surprisingly, the ratio of 4-hydroxylation pathway to parent estrogens was reported to be inversely related to breast cancer risk (HR$_{top\ vs.\ bottom\ quintile}$ =0.61; 95% CI, 0.40-0.93; P_{trend}=0.004), which is not in agreement with the findings from previous studies. In contrast to Fuhrman and Dallal studies, no statistically significant results were observed in another nested case-control study by the same lab using blood samples from the Columbia Missouri Serum Bank [253]. The reasons for the discrepancies among these results are not quite clear, however, the investigators believe they might be due to differences in *COMT* polymorphisms or simply due to chance alone.

The association between circulating endogenous estrogens and breast cancer risk in postmenopausal women has been conclusively established, and compelling evidence exists to support a causal relationship. Epidemiological studies have consistently shown a 2-3 fold increase in breast cancer risk in women with elevated blood estradiol levels. On the other hand, findings from estrogen metabolite investigations are mixed. This inconsistency in estrogen metabolite results may be due to methodological differences in participant characteristics, study design and follow-up length (for some studies insufficient), number of cases (some studies under-powered), and high inter-individual variation in serum and urinary concentrations of estrogen metabolites, or limitations associated with estrogen metabolite measurement. Of particular note are differences in assay methodologies. Until recently, the leading methodology for measurement of estrogen metabolites was ELISA, a method that has limited specificity and sensitivity. This is of particular importance for analysis of samples from postmenopausal women, whose levels are extremely low. Recently, some groups have used liquid chromatography–tandem mass spectrometry (LC–MS/MS), which has much higher sensitivity and specificity.

A. 3. Circulating androgens. Androgen hormones including testosterone and androstenedione have been positively associated with risk of breast cancer in postmenopausal women. Combined analysis from nine prospective studies in the Endogenous Hormones and Breast Cancer Collaborative Group [221] demonstrated a significantly higher breast cancer risk in women with higher circulating levels of testosterone (RR_{Q5} vs. $_{Q1}$= 2.2; 95% CI, 1.6- 3.1). Following this pooled analysis, two of the included studies [223, 254] updated their results with new cases of breast cancer, which yielded the same pattern of results. Moreover, findings from the EPIC study that was published later [224] showed women at the highest quintile of testosterone had a 1.9 times greater risk of developing breast cancer than those in the lowest quintile (RR_{Q5} vs. $_{Q1}$= 1.9; 95% CI, 1.3- 2.6). The most recent results from the NHS [233] showed that women in the top vs. bottom quartile of testosterone levels were at a 50 % higher risk of breast cancer (RR= 1.5; 95% CI, 1.2- 1.9; P $_{trend}$<0.001). The observed association was

stronger for ER+/PR+ breast cancer cases compared to the ER-/PR- counterparts (RR= 1.8; 95% CI, 1.3- 2.5; P trend<0.001 vs. RR= 0.6; 95% CI, 0.3- 1.2; P trend=0.35, respectively). Adjusting for estradiol levels in some of the studies discussed earlier resulted in either no significant changes in the risk estimates [221, 224], or significant attenuation of relative risks [233]. Therefore, it remains to be elucidated whether the association between testosterone and breast cancer is independent of estradiol or through aromatization of estradiol.

Androstenedione is produced in the adrenal glands and ovaries in women, and is a precursor for estrone and testosterone hormones. Androstenedione is also directly associated with the risk of breast cancer, but its magnitude is slightly smaller than the testosterone. Findings from pooled analysis of 9 cohort studies [221] showed that top vs. bottom quintile of blood level of androstenedione was related with 115 % higher risk of breast cancer (RR$_{Q5}$ vs. $_{Q1}$= 2.15; 95% CI, 1.4- 3.2; P trend<0.001). Kaaks *et al* [224] reported later more modest effect of androstenedione on breast cancer in the EPIC study (RR$_{Q5}$ vs. $_{Q1}$= 1.94; 95% CI, 1.4- 2.7; P trend<0.0001). Although androstenedione was not reported by the NHS, plasma levels of DHEAS was significantly associated with breast cancer (RR$_{Q4}$ vs. $_{Q1}$= 1.7; 95% CI, 1.3- 2.3; P trend<0.001). Corresponding risk estimate was stronger for the ER+/PR+ cases than women with the ER-/PR- (RR of 2.1 vs. 1.1) [255].

Taken together, breast tumor development looks to be influenced more by estrogens than androgens. By shifting into menopause stage, androgens are mainly produced by adrenal glands and peripheral tissues rather than ovaries. Although bilateral oophorectomy has been shown to reduce the blood concentrations of androgens in postmenopausal women [256], risk of breast cancer is not reduced with the oophorectomy in years at or close to menopause [257]. This implies the importance of androgen synthesis in organs other than ovaries. Androgen levels are positively correlated with the levels of estrogens and this makes it challenging to draw a conclusion to what extent androgens exert their breast carcinogenic effect independently.

B. Premenopausal Women

B.1. Circulating primary hormones. Much less research has been performed on the effects of endogenous estrogens on breast cancer risk in premenopausal women than in postmenopausal women. Thus, the role of estrogens in breast carcinogenesis in this population is not thoroughly understood and remains relatively unclear. This is likely due to the much smaller number of breast cancer cases in premenopausal women. Another potential reason may be the large inter- and intra-individual variations in sex hormone concentration during the menstrual cycle. To the best of our knowledge, only nine prospective studies [258-267] have evaluated the associations between serum estrogens and breast cancer risk in premenopausal women.

Hankinson and Eliassen reviewed the results of seven of these studies in 2010 [268]. Briefly, three cohort studies [258, 259, 262] failed to show significant associations between breast cancer risk and estradiol, estrone, or estrone-sulfate, possibly due to the fact they were small studies with less than 50 cases, or had not adjusted for the timing of the menstrual cycle at the blood draw. Although the EPIC cohort [263], Guernsey cohort [261], and Rosenberg studies [260] had larger sample sizes with 285, 62 and 79 cases respectively and accounted for the phase of menstrual cycle, no statistically significant results were found for associations between breast cancer risk and estrone, estradiol, or SHBG.

On the other hand, early follicular blood samples from breast cancer cases (n=197) in the Nurses' Health Study II (NHSII) demonstrated a significantly elevated breast cancer risk in women with higher total and free estradiol ($RR_{Q4 \text{ vs. } Q1}$= 2.1; 95% CI, 1.1- 4.1; and, $RR_{Q4 \text{ vs. } Q1}$= 2.4; 95% CI, 1.3- 4.5; respectively). Importantly, the magnitude of the effect estimate was more pronounced among the ER+/PR+ cases compared with all breast cancer cases ($RR_{Q4 \text{ vs. } Q1}$= 2.7; 95% CI, 1.2- 6.0 for follicular total estradiol) consistent with the classical role of ERs in stimulating higher cell proliferation and mutagenesis [269]. On the other hand, no evidence of a relationship between breast cancer risk and estrone, estrone-sulfate, or SHBG was seen [265].

In a 2011 meta-analysis of those seven nested case–control studies with a total of 693 cases and 1609 controls, only a weak relationship between circulating estradiol and breast cancer risk was reported (OR for a doubling of estradiol=1.10; 95% CI, 0.96- 1.27) (**Table 3**) [270]. Following this meta-analysis, results of three studies and a new systematic review have been published. Dorgan *et al*. [264] reported no association between total or bioavailable estradiol or SHBG and breast cancer risk, in a prospective case-control study of 98 breast cancer cases nested in the Columbia, MO, Serum Bank cohort matched with 168 controls on factors such as the day of blood draw and menstrual cycle phase. Similarly, data from 104 cases of the Italian Hormones and Diet in the Etiology of Breast Tumors (ORDET) cohort did not suggest any associations with risk when the highest tertiles of estradiol and SHBG levels were compared with the lowest tertiles [266]. The NHS II has recently published updated data regarding the relationship of plasma sex hormones to breast cancer risk [267]. Since the last data were published in 2006, more than 400 additional cases were identified, bringing the total number of cases to 634. Estrogens were measured by radioimmunoassay or LC–MS/MS, and SHBG was measured by chemiluminescence immunoassay. Overall, after adjusting for known breast cancer risk factors, no significant associations were reported between breast cancer risk and early follicular or mid-luteal total estradiol, free estradiol, estrone, or SHBG. There was weak evidence indicating that mid-luteal estradiol was positively related with ER+/PR+ breast cancer ($OR_{Q5\ vs.\ Q1}$= 1.7; 95% CI, 1.0- 2.9; P_{trend} = 0.02).

Interestingly, results from the most recent pooled analysis of data by the Endogenous Hormones and Breast Cancer Collaborative Group [271] were not consistent with the findings of the previous studies. This inconsistency may be due to the exclusion of data from four of the previously discussed studies [258, 260, 262, 266]. Participants included in the analyses were premenopausal women below 50 years of age who were not using any exogenous sex hormones at the time of blood collection. Cases and controls were matched for age, menstrual cycle day, and blood draw date, and an odds ratio associated with a doubling in hormone levels was calculated separately for each hormone. Data from 600 women with incident breast cancer and 1375 controls were used

to calculate the odds ratio for breast cancer in relation to serum estradiol. The odds ratio for circulating estrone was estimated based on data from 477 cases and 933 controls. Findings showed that a doubling in concentrations of estradiol and estrone was associated with 19% and 27% elevated risk of breast cancer, respectively (OR for estradiol= 1.19; 95% CI, 1.06–1.35; P_{trend}=0.004; OR for estrone= 1.27; 95% CI, 1.05–1.54; P_{trend}=0.01) (**Table 3**). SHBG was not related to breast cancer risk. These risk estimates were not changed significantly after adjustment for hormonal breast cancer risk factors. When data were evaluated based on the tumor ER subtype, no significant differences in results were observed between ER+ or ER- cases, although odds ratios were generally larger among the ER+ subjects.

Overall, observational data regarding the associations of circulation estrogen concentrations with breast cancer risk in premenopausal are not as strong as postmenopausal women. Therefore, no conclusions can be drawn until larger well-designed studies with enhanced analytical methods are conducted.

B .2. Urinary and circulating estrogen metabolites. Four prospective studies have investigated the relationships between breast cancer incidence and concentrations of individual estrogen metabolites, their ratios or metabolic pathways in premenopausal women. The ratio of 2/16-hydroxyestrone has been studied in either urine or blood samples with inconsistent results. Muti et al. [272] reported that relative to the lowest quintile, cases in the highest quintile of luteal phase urinary 2/16-hydroxyestrone had a 45% reduction in breast cancer risk (n =67 cases; adjusted RR= 0.55; 95% CI, 0.23-1.32). Additionally, both 2-hydroxyestrone and 16α-hydroxyestrone were positively but non- significantly linked with risk. Consistent with these results was a study conducted by Meilahn et al. [248] in which 60 breast cancer cases were matched to 184 controls on age, baseline visit date, and menstrual cycle phase. Results showed that women in the top tertile of the 2/16-hydroxyestrone ratio had a lower breast cancer risk compared to those in the bottom tertile (OR= 0.75; 95% CI, 0.35-1.62; P value= 0.46).

The New York University Women's Health Study [273] is the only trial that has reported circulating levels of the 2/16-hydroxyestrone ratio. In this study, 377 cases were matched with 377 controls on day and phase of menstrual cycle. Results revealed no significant associations between breast cancer risk and 2-hydroxyestrone or 16α-hydroxyestrone, or their ratio (OR for 2-hydroxyestrone: 16α-hydroxyestrone =1.13; 95% CI, 0.68-1.87; P_{trend}=0.5). Along the same lines, in the subgroup analysis based on ER status when comparing the highest quartile with the lowest quartile among ER+ cases, the 2/16-hydroxyestrone ratio was associated with more than doubling of breast cancer risk (OR=2.15; 95% CI, 0.88- 5.27; P_{trend}=0.09).

Contrary to the previous studies that had analyzed 2-hydroxyestrone, 16α-hydroxyestrone, and their ratio with the ELISA method, the NHSII quantitated a comprehensive list of 15 urinary estrogens and estrogen metabolites utilizing the gold standard method, LC-MS/MS [274]. Mid-luteal urine samples were collected by 247 cases and 485 matched controls. Findings indicated that 2- and 4-hydroxylation pathway estrogen metabolites, but not 16-hydroxylation pathway, were inversely associated with risk, but results did not reach statistical significance. Likewise, the 2/16-hydroxyestrone ratio was not linked to risk ($RR_{Q4\ vs.\ Q1}$=0.90; 95% CI, 0.57- 1.41; P_{trend} = 0.86). The only estrogen metabolite positively associated with risk was 17-epiestriol, which is a metabolite in the 16-hydroxylation pathway ($RR_{Q4\ vs.\ Q1}$= 1.74; 95% CI, 1.08- 2.81; P_{trend} = 0.01). Interestingly, when comparing the highest quartile with the lowest quartile, elevated urinary concentrations of estrone and estradiol were associated with 48% and 49% reductions in risk, respectively (RR for estrone = 0.52; 95% CI, 0.30- 0.88; RR for estradiol = 0.51; 95% CI, 0.30- 0.86). Additionally, elevated ratio of 16-pathway estrogen metabolites to parent estrogen metabolites was associated with higher risk ($RR_{Q4\ vs.\ Q1}$= 1.61; 95% CI, 0.99- 2.62; P_{trend} = 0.04), and parent estrogen metabolites to non–parent estrogen metabolites ratio was negatively linked to risk ($RR_{Q4\ vs.\ Q1}$= 0.58; 95% CI, 0.35-0.96; P_{trend} = 0.03). It is difficult to interpret these findings since the directions of the associations for the primary estrogens in the urine are not in agreement with that observed in circulating primary estrogens. Also, due to a lack of research done in this

area, sufficient data do not exist for comparison. One possible explanation for these results is that higher levels of primary estrogens are excreted into urine prior to finding any chance to convert into genotoxic metabolites. Also, one may speculate that urine is not as relevant as blood to breast tissue exposure.

Recently, two systematic reviews and combined analyses have examined the relationships between circulating or urinary 2-hydroxyestrone, 16α-hydroxyestrone, and their ratio with breast cancer risk. Obi *et al*. [250], in a study of 682 premenopausal cases and 1027 matched controls, concluded that the urinary 2/16-hydroxyestrone ratio, but not circulating levels, is non-significantly associated with lower risk of breast cancer (range of ORs=0.5-0.75; 95% CI, 0.25- 1.01 and 0.35- 1.62; respectively) (**Table 3**). Similarly, Dallal *et al*. [249], in a combined analysis of 726 women (*n*=183 cases) demonstrated that elevated urinary 2/16-hydroxyestrone is suggestive of lower breast cancer risk ($OR_{top\ tertile\ vs.\ low\ tertile}$= 0.74; 95% CI, 0.45-1.23) (**Table 3**). Additionally, data from the same analysis showed that higher urinary 2/16-hydroxyestrone is indicative of decreased risk of breast cancer for ER- cases ($OR_{top\ tertile\ vs.\ low\ tertile}$= 0.33; 95% CI, 0.13- 0.84). This latter finding is based on small number of 31 cases; therefore, it may be due to chance and needs more research to be confirmed. A summary of data for both circulating and urinary estrogens and their metabolites is presented in **Table 3**.

CONCLUSIONS

There is currently convincing evidence to infer a causal relationship between endogenous estrogens and breast cancer in postmenopausal women; however, this relationship has not been firmly established in premenopausal women, possibly due to the large variations in hormone levels during the menstrual cycle, the small number of studies that have been performed, and the small number of cases. Of recent interest are genetic polymorphisms in the enzymes involved in estrogen metabolism that may modify breast cancer risk in relation to sex hormones.

The role of estrogen metabolites in the etiology of breast cancer has been studied, but available data are mixed and no firm conclusion can be drawn in either pre- or postmenopausal women. Developments in mass spectrometry have greatly enhanced the sensitivity and specificity in the quantification of estrogens and estrogen metabolites in both blood and urine.

Further studies with particular attention to factors such as hormone receptor subtype, controlling for hormones variations during menstrual cycle in premenopausal women, are needed to improve our understanding of the importance of estrogen to breast cancer risk. Taken together, there are not sufficient data to confirm the role of estrogen metabolites as predictors of breast cancer, but it can be concluded at this point that any intervention which leads to reduced circulating levels of primary estrogens has the potential to lower the risk of breast cancer in postmenopausal women.
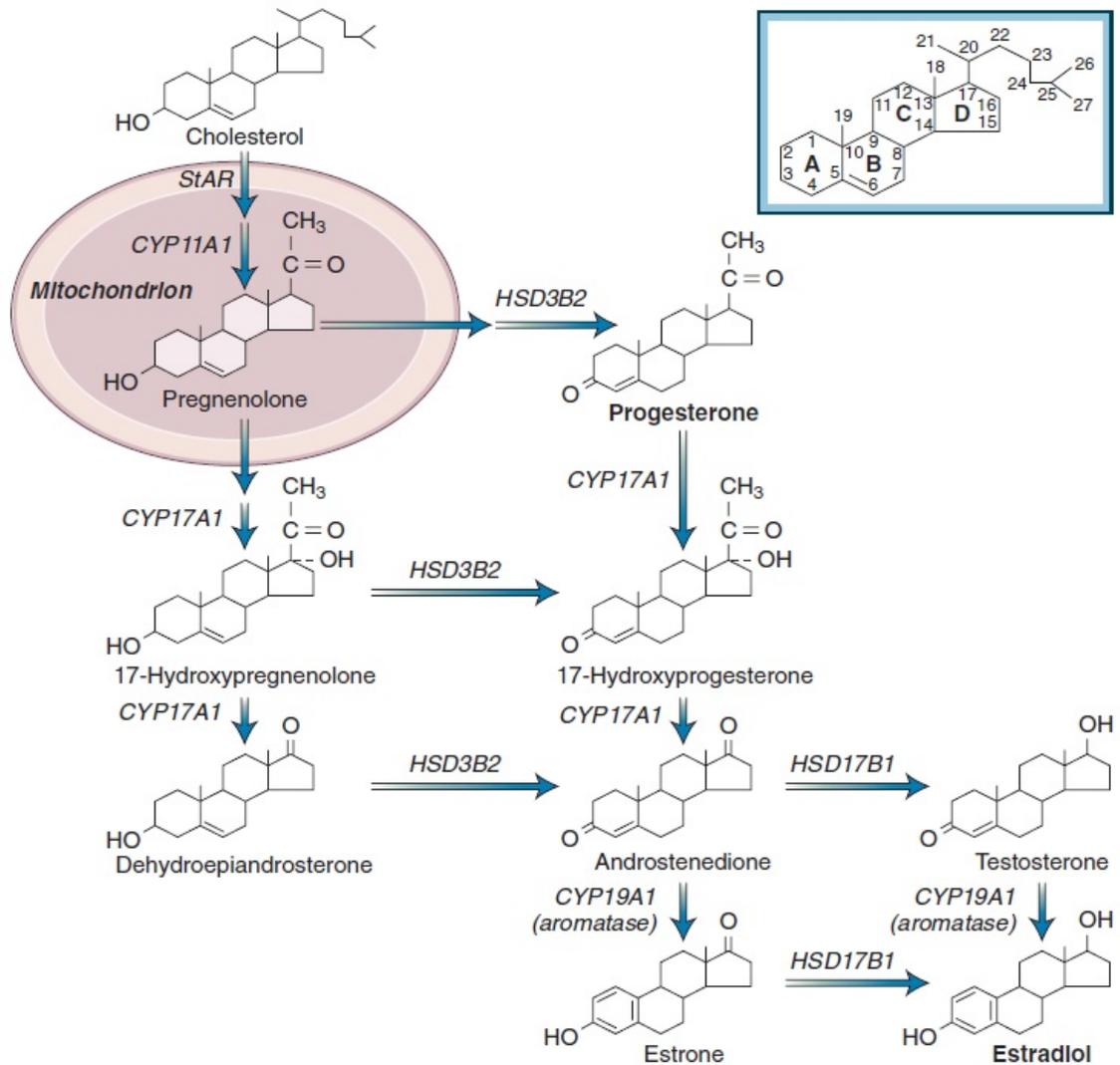
Figure 5. Pathways of steroid hormone synthesis in humans.

Abbreviations: StAR , steroidogenic acute regulatory protein; CYP11A1, side-chain cleavage of P450; CYP17A1, 17-hydroxylase/17,20- lyase; HSD3B2, 3β-hydroxysteroid dehydrogenase-$\Delta_{5,4}$ isomerase type 2; ; CYP19A1, aromatase; HSD17B1, 17β-hydroxysteroid dehydrogenase type 1 [150].
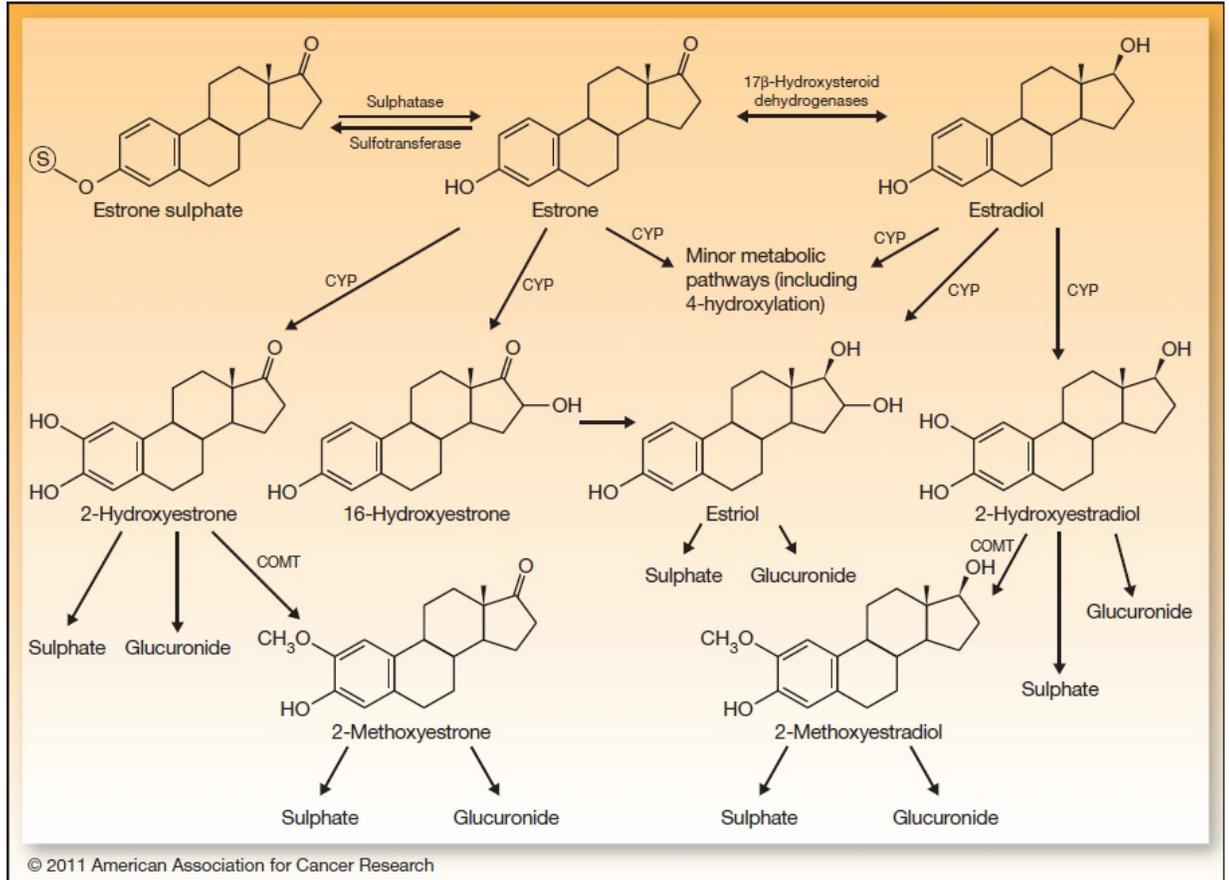
Figure 6. Endogenous estrogen metabolism in human.

The parent estrogens estrone and estradiol are reversibly inter-converted, catalyzed by the 17β-hydroxysteroid dehydrogenase enzyme. They are also converted to catechol estrogens including 2-hydroxestrogens, 4-hydroxestrogens, 16-hydroxestrone, or estriol through the action of CYP enzymes. Catechol estrogens, in turn, are metabolized to 2-methoxyestrogens and 4-methoxyestrogens. Estrone, catechol estrogens, and methoxyestrogens can be conjugated to glucuronic acid and sulfate.
Abbreviations: COMT, catechol-O-methyltransferase; CYP, cytochrome P-450 enzyme [245].
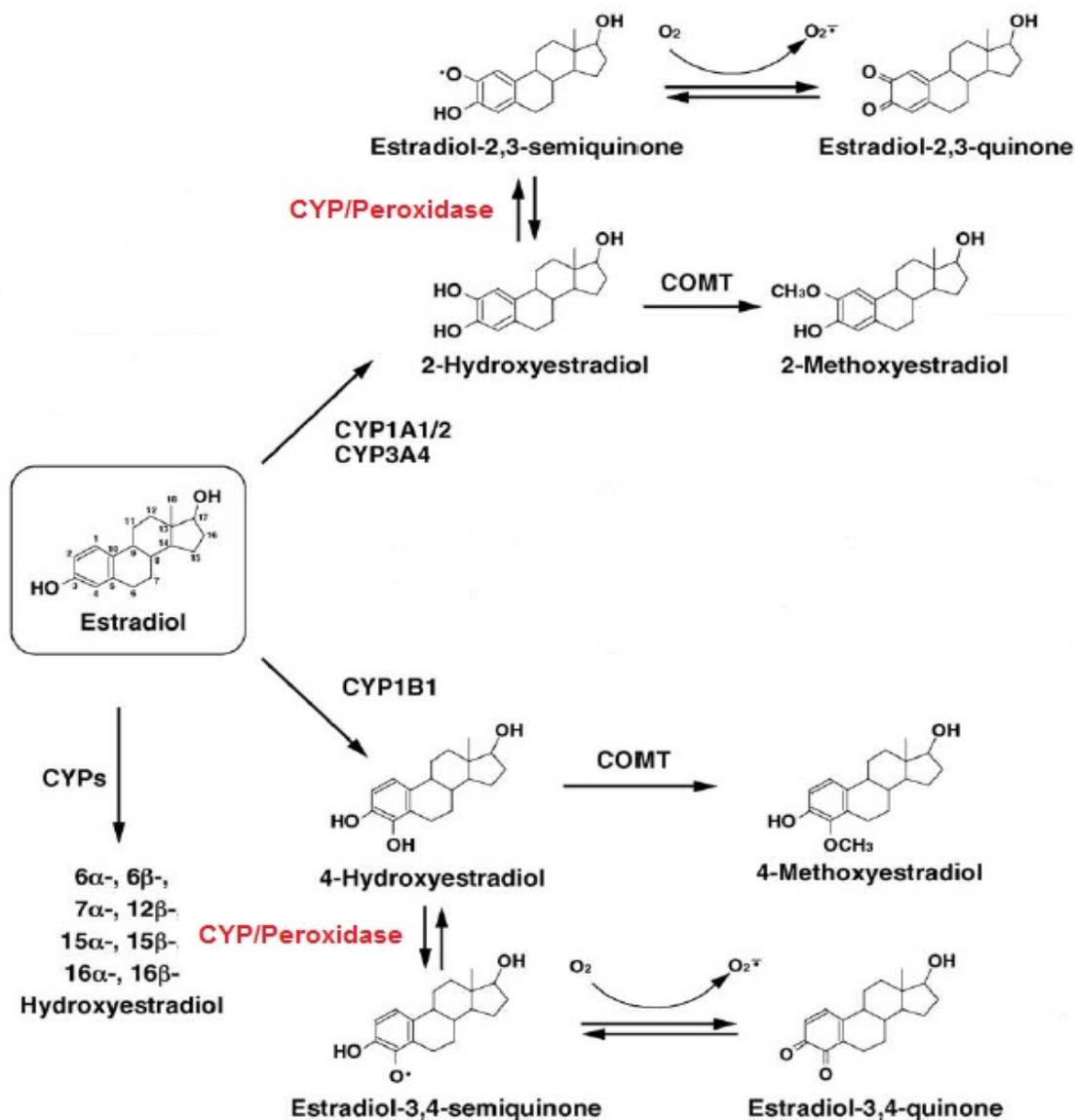
Figure 7. Estradiol metabolism and DNA adduct formation.

Estradiol catechol estrogens, including 2-hydroxyestradiol and 4-hydroxyestradiol, can go through reductive-oxidative cycling and produce mutagenic free radicals. These reactions are catalyzed by CYP and peroxidase enzymes. Estrogen semiquinones and quinones are reactive and carcinogenic intermediate metabolites of redox cycling pathways and can cause DNA damage.
Abbreviations: CYP, cytochrome P-450 enzyme; COMT, catechol-O-methyltransferase [153].

Table 1. Risk estimates for estrogens and breast cancer risk in postmenopausal women in studies of over 300 cases only.

First author, year	Hormone	Cases/controls	Design/ Biospecimen	RR (95% CI) for the top vs. bottom hormone category level	P_{trend}
Key, 2002[*][†] [221]	Total estradiol	656/1709		2·00 (1·47- 2.71)	<0.001
	Free estradiol	478/980		2·58 (1·76- 3.78)	<0.001
	Estrone	469/1188		2·19 (1·48- 3.22)	<0.001
	Estrone-sulfate	310/651		2·00 (1·26- 3.16)	<0.001
	SHBG	373/1160	Pooled analysis/blood	0.66 (0.43- 1.00)	0.041
	Androstenedione	375/1000		2·15 (1·44- 3.21)	<0.001
	DHEA	231/423		2·04 (1·21- 3.45)	0.018
	DHEAS	578/1501		1.75 (1·26- 2.43)	0.002
	Testosterone	585/1574		2.22 (1·59- 3.10)	<0.001
Missmer, 2004[††] [223]	Estradiol			2.1 (1.5- 3.2)	<0.001
	Free estradiol			1.9 (1.2- 2.9)	<0.001
	Estrone		Nested case-control study within the Nurses' Health Study/blood	1.7 (1.1- 2.6)	<0.001
	Estrone-sulfate			2.4 (1.6- 3.8)	<0.001
	SHBG	322/643		0.8 (0.6- 1.3)	0.14
	Androstenedione			1.5 (1.0- 2.3)	0.04
	DHEA			1.4 (0.9- 2.2)	0.02
	DHEAS			1.7 (1.1- 2.7)	0.003
	Testosterone			1.6 (1.0- 2.4)	<0.001

	Treatment intervention	# of BC events in treatment/placebo	Design	HR (95% CI)	P value
Manson, 2013[†††] [230]	CEE (Premarin)	168/216	RCT	0.79 (0.65- 0.97)	0.02
	CEE+MPA(Prempro)	434/323		1.28 (1.11- 1.48)	<0.001

Abbreviations: SHBG, sex hormone binding globulin; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; RR, relative risk; HR, hazard ratio; CI, confidence interval; CEE, conjugated equine estrogens; MPA, medroxyprogesterone acetate; RCT, randomized controlled trial; BC, breast cancer.

Note: Results from Manjer [222], Kaaks [224] , Eliassen [225], Baglietto [226], and Beattie [229] studies are not shown in this table. Number of cases in these studies includes 173, 285, 197, 197, and 135, respectively.

[*] From individual studies cited in references [213-220], [259], [262], and [275-277]. Reported relative risks are based on quintile comparisons.

[†] Cases and controls are matched on different factors such as age, date of blood collection, etc.

[††] Cases and controls are matched on age and date and time of blood collection. Reported relative risks are based on quartile comparisons.

[†††] With a median cumulative follow-up of 13 years. Median intervention was 7.2 year for CEE trial and 5.6 years for CEE+MPA trial.

Table 2. Risk estimates for estrogen metabolite pathways or ratios and breast cancer risk in postmenopausal women in studies of over 300 cases only.

First author, year	Estrogen metabolite pathways or ratios[e]	Cases/controls	Design/ Biospecimen	Risk estimates[f] (95% CI) for the top vs. bottom hormone category level	P_{trend}
Dallal, 2013[a] [249]	2-OHE$_1$/16α-OHE$_1$ Ratio	385/723	Combined analysis/ urine	1.02 (0.71- 1.48)	0.9
Obi, 2011[b,c] [250]	2-OHE$_1$/16α-OHE$_1$ Ratio	1189/1888	Systematic review/ urine& blood	0.75 (0.35- 1.62) to 1.31 (0.53- 3.18)	N/A
Dallal, 2014[d] [252]	2-OHE$_1$/16α-OHE$_1$ Ratio	407/496	Case-cohort study within the B~FIT/blood	0.88 (0.59- 1.32)	0.8
	2-pathway/16-pathway			0.6 (0.40- 0.90)	0.002
	2-pathway/parent estrogens			0.69 (0.46- 1.05)	0.01

Abbreviations: N/A, not available; 2-OHE$_1$, 2-hydroxyestrone; 16α-OHE$_1$, 16-alpha-hydroxyestrone; CI, confidence interval; PLCO, the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial; B~FIT, the Breast and Bone Follow-up to the Fracture Intervention Trial.

Note: All studies adjusted or matched for different covariates affecting breast cancer risk. Also, results from Kabat [247], Fuhrman [251], and Falk [253] studies are not shown in this table. Number of cases in these studies includes 42, 277, and 215, respectively.

[a] From individual studies cited in references [248], [272], and [278-280]. Reported odds ratio is based on tertile comparison.

[b] Range of odds ratios with respective CIs. Reported odds ratios are based on tertile and quintile comparisons, respectively.

[c] From individual studies cited in references [248], [272], and [279-283] .

[d] Reported hazard ratios are based on quintile comparisons.

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Table 3. Risk estimates for estrogens and their metabolites and breast cancer risk in premenopausal women: results from meta-analysis, systematic review, or individual studies of over 300 cases only.

First author, year, reference	Hormone	Cases/ controls	Design/ Biospecimen	Odds ratio (95% CI) for the top vs. bottom hormone category level	P_{trend}
Fortner, 2013[a] [267]	Estradiol				
	Follicular	462/909		1.0 (0.7 to 1.5)	0.76
	Luteal	479/959		0.9 (0.6 to 1.4)	0.65
	Free estradiol				
	Follicular	447/886	Nested case-	0.8 (0.5 to 1.2)	0.48
	Luteal	469/944	control/blood	1.0 (0.7 to 1.5)	0.99
	Estrone				
	Follicular	469/920		1.0 (0.7 to 1.4)	0.62
	Luteal	500/1005		0.9 (0.7 to 1.3)	0.89
	SHBG	624/1246		1.2 (0.8 to 1.6)	0.23
Key, 2013[b, c] [271]	Total estradiol	600/1375		1·19 (1·06- 1·35)	0·0042
	Free estradiol	587/1341		1·17 (1·03- 1·33)	0·014
	Estrone	477/933	Pooled analysis/blood	1·27 (1·05- 1·54)	0·014
	SHBG	767/1699		1·07 (0·94- 1·23)	0.29
	Androstenedione	569/1177		1·30 (1·10- 1·55)	0·0026
Walker, 2011[d] [270]	Estradiol	693/1609	Meta-analysis/blood	1.10 (0.96- 1.27)	N/A
Dallal, 2013[e] [249]	2-OHE1/16α- OHE1 Ratio	183/543	Combined analysis/ urine	0.74 (0.45- 1.23)	0.25

| Obi, 2011[f, g] [250] | 2-OHE1/16α-OHE1 Ratio | 682/1027 | Systematic review/ urine | 0.5 (0.25- 1.01) to 0.75 (0.35- 1.62) | 0.05, N/A |
| Arslan, 2009[h] [273] | 2-OHE1/16α-OHE1 Ratio | 377/377 | Nested case-control/blood | 1.13 (0.68- 1.87) | 0.51 |

Abbreviations: N/A, not available; SHBG, sex hormone binding globulin; 2-OHE1, 2-hydroxyestrone; 16α-OHE1, 16-alpha-hydroxyestrone; OR, odds ratio; CI, confidence interval.

[a] Reported odds ratio is based on quintile comparisons.

[b] ORs for a doubling in concentrations of hormones.

[c] From individual studies cited in references [259, 261], and [263-267] .

[d] From individual studies cited in references [258-263], and [265].

[e] From individual studies cited in references [248, 272], and [278]. Reported odds ratio is based on tertile comparison.

[f] Range of ORs with respective CIs. Reported odds ratios are based on tertile comparisons.

[g] From individual studies cited in references [248], [272], and [281].

[h] Reported odds ratios is based on quartile comparisons.

Note: All studies adjusted or matched for different covariates affecting breast cancer risk, but not necessarily for phase of menstrual cycle. Studies with less than 300 cases are part of meta-analysis or systematic review studies reported above.

Chapter 2: Study design and methods

Manuscript 2: The Minnesota Green Tea Trial (MGTT), a randomized controlled trial of the efficacy of green tea extract on biomarkers of breast cancer risk: Study rationale, design, methods, and participant characteristics

A shorter version of the following manuscript has been published by Cancer Causes and Control journal, 2015 Jul 24 (Epub ahead of print). This work has been used here with permission from this journal.

SUMMARY

Green tea intake has been associated with reduced risk of chronic diseases including cancer. The Minnesota Green Tea Trial (MGTT) was a randomized, placebo-controlled, double-blinded trial investigating the effect of daily green tea extract consumption for 12 months on well-established biomarkers of breast cancer risk. Participants were healthy postmenopausal women at high risk of breast cancer due to dense breast tissue with differing catechol-O-methyltransferase (*COMT*) genotypes. Other eligibility criteria were ages 50-70 years, non-smoker, body mass index between 19 and 40 kg/m^2, and not a regular green tea consumer. The intervention was a green tea catechin extract containing 843.0 ± 44.0 mg/day epigallocatechin gallate or placebo capsules for one year. Annual digital screening mammograms were obtained for each participant at baseline and month 12, and fasting blood and 24-hour urine samples were provided by all women at baseline, months 6 and 12 months. Primary endpoints included change from baseline in percent mammographic density, circulating endogenous sex hormones and insulin-like growth factor axis proteins; secondary endpoints were change from baseline in urinary estrogens and estrogen metabolites and circulating F2-isoprostanes, a biomarker of oxidative stress. The MGTT screened more than 100,000 mammograms and ultimately randomized 1075 participants based on treatment (green tea

extract vs. placebo), and stratified by *COMT* genotype activity (high *COMT* vs. low/intermediate *COMT* genotype activity). Among enrolled participants, 937 women successfully completed the study and 138 dropped out from the study (overall dropout rate= 12.8%). In this paper we report the rationale, design, recruitment, participant characteristics, and methods for biomarker and statistical analyses.

INTRODUCTION

Breast cancer is the most commonly diagnosed malignancy and the second leading cause of cancer-related death among women in the United States [138]. In the U.S., it is expected that one out of eight women will develop breast cancer throughout her lifetime [284]. Diet is one of the modifiable factors considered to play an important role in the prevention of several types of cancer, including breast cancer [143, 285]. Among dietary factors suggested to affect breast cancer risk, green tea has been the subject of a great deal of research within the last two decades.

Tea is a commonly consumed beverage worldwide – second only to water - and is made from leaves of the *Camellia sinensis* plant. Differentiated by the processing method and fermentation level, black, green, and oolong tea are the three main varieties. Green tea is a non-fermented tea most popular in Eastern Asia, and accounts for nearly 20% of total tea consumption [286]. There is convincing evidence from in vitro and animal studies that green tea has preventive effects against cancer, although epidemiological studies are inconsistent [12, 59, 287, 288]. The chemoprotective effects of green tea are primarily attributed to bioactive polyphenolic compounds known as catechins, wherein (-)-epigallocatechin-3-gallate (EGCG) is the most abundant and active [286]. Some of the purported mechanisms by which green tea intake is believed to influence breast cancer risk include changes in well-recognized breast cancer biomarkers such as mammographic density [85], circulating sex hormone or urinary estrogen metabolite levels [80, 100], as well as the insulin-like growth factor (IGF) system [132]. Tea catechins also possess potent antioxidant activities [289] and the role of oxidative stress in carcinogenesis has

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been established [290]; however, current human clinical trial findings are mixed and further research is needed to clarify this discrepancy [291, 292].

The catechol-O-methyltransferase (COMT) enzyme is involved in both estrogen and tea catechin metabolism. The *COMT* gene is polymorphic; a G to A polymorphism at codon 108/158 of *COMT* (SNP rs4680) causes a valine to methionine substitution in the cytosolic or membrane-bound form of this enzyme, which results in a 3- to 4-fold decrease in enzymatic activity in individuals possessing homozygous variant alleles A/A relative to wild-type homozygous wild-type alleles G/G, and intermediate levels of COMT activity in individuals with heterozygous variant alleles A/G [20, 21]. It has been demonstrated that individual genetic variability in the COMT enzyme influences the excretion rate of tea polyphenol metabolites from the body and conversion of catechol estrogens to methoxyestrogens [23, 27]. In addition, Wu, *et al* [22] found that the inverse association between green tea intake and breast cancer risk is significantly more prominent in women with the *COMT* homozygous mutant variant or low-activity alleles (A/A) than those with the homozygous wild-type or high-activity alleles (G/G). These findings suggest that those with the low-activity COMT enzyme may metabolize tea catechins more slowly and retain these bioactive components *in vivo* longer than those with the high-activity enzyme, which consequently may contribute to a greater health benefit of green tea intake. This hypothesis has been further investigated in a few studies reporting that individuals with low-activity *COMT* genotype have lower urinary levels of tea polyphenols compared to those with high-activity *COMT* genotype [23, 293] although this evidence is not completely consistent [294].

The MGTT was a randomized, double blind, controlled trial designed to determine the effects of 12-month daily green tea catechin supplementation on recognized biomarkers of breast cancer risk in 937 high-risk postmenopausal women. Breast cancer biomarkers to be evaluated in this trial include mammographic density, endogenous sex hormones and their metabolites, IGF axis proteins, and F2-isoprostanes, recognized biomarkers of oxidative stress. This paper describes key aspects of the trial

including its rationale, design, methods, response rate and the demographic characteristics of the participants. Subsequent publications will address specific research questions based on the collected data.

METHODS

Objectives

The primary objectives of this trial were to investigate the effects of consumption of green tea extract (GTE) containing 800 mg EGCG daily for one year on (i) mammographic density (ii) circulating levels of reproductive hormones including estrone, estradiol, testosterone, androstenedione, and sex hormone binding globulin (SHBG) (iii) blood concentrations of insulin-like growth factor (IGF) axis proteins including IGF-1 and IGF binding protein 3 (IGFBP-3) among healthy postmenopausal women at high risk of breast cancer due to dense breast tissue. We hypothesized that consumption of GTE would reduce mammographic density and circulating concentrations of IGF-1, estrone, estradiol, testosterone, and androstenedione, and increase blood levels of IGFBP-3 and SHBG, in the direction associated with breast cancer prevention.

Secondary endpoints included assessment of urinary concentrations of estrogen metabolites (estrone, estradiol, and their 2-hydroxy, 4-hydroxy, 2-methoxy, and 4-methoxy metabolites, estriol, and 16-αhydroxyestrone) and circulating levels of F2-isoprostanes. The MGTT also aimed to (i) determine whether the effect of GTE supplementation on the primary outcomes described above differs by *COMT* genotype and (ii) evaluate whether *COMT* genotypes alter tea catechin metabolism and urinary excretion. We hypothesized that the low-activity *COMT* genotype would show the greatest response to catechin consumption and would have lower concentrations of urinary methylated catechins and methoxyestrogens, and higher circulating levels of unmethylated catechins.

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This study was approved by the Institutional Review Boards (IRB) of the University of Minnesota, Park Nicollet Institute, the University of Southern California, and the University of Pittsburgh.

Study participants

The eligibility and exclusion criteria of the MGTT are listed in **Table 4**. The eligibility criteria were based on scientific and ethical considerations. Eligible participants were healthy, non-smoking postmenopausal women at high risk of breast cancer due to high mammographic density (defined as heterogeneously or extremely dense breasts, and assessed by radiologists on routine mammograms), aged 50-70 years, non-regular green tea drinkers (less than one cup per week) and noncurrent (i.e., within 6 months of enrollment) use of any hormone therapy including aromatase inhibitors, selective estrogen receptor modulators (SERMS), systemic hormone replacement therapy (HRT), or chemopreventive agents. Additional eligibility criteria included alcohol consumption equal to or less than 7 servings/week, body mass index (BMI) 18.5-40 kg/m^2, stable weight for the past year (less than 10 pounds change), and normal liver alanine aminotransferase (ALT) levels (below 1.5 times of the upper limit of normal, defined as 60 U/L). We further excluded women with a history of breast cancer, breast proliferative disease or ovarian cancer over their lifetime or any other cancer in the past 5 years except for non-melanoma skin cancer; those who tested seropositive for hepatitis B surface antigen (HBsAg) or who had antibodies to hepatitis C virus (anti-HCV) (see details in **Table 4**).

Recruitment

Recruitment for the MGTT took place in the Minneapolis-St. Paul metropolitan area and began in August 2009 at the University of Minnesota Medical Center (UMMC), Fairview Southdale, and Fairview Maple Grove Breast Clinics. Recruitment from Park Nicollet Institute Breast Clinics, (St. Louis Park*, Burnsville*, Maple Grove*, Wayzata*, and Shorewood) began* in November 2011. Recruitment concluded in April 2013. Study staff

identified potential participants by reviewing routine mammogram reports for breast density and age criteria.

Study staff identified potential participants by reviewing routine screening mammogram reports. Women aged 50 - 70 with breast density assessments on screening mammography radiology reports as either "heterogeneously dense" or extremely dense", i.e., the breast tissue estimated as 51-75% glandular or more than 75% glandular, respectively, as specified by Breast Imaging and Reporting Data System (BI-RADS) criteria [295], were eligible for further study screening. Potential participants received a recruitment letter describing the intention and basic requirements of the study. If interested, prospective participants either called the study screening hotline or visited the study website to complete a brief screening questionnaire for further assessment of their eligibility. If they qualified for the study, research staff scheduled an in-person one-hour orientation session in which the study was explained in detail. At the end of the orientation, women signed and provided written informed consent. A screening clinic visit was subsequently scheduled to obtain anthropometric measurements, blood pressure, and a blood draw to assess *COMT* genotype and hepatic function as well as serological markers of hepatitis B and C virus infection to avoid potential hepatotoxicity risk in women with compromised liver function. Participants were notified of their eligibility status within 3-4 weeks after their initial screening clinic visit. If eligible, participants were randomized into the study and a baseline clinic visit was scheduled. All randomized participants completed the baseline clinic visit within 3.5 months from the date of their baseline mammogram (see Figure 8 for details of recruitment).

Randomization

Figure 8 depicts the randomization scheme. Randomization was performed by the Investigational Drug Services (IDS) pharmacy at University of Minnesota Medical Center -Fairview, which was not involved in either subject recruitment or assessment of study endpoints. The IDS pharmacy utilized a computer generated randomization scheme

using the permuted block method and randomized participants to GTE or placebo in blocks of 8 stratified by *COMT* genotype (high activity = G/G or low/intermediate activity = A/A + A/G). A/A and A/G were combined in the low activity group based on previous studies [22]. Accordingly, participants were randomized and stratified into one of four groups: GTE/low activity *COMT* (*n*= 394); GTE/high activity *COMT* (*n*= 144); placebo/low activity *COMT* (*n*= 396); and placebo/high activity *COMT* (*n*= 141). Participants in the low activity *COMT* genotype arm were further divided, but not randomized, into low and intermediate *COMT* genotype activity following recent studies suggesting that the heterozygous *COMT* genotype may show intermediate levels of enzymatic activity [23, 294].

Blinding

In this double blinded study, study staff, participants, laboratory personnel, and all parties involved with assessment of the study endpoints were blinded to treatment assignment. The treatment codes were only available to the IDS pharmacy staff in charge of randomization and a study biostatistician. In addition, the assignment code was only broken for the members of the Data and Safety Monitoring Board (DSMB) at annual meetings. We also unblinded the assignment code of one participant who had a serious adverse event and subsequently terminated the study intervention for this participant.

Study design, data collection and processing

Table 5 demonstrates the data collection schedule of the trial. The MGTT was a single-center, phase II, randomized, placebo-controlled, double-blind, parallel-arm trial. Participants consumed two GTE capsules (containing an average of 330 ± 29.0 mg total catechins and 211 ± 11.0 mg EGCG per capsule) or two placebo capsules twice daily for 12 months, for a total of 1315 ± 115.0 mg/day total catechins containing 843.0 ± 44.0 mg/day EGCG.

Blood draws were divided into fasting and non-fasting visits. At months 0 (i.e., baseline), 6 and 12, fasting blood draws were scheduled for measurement of trial endpoints. The remaining blood draws were non-fasting and exclusively planned for the purpose of ALT evaluation. During the first 2 years of the study, participants came to the clinic monthly for monitoring of liver function. Because very few women developed elevated liver ALT, especially after month 6 of the study, clinic visits at months 7, 8, 10, and 11 were omitted for the rest of the study upon receiving FDA and IRB approval in September 2011. As a result, clinic visits at those four time points only took place for 24% of the participants who completed the study.

In order to retain the participants in the study and keep the dropout rate low, study staff helped participants to schedule their next clinic visit when they were coming for each visit. In addition, participants were contacted via email or phone call one week in advance to remind them of their upcoming clinic visit time.

Biospecimens.

Blood. Whole blood was collected by a trained nurse or phlebotomist via venipuncture. For fasting blood collections, participants were instructed to refrain from eating and drinking anything other than water for 10 hours prior to blood draw, which occurred between 06:30am and 12:00pm. Whole blood (65 mL) was drawn into a total of 10 tubes containing sodium heparin (BD, Franklin Lakes, New Jersey) for plasma and serum separator tubes (BD, Franklin Lakes, New Jersey) with clot activator and gel for serum. Samples were delivered to the lab within 15 minutes and were centrifuged for 10 minutes at 4°C and 3000 rpm. Serum and plasma were separated, aliquoted in 1.5 mL volumes tubes (Sarstedt Inc., Newton, NC), and stored at -70°C. For blood samples designated for catechin measurement, 100 μl of ascorbic acid-ethylenediaminetetraacetic acid (EDTA) solution (20% ascorbic acid, 0.1% EDTA, 5.52% sodium phosphate monobasic monohydrate) was added to 1.0 mL plasma aliquots. In addition, fasting blood

samples were collected in Cell Preparation tubes (CPT) and PAX gene Blood RNA tubes (BD, Franklin Lakes, New Jersey) from nearly 850 participants for future analyses.

For the non-fasting blood collections performed at the screening visit, tubes containing EDTA (Becton, Dickinson and Company (BD), Franklin Lakes, New Jersey) were extracted for buffy coat, and serum separator tubes (BD, Franklin Lakes, New Jersey) were used for hepatic panel evaluation and screening for chronic infection with hepatitis B and C virus (Quest Diagnostics, Wood Dale, IL). Samples were transported and centrifuged as described above. Buffy coat was collected by removing plasma from whole blood and adding 0.5 mL 0.9% sodium chloride to each 0.5 mL aliquot, then stored at -70°C until genotyping. Samples collected for ALT assessment were centrifuged and stored at refrigerator temperature within 72 hours for pick up by the external laboratory conducting the hepatic function panel and serological status of HBsAg and anti-HCV (Quest Diagnostics, Wood Dale, IL).

Urine. Participants collected 24-hour urine specimens at months 0, 6, and 12. All women were instructed to avoid drinking alcohol one day prior to the start of collection, and collected all urine for 24 hours in a 3-liter plastic container containing 3 g of ascorbic acid as preservative. For those participants collecting urine away from home, a 1-liter plastic bottle, an insulated bag and two ice packs were also provided and they were asked to transfer collected urine to the 3-liter bottle as soon as they returned home. Urine was kept refrigerated until it was brought to the clinic on the following day. Urine volume was recorded using a plastic graduated cylinder and aliquots of 1.0 mL and 10.0 mL were saved. For catechin measurement, 10 μl of ascorbic acid-EDTA solution was added to 1.0 mL aliquots before storage at -70°C, while 10 mL aliquots without this additive were stored at -20°C.

Spot urine samples were also collected at the clinic at months 3 and 9 for evaluation of compliance by catechin analysis. Participants were instructed to collect 25 mL midstream urine in a small plastic container with prior notice. Samples were

separated into 1.0 mL aliquots, and 10 μl of ascorbic acid-EDTA solution was added before storage at -70°C.

Health History Questionnaire (HHQ). At the baseline visit, each participant completed an in-depth health survey including information about demographics, lifestyle factors (physical activity, smoking history, and alcohol intake), medical history, medication use (current and former), and full reproductive history.

Diet History Questionnaire I (DHQI). This validated food frequency questionnaire was developed by the National Cancer Institute (NCI). The DHQI includes 124 food items and inquires about the past 12 months of food intake with details regarding portion size and dietary supplement use. Average daily food and nutrient intake were estimated using NCI DietCalc software.

Menopause-Specific Quality of Life Questionnaire (MENQOL). Quality of life with particular emphasis on menopausal symptoms was assessed using the validated MENQOL at baseline, months 6 and 12. The MENQOL questionnaire is a self-administered questionnaire developed by Hilditch *et al* [296, 297], that consists of 32 questions separated into four domains (vasomotor, psychosocial, physical, and sexual). Participants were asked about symptoms occurring during the past week.

Anthropometric measurements.

Anthropometric measurements including weight, height, and waist and hip circumferences were taken for all participants by trained clinic staff. Participants were asked to wear minimal clothing and no shoes. Weight was measured to the nearest 0.1 kg using a digital stand-on scale (Scale-Tronix Inc., White Plains, NY) at screening, baseline and at 3-month intervals. Standing height was determined to the nearest 0.1 cm at baseline and month 12 with a wall-mounted stadiometer (Seca, Hanover, MD). BMI was

calculated as weight in kilograms divided by height in meters squared (kg/m^2). The scale and stadiometer were calibrated annually by an external local biomedical services company. In order to assess body fat distribution, waist and hip circumferences were measured using a flexible body tape at baseline and at the end of study. Waist circumference was measured at the uppermost lateral border of the iliac crest at the narrowest point of torso, and hip circumference was measured at the widest part of the buttocks. Both measurements were repeated twice and the average measure was recorded to the nearest 0.1 cm. Waist-to-hip ratio (WHR) was calculated by dividing the waist circumference (in cm) by the hip circumference (in cm).

Vital signs.

Vital signs including heart rate, blood pressure, respiration rate, and body temperature were measured and monitored at each clinic visit. Blood pressure and heart rate were measured using an automated digital vital signs monitor (Philips Healthcare, Eindhoven, The Netherlands). Participants were asked to sit with legs and ankles uncrossed following resting and relaxing for 5 minutes. If the blood pressure value was above 120 mm Hg (systolic) or 80 mm Hg (diastolic), it was measured again to confirm the accuracy. Body temperature was taken orally by a digital thermometer after placing a disposable probe under the tongue (Welch Allyn, Inc. Skaneateles Falls, NY). Respiration rate was estimated at rest by counting the number of breaths for 20 seconds and multiplying it by three to obtain the rate per minute.

Intervention

The full composition of the study great tea extract supplement is shown in **Table 6**. Green Tea Extract Catechin Complex (Corban complex GTB; referred to as green tea extract or GTE in this paper; Investigational New Drug #103,431) is a decaffeinated green tea extract with roughly 330 ± 29.0 mg catechins per capsule, including 211 ± 11.0 mg epigallocatechin gallate (EGCG), 27 ± 30.0 mg epigallocatechin (EGC), 51 ± 19.0 mg

epicatechin gallate (ECG), 27 ± 6.0 mg epicatechin (EC), and other catechins. Participants were instructed to take two capsules, twice daily with breakfast and dinner, for a total of 1315 ± 115.0 mg catechins containing 843.0 ± 44.0 mg EGCG. According to the USDA Database for the Flavonoid Content of Selected Foods [298], there is 70.2 mg of EGCG in 100 mL of brewed green tea. The dose of 843 mg EGCG used in this study thus was equivalent to approximately 10 grams of dry tea leaves or five 8-ounce cups of brewed green tea ((843 mg/70.2 mg *100mL/237 mL).

The study supplement was administered in opaque, size 0 hard gelatin capsules that were light yellow to brown in color. Placebo capsules were identical in appearance to the study supplement and consisted of maltodextrin (50%), cellulose (49.5%), and magnesium stearate (0.5%). Both study supplements and placebo were supplied by Corban Laboratories /Eniva Nutraceutics (Plymouth, MN), and stored at ambient temperature and moisture conditions at the University of Minnesota IDS pharmacy.

Eight batches of GTE and placebo were used in this study. GTE catechins in each batch were analyzed by high-performance liquid chromatography (HPLC) in the laboratory of C.S. Yang (Rutgers University, Piscataway, NJ). Catechins were stable for at least one year after production. Although the original intent of dose of this trial for EGCG level was 800 mg, the average EGCG content in each capsule from eight batches was 211 mg equivalent to a daily dose of 843 mg EGCG.

The IDS pharmacy dispensed study product for each participant every three months. Clinic staff distributed capsules to participants at baseline, month 3, month 6 and month 9 of the study. Participants were asked to store pills in a cool and dry place and to return all unused capsules in the original bottles to the study staff for disposal at their clinical visits.

Compliance assessment

Compliance with treatment was assessed using three different approaches. First, participants were asked to return all unused capsules in the original bottles every 3

months. Clinic staff counted the number of returned capsules, calculated the number of unreturned capsules from the number originally dispensed, and calculated compliance as the number of capsules actually consumed divided by the number of capsules the participant should have consumed. Each participant also completed a study log indicating the time and date of capsule consumption, and returned the study log to clinic staff at each visit. As the final compliance check, urinary levels of EGC and EC, expressed in mg creatinine to control for urine volume, were measured at baseline, months 3, 6, 9 and 12 in a randomly selected 10% of participants ($n = 90$; 43 participants in the GTE group and 47 participants in the placebo group).

Participant compensation

All study participants received modest financial compensation for their study participation. Reimbursement amount was paid based on completed clinic visits during the course of the study. Participants who were found ineligible during the study received pro-rated compensation. Following all the study grant endpoint measurements were completed, the study staff were unblinded to the participants supplement codes. All participants will receive soon reports containing information on their treatment group, body weight, body fat distribution and blood pressure, and changes in these measurements over their 1 year participations, as well as analysis of dietary practices.

Endpoint measurement methods

Mammographic density.

Digital mammograms at baseline and month 12 were assessed for % mammographic density (%MD) by an experienced researcher on scanned images using a validated, highly reproducible computer-assisted, quantitative method, Madena, developed at the University of Southern California [299, 300]. The density readings were done as follows: The reader drew a region of interest that excluded the pectoralis muscle, major veins, and other artifacts and then used a tinting tool to color the areas within the

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region of interest considered to contain mammographic densities. The software counted the number of tinted pixels within the region of interest. Absolute density represents the count of tinted pixels with the region of interest. The total area of the breast was assessed using the same program, but by another researcher trained by GU. This was done by the reader outlining the entire breast using an outlining tool on the computer screen, and the computer counted the total number of pixels in the breast. Percent MD or the fraction of the breast with densities is the ratio of absolute density to the total breast area multiplied by 100. Baseline and month 12 mammograms from a given study participant were read in the same batch as a set but the order of the mammogram and the participant's assignment to GTE or placebo group were blinded to the reader.

Circulating sex steroids and SHBG.

Estradiol, estrone, androstenedione, and testosterone were analyzed in fasting serum at the Quest Diagnostics Nichols Institute (San Juan Capistrano, CA). All samples for a given participant with an equal number of participants in the two treatment groups were analyzed in the same batch. These assays were done on serum samples collected at baseline and month 12 on all participants. Samples at month 6 were also analyzed on a subset of 371 participants (184 in GTE and 187 in placebo group). Since there were no differences between the months 6 and 12 hormone levels, for the remainder of the participants, analyses were done for 0 and 12 months only. All hormone levels were measured by liquid chromatography/tandem mass spectrometry (LC/MS/MS) on a Finnigan TSQ Quantum Ultra LC/MS/MS instrument (ThermoFisher; San Jose, CA) following extraction and separation procedures [301]. Free and bioavailable concentrations of estradiol and testosterone were calculated from total estradiol and testosterone, and SHBG using a validated algorithm and absolute individual serum albumin levels [302, 303]. All laboratory staff were blinded to the treatment code status, and all biospecimen were quantified in 2014. Assay sensitivities were 2 pg/mL for estradiol, 10 pg/mL for estrone, 5 ng/dl for androstenedione, and 1 ng/dl for testosterone. In each batch, 3-4 blinded quality control serum samples were inserted to evaluate

laboratory assay variability. The average intraassay coefficient of variation (CV) across all batches were 11.0 % for estrone; 20.5 % for estradiol; 11.6 % for androstenedione; and 13.1 % for testosterone. Interassay variabilities were 14.4 %, 26.1 %, 13.7 %, and 15.9 % for estrone, estradiol, androstenedione, and testosterone, respectively.

Concentration of SHBG was quantified by Human SHBG Quantikine ELISA Kit DSHBG0 (R&D Systems, Inc., Minneapolis, MN) in the laboratory of Dr. Mindy Kurzer on fasting serum samples collected a baseline and month 12 on all participants and a subset samples at month 6 (187 in GTE and 187 in placebo group). The final colorimetric analysis was done on a Synergy H1 Plate Reader (Biotek, Winooski, VT). Plates were read at 450nm with a background wavelength of 570 nm and results were calculated using linear regression analysis following log/log transformation. Each sample was analyzed in duplicate, and their average value was used in the final statistical analysis. In addition, a quality control sample was analyzed with each batch to determine reproducibility of the method. Intra- and interassay CVs were 10.8 % and 6.9 %, respectively. All samples from a given participant were run in the same analysis batch with laboratory technicians blinded to the study treatment status.

Urinary estrogen metabolites.

Urinary estrone (E_1), estradiol (E_2), and their metabolites were quantified in 24-hour urine samples collected at baseline and month 12 from all participants, in the laboratory of Dr Mindy Kurzer. The metabolites measured were 2-hydroxy E_1 (2-OHE$_1$), 2-hydroxy E_2 (2-OHE$_2$), 4-hydroxy E_1 (4-OHE$_1$), 4-hydroxy E_2 (4-OHE$_2$), 2-methoxy E_1 (2-MeOE$_1$), 2-methoxy E_2 (2-MeOE$_2$), 4-methoxy E_1 (4-MeOE$_1$), 4-methoxy E_2 (4-MeOE$_2$), estriol, and 16α-hydroxy E_1 (16α-OHE$_1$) using a modification of the method developed by Xu *et al* [304].

Urine was collected in the presence of 0.1% ascorbic acid, divided into aliquots, and frozen at -20 ℃. An aliquot of the urine was then thawed at room temperature, and centrifuged 5 minutes at 1500 x g in order to remove any precipitate. One ml of urine was

pipetted into each of 2 silanized glass 16x125 mm screw cap test tubes, followed by 10 ng each of the internal standard mixture in 10 ul methanol. One ml of digestion mixture was then added to each tube, mixed briefly, capped, and then incubated overnight at 37 ºC. The digestion mixture contained 4 mg/ml ascorbic acid and 10 u/ml of glucuronidase (Sigma G7770) in 0.15 M sodium acetate buffer, pH = 4.1.

After hydrolysis, the sample was purified by solid phase extraction with Strata X columns (8B-S100-TBJ, phenomenex corp. Torrence California, USA). The column was washed with 1.5 ml of methanol, followed by 1.5 ml of HPLC water. The sample was then applied and allowed to pass through the column. The column was washed with 1.5 ml HPLC water and then 1.5 ml of 65% methanol in water. A variety of concentrations of methanol were tested for washing, and 65% was chosen to give an optimal balance between recovery and removal of contaminants. After the 65% methanol wash, the column was dried 1 minute under full vacuum. The metabolites were then eluted with 1.5 ml of methanol. The methanol eluate was evaporated to dryness in an n-Evap, redissolved in 0.15 ml methanol, and transferred to a conical 0.3 ml silanized autosampler vial and stored at -20 ºC until the day of analysis. Standards were diluted in methanol, evaporated and reconstituted into autosampler vials as for the samples.

On the day of analysis, the sample was evaporated to dryness in a rotary evaporator, and 25 ul of derivatization buffer (0.1% Ascorbic acid in 0.1M sodium bicarbonate) and 25 ul dansyl chloride (3.0 mg/ml in acetone) were added, mixed, and incubated 6 minutes at 65 ºC. The vials were then cooled to 4 ºC, and placed in a cooled autosampler for injection. The sample was next analyzed by LC/MS/MS on a Thermo Electron Quantum Discovery Max triple quadrupole instrument, using electrospray ionization in the positive mode. Separation was performed using a gradient elution of water/acetonitrile on a SB-C18 column, 0.5mm x 100mm, 1.8 micron particle size (Agilent Technologies, Wilmington, DE.) Quantitation was performed with Xcalibur software.

Baseline and month 12 samples from each participant were analyzed in duplicate in the same batch. Quality control was monitored by analysis of a spiked charcoal stripped urine sample analyzed in duplicate for each run. Intraassay CV was less than 6.5% for all analytes. Interassay CV ranged from 5.3% to 13.3%, with an average for all analytes of 8.45%. Assay sensitivity varied from 5 to 10 pg/ml for all 12 urinary analytes.

Insulin-like growth factor axis proteins.

Concentrations of IGF-1 and IGFBP-3 were quantified using commercially available enzyme-linked immunosorbent assay kits (R&D Systems, Inc., Minneapolis, MN) on fasting heparinized plasma samples collected at baseline, month 6 and month 12 on all participants. IGF-1 was analyzed using the Human IGF-1 Quantikine ELISA Kit #DG100. IGFBP-3 was analyzed using the Human IGFBP-3 Quantikine ELISA Kit # DGB300. IGF-1 result was calculated using linear regression analysis following log/log transformation, while IGFBP-3 was quantified by a 4 parameter curve fit. IGF1: IGFBP-3 ratio was subsequently calculated from the concentrations of IGF-1 and IGFBP-3.

All samples from a given participant were measured in the same batch in duplicate with a quality control sample in each kit. Each kit included an equal number of samples from active treatment and placebo participants. Duplicate samples with mean values varying more than 15% were repeated. Measurements were conducted at the laboratory of Dr. Mindy Kurzer at the University of Minnesota with laboratory personnel blinded to the participant treatment assignment. The intra- and interassay CVs were as follows: 6.5% and 2.0% for IGF-1, and 4.4% and 2.0% for IGFBP-3. The assay minimum detectable dose for IGF-1 was 0.026 ng/ml and for IGFBP-3 was 0.05 ng/ml.

F2-isoprostanes.

Free F2-isoprostanes (a collection of isomers) were measured in EDTA plasma in the University of Minnesota Molecular Epidemiology and Biomarkers Research Laboratory by a gas chromatography-mass spectrometry (GC-MS)-based method as

described by Morrow [305-307] and Gross [308]. Isoprostanes were extracted with 4 deuterium-labeled 8-iso-prostaglandin F2 alpha used as an internal standard. Unlabeled purified F2-isoprostane was used as a standard for the F2-isoprostanes measured in the samples. This F2-isoprostane standard co-migrates and, as expected, has the same molecular weight as the F2-isoprostanes measured in the samples. Thus, the F2-isoprostane standard is a good marker for the assay of F2-isoprostanes by GC-MS that measures a well-defined set of F2-isoprostane isomers. This laboratory has measured over 10,000 samples and this method has an analytical variation of 10% in control pools at 3 concentrations. All samples were analyzed within one year of the time that the last participant completed the trial. Long-term studies and previous results have shown that the levels of plasma F2-isoprostanes are stable for >60 months when stored at -70°C.

Catechins.

Tea catechins and their metabolites were quantified in both plasma and urine samples collected from participants in the GTE group at month 12. Urinary excretion of EGC, methylated-EGC (4′-MeEGC), EC, and their corresponding ring-fission microbial metabolites 5-(3′, 4′, 5′-trihydroxyphenyl)-γ-valerolactone (M4) and 5-(3′, 4′-dihydroxyphenyl)-γ-valerolactone (M6), and plasma concentrations of EGCG, EGC, ECG, and EC were measured using validated methods [31, 309]. Urinary creatinine was analyzed in these samples via a modified method as described previously [310].

To measure compliance, urinary levels of EGC and EC were measured at baseline, months 3, 6, 9 and 12 months in a randomly selected 10% of participants. In addition, for a subsample of 180 participants (90 GTE and 90 placebo) urinary and plasma catechins were quantified from samples collected at baseline, months 6 and 12. All catechin analyses were completed in the laboratory of Dr. C.S. Yang (Rutgers University, Piscataway, NJ).

COMT, SULT, and UGT genotyping.

DNA was extracted from buffy coat samples using the Qiagen DNAeasy Blood and Tissue Kit method (Qiagen Inc., Gaithersburg, MD, USA) according to the manufacturer's protocol. A TaqMan assay was developed for determining the *COMT* A/G polymorphism using a TaqMan PCR Core Reagent kit (Applied Biosystems, Foster City, CA). Controls from Corriell cell lines with known *COMT* genotype were used as quality control with each PCR run. Forty-six known tag SNPs involved in glucuronidation and sulfation pathways were chosen for exploratory analyses of *SULT1A1*, *SULT1E1*, *UGT1A1*, *UGT1A4*, *UGT1A6*, *UGT1A8*, and *UGT2B7* genes. SNP analysis was performed on Sequenom iPlex platform (Sequenom, San Diego, CA) by the University of Minnesota Genomics Center.

Data and safety monitoring

An independent external data and safety monitoring board (DSMB) monitored and evaluated the integrity of the trial, data collection and study progress, and adverse events during the study. The DSMB consisted of a medical oncologist, a biostatistician, and a community representative, who met annually through conference calls with the study investigators and staff. Additional telephone conferences were held with study investigators as needed. A biostatistician performed all requested analyses for the DSMB and provided them with the randomization code. Aside from the DSMB, all serious adverse events were reported to the NIH, FDA, the supplement manufacturer, and the IRB of the University of Minnesota within the required time frame specified by each organization. The DSMB chair also prepared an annual report evaluating the progress of the study and making recommendation for improvement. Additionally, a trained clinical monitor from the University of Minnesota periodically audited trial compliance with the protocol approved by the IRB, and ensured that the study was conducted and reported in accordance with FDA Good Clinical Practice.

Sample size estimate and statistical analysis

Sample size was estimated based on the study primary endpoint, change in %MD. With the originally planned sample size of 800 (400 in GTE and 400 in placebo), the MGTT had 81% statistical power to detect 3.4% reduction in the %MD between the GTE vs. placebo groups. Additionally, we had 87% and 100% power to detect 13% change each in circulating estrone and IGF-1 levels, respectively. The calculations assumed a two-sided significance level of 5% ($\alpha =0.05$). Since the MGTT over recruited and completed the study for 937 rather than 800 participants, this enhanced our study power to 86% and 91% to detect desired changes specified above in the %MD and circulating estrone level, respectively, in the GTE vs. placebo study participants.

The intention-to-treat (ITT) principle was used for the analysis of the study results. Participants who withdrew or were suspended from the study due to experiencing ALT elevation were invited to remain in the study and follow all study guidelines except taking the study supplements.

Data analysis started with inspecting the distribution of all variables by using descriptive statistics and histograms. Comparisons shown in this paper include all participants. All comparisons between GTE and placebo participants for baseline demographic, dietary intake, or withdrawal reasons were based on Student t-tests for continuous variables and chi-square or Fisher's exact tests for categorical variables. Study primary and secondary outcome variables, including circulating sex hormones, IGF proteins and urinary estrogens and estrogen metabolites, were not normally distributed; therefore, hormone concentrations were log-transformed and geometric means and 95% CI were calculated for the treatment groups.

Given that no significant differences were observed for baseline demographics and characteristics of two groups (other than vitamin intake) and considering the randomized design of the study, it was assumed that potential confounding variables were equally distributed between two treatment groups; therefore, no further adjustment was performed. We only stratified hormone results by *COMT* genotype status as this was part of the trial's major questions and *a priori* hypothesis.

General linear mixed model (PROC MIXED in SAS) was used to compare hormones for baseline and month 12 values. All between- and within groups comparisons including pairwise tests were based on repeated measures analysis of variance (ANOVA). For stratified analyses, one-way ANOVA test was used first; if the result was significant, comparison between all three groups was performed using the Tukey's test for multiple comparisons. We decided to show the change in hormones after 12 months GTE supplementation by using a "ratio of change" concept, which is simply divining values of month 12 by month 0. Using ratio of change looked more meaningful than using absolute change or percent change from baseline considering the distribution of our data. For comparisons of ratio of change, PROC GLM was used as it is not repeated measure. We also assessed potential statistical interactions between treatment and time, and *COMT* genotype and time.

Spearman correlation coefficients were estimated to measure the strength of the linear relationship between selected baseline characteristics and hormones, as well as urinary and blood estrogens associations.

We used continuous model for primary and secondary outcome variables. When circulating sex hormones or urinary estrogens or its metabolites levels were below the detection limit and were reported as "non-detected", the following approaches were taken. For blood sex hormones, a value half the minimum detectable level was assigned to the respective analyte. For urinary estrogens and its metabolites, a value that was half the lowest standard (i.e. 0.004) was set for the analyte. Following is a list of analytes in which 20% or more of its samples had levels lower than the detectable limit: circulating estradiol (35.6%), urinary 4-OHE$_2$ (61.8%), 2-MeOE$_1$ (20.9%), 2-MeOE$_2$ (53.0%), 4-MeOE$_1$ (49.8%), and 4-MeOE$_2$ (54.6%).

For compliance assessment, data were analyzed on a log-scale and were reported as geometric means and 95% confidence intervals for their approximate normal distribution. All data were analyzed using SAS software, version 9.3 (SAS Institute Inc., Cary, North Carolina); all statistical tests were two-sided, and the value of $P < 0.05$ was considered statistically significant.

RESULTS

Enrollment

The flow of potential participants through the recruitment and intervention procedures is presented in Figure 8. The radiology reports of approximately 107,000 digital mammograms were reviewed. Mammograms taken for the purpose of diagnosing breast cancer or proliferative breast disease, composed almost entirely of fat or scattered fibroglandular tissue, or taken from participants out of the age range were not considered eligible. Approximately, 24,400 letters of invitation were mailed to potentially eligible women. Participants who responded to the letter of invitation were further screened via telephone (n= 5,473; response rate: 24.2%). Women were ineligible mainly due to not being postmenopausal, current or recent use of HRT, weight change more than 10 pounds within last year, or difficulty with time commitment. Of the 1801 participants who consented and attended a study orientation session, 1075 women were randomly assigned to either GTE (n = 538) or placebo (n = 537) and stratified by *COMT* genotype to one of four treatment/genotype groups: 1) GTE/low or intermediate-activity *COMT* genotype (*n* = 394, G/G + A/G); 2) GTE/high-activity *COMT* genotype (*n* = 144, A/A); 3) Placebo/low-activity *COMT* genotype (*n* = 396, G/G + A/G); 4) Placebo/high-activity *COMT* genotype (*n* = 141, A/A). A total of 937 women completed the study, 59 of which (6.7% of completers) went off study product during the study period but chose to remain in the study, in accordance with the ITT model. In addition, 138 participants (12.8%) dropped out of the study.

Baseline characteristics

Table 7 summarizes baseline characteristics for the GTE and placebo completer groups. The majority of participants were white, non-Hispanic, never-smokers, past users of oral contraceptives, and had some college level education. There were no statistically significant differences in baseline characteristics between the two groups except for use of vitamin supplements. Individuals in the GTE group reported higher intake of vitamin supplements compared with those in the placebo group (90.7% vs. 86.1%, *P* =0.04).

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Women assigned to the GTE arm had a slightly younger age at first live birth relative to those in the placebo group (27.9 years vs. 28.6 years; $P = 0.07$). The *COMT* G/G, G/A, and A/A genotypes were distributed as follows: 26.5%, 41.8%, and 31.7%, respectively, which were close to their distributions in Caucasian women reported previously [311]. The distributions of age, BMI, race, ethnicity, level of education, smoking status, alcohol consumption, and characteristics of menstrual and reproductive history were similar between the completers of GTE and placebo groups.

Table 8 also shows the baseline characteristics of all participants who were randomized into the study. Results are mostly similar to the Table 7 with the exception that randomized placebo participants were significantly taller than women in the GTE group.

Baseline dietary intake

Women assigned to the placebo arm were more likely to consume soy on a weekly basis than those in the GTE arm ($P = 0.09$). The intakes of major food groups, macro- and micro-nutrients, as well as total calories were comparable between the GTE and placebo groups (**Table 9**).

Compliance

On average, participants in both treatment and placebo groups took 96.5% of prescribed capsules based on pill count. As expected, urinary levels of catechins including EGC and EC were similar between two groups at baseline, but were significantly higher in the GTE participants compared with those in the placebo group at all time points thereafter. Participants in the GTE group experienced, on average, a 10.6-fold increase in urinary levels of EGC and 16.5-fold increase of EC concentrations compared with placebo (**Table 10**). Significant positive correlations were noted between the pill counts and urinary levels of EGC and EC among participants in treatment group during the first 3 months of the intervention (Spearman correlation coefficient (r) = 0.33,

$P = 0.03$ for EGC; and r = 0.35, $P = 0.02$ for EC). Participants who remained in the study but stopped receiving test pills at some point during the trial were excluded from compliance analysis.

Dropouts

By the end of the intervention, 138 participants dropped out of the trial: 75 (13.9%) from the GTE group and 63 (11.7%) from the placebo group. The overall dropout rate of this study was 12.8%, and characteristics did not differ between the GTE and placebo dropouts except for higher weight in placebo-dropouts than GTE-dropouts (**Table 11**). Dietary intake did not differ between GTE and placebo dropouts, with the exception of higher weekly intake of soy in the placebo-dropouts compared to the GTE-dropouts ($P = 0.02$) (**Table 12**).

Relatively high proportions of dropouts in both GTE and placebo groups had missing values on demographics and lifestyle factors due to their withdrawal from the study shortly after they consented. However, the proportions with missing values between the dropouts of GTE and placebo groups were comparable.

The most common reasons for dropout were participant request (67.4%), self-perceived or objective side effects such as gastrointestinal distress or elevated liver enzyme levels (15.9%), and lost to follow-up or no contact. Withdrawals due to adverse events were significantly more frequent in GTE than placebo group. (24.0% vs. 6.4%, $P = 0.005$) (See **Table 13**). The majority of dropouts (130 of 138, 94.2%) withdrew from the study within the first 6 months of the intervention. In addition, 59 participants (39 GTE and 20 placebo) chose to continue the trial guidelines even after they stopped taking the study supplements according to the ITT basis. The main reasons for continued follow-up but discontinued intake of study capsules were self-perceived or observed side effects, (84.7%), participant's request (10.2%), and protocol deviation (5.1%).

DISCUSSION

Existing evidence supports the notion that green tea, when consumed on a daily basis, supports health and may prevent chronic diseases such as cancer, cardiovascular, and inflammatory diseases [2, 312]. Green tea and its polyphenolic components (catechins) have been associated with lower breast cancer risk; however, current findings from epidemiological studies are inconclusive. This inconsistency may be due to the fact that most available studies were not originally designed to evaluate the role of green tea intake on breast cancer risk; hence, they failed to accurately estimate the amount, frequency and duration of tea intake. To date, only two relatively small human intervention trials have examined the effects of green tea intake for 2-6 months on biomarkers of breast cancer risk in either healthy postmenopausal women (n= 103) [88] or breast cancer survivors (n= 40) [89]. To the best of our knowledge, the MGTT is the first double-blind, placebo-controlled, randomized intervention study with a very large sample size, long treatment period in humans that specifically evaluated the effects of oral green tea extract containing defined quantities of EGCG on established biomarkers of breast cancer risk in postmenopausal women at high risk of breast cancer and with differing *COMT* genotypes.

The rationale for the dose of GTE in this trial is based on its safety and efficacy described in earlier pharmacokinetic studies and the common upper end of green tea consumption in Asian populations. Chow *et al* have [41, 48] previously shown that single or 4-week daily doses of 800 mg EGCG or Polyphenon E (a GTE similar to our study supplement) were well tolerated in healthy participants, and also lead to elevated systemic availability of EGCG. More recently, Crew *et al* [89] reported that daily consumption of 600 mg Polyphenon E twice daily (for a total of 1200 mg EGCG) was the maximum tolerated dose in women with a history of breast cancer. In addition, daily intake of 800 mg EGCG for two months has been shown to be safe and well tolerated in healthy postmenopausal women with very few adverse events (mostly gastrointestinal in

nature) [88]. Observed adverse events in the MGTT were mild and have been reported in a separate recent manuscript [313].

Although we observed several statistically significant differences when comparing baseline demographic and clinical characteristics of completers or compliant participants with those who dropped out of the study within either GTE or placebo groups, most of these significant differences were due to unknown or missing data mainly from 26 participants who were randomized but withdrew from the study prior to their baseline clinic visits when demographic data were collected. When unknown data were excluded from the statistical analyses, most of these differences disappeared or were no longer statistical significant.

There are a number of strengths of this study. The large sample size, 12 month intervention period, and randomized, double-blind, placebo controlled design are among important improvements compared to the previous two trials that investigated the impact of GTE on biomarkers of breast cancer risk (42, 43). Study participants showed excellent compliance as confirmed by pill count, study log records, and objective measures from urinary catechin levels are strengths of this study. We also took advantage of a unique nutrigenetic approach to determine individual differences in metabolism of tea catechins based on *COMT* genotype as well as the state-of-the-art methodologies to ascertain the study outcomes such as circulating and urinary levels of estrogens and their metabolites. Furthermore, the study supplements were repeatedly checked to assure catechin stability. Finally, we were successful in retaining the participants in the study, as demonstrated by a dropout rate of just 12.8%.

This study has some limitations as well. We used one dose of an extract of tea catechins rather than a brewed green tea infusion for dose standardization purposes; therefore, the impact of this dose of GTE on breast cancer risk factors may not be extrapolated to other forms of catechin administration or to other doses of GTE. We had limited success in recruiting minority populations, and baseline measurement of study biomarkers was based on single sample collection. A further limitation of our study was that we did not capture information on frequency, amount, or duration of green tea

drinking prior to the study, thus we cannot rule out potential effect of chronic past green tea intake on our study endpoints. Finally, we cannot completely rule out the possibility of a collinearity effect of catechins from other dietary sources such as black tea, dark chocolate, apples, or wine; however, it should be noted that the quantity of catechins provided in the study green tea capsules is much higher than what is usually found in other catechin-containing foods. For example, provided amount of EGCG from the GTE treatment was more than hundred times of amount usually found in 100 g of normal dietary sources of raw Fuji apples, hazelnuts, or pecans; similarly catechin content of the GTE was more than 5-time than 100 g of dark chocolate, which is one of the best dietary sources of catechin [298, 314].

In summary, the MGTT enrolled 1075 participants and completed the trial for 937 women residing in Minnesota during 2009-2014. The MGTT is the largest long-term study investigating the effects of green tea catechins on well-known biomarkers of breast cancer among postmenopausal women at high risk of breast cancer. This study aims to elucidate the mechanisms by which green tea may reduce breast cancer risk, potentially identifying subgroups of women who may benefit from green tea intake, and lead to improved dietary recommendations for breast cancer prevention.

106,758 women's mammograms screened

82,342 women ineligible due to not meeting age and breast density criteria

24,416 invitations mailed to eligible women

5,473 women further assessed for eligibility via phone screening

3,156 Ineligible
  628 Not post-menopausal
  438 Current or within 6 mos. HRT
  817 Declined to participate
  280 Weight change >10 lbs. in
      past year
  126 Not within BMI range
  116 Mammogram out of date
  120 Current Smoker
   37 Diabetes mellitus dx
   23 Current or within 6 months
      Raloxifene use
   15 On Warfarin/anti-coagulant
   36 Regular green tea drinker
   65 History of breast cancer or
      proliferative breast disease
    4 History of liver conditions
  451 Other

516 non-respondents

1,801 consented

328 ineligible

398 dropped out

1,075 Randomized

85

Figure 8. Flow diagram of participant screening, enrollment, randomization, and follow-up of the Minnesota Green Tea Trial, Minnesota, 2009-2014 [313].

Abbreviations: BMI, body mass index; COMT, catechol-O-methyltransferase; GTE, green tea extract; HRT, hormone replacement therapy; ITT, intention-to-treat

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Table 4. Inclusion and exclusion criteria of the MGTT, Minnesota, 2009-2014.

Inclusion criteria
50-70 years old
Generally healthy postmenopausal women[a]
Heterogeneously (51-75% glandular) or extremely (>75% glandular) dense breasts[b]
Planning to reside in or near Minnesota for study duration
Willing to give written informed consent

Exclusion criteria
Regular green tea intake (i.e. more than one cup per week)
Hepatitis B or C viral infection indicated by the presence of hepatitis B surface antigen or antibodies to hepatitis C virus, respectively
Elevated liver enzymes level > 1.5 times the upper limit of normal
Current or prior (within last 6 months) use of hormone therapy, including systemic postmenopausal hormone therapy (such as oral pills, patch, or gel), chemopreventive agents such as selective estrogen receptor modulators (Tamoxifen, Raloxifene) or aromatase inhibitors
Previous diagnosis of breast cancer, proliferative breast disease, or ovarian cancer
History of any other malignancy in the past 5 years (apart from non-melanoma skin cancer)
Presence of breast implants
Currently taking methotrexate or etanercept (Enbrel)
BMI <18.5 or >40 kg/m^2
Ongoing enrollment in a weight loss or weight gain program
Weight change > 10 lbs during the previous year
Alcohol intake >7 drinks per week
Current smoker of cigarettes or other tobacco products

Abbreviation: MGTT, Minnesota Green Tea Trial.
[a] Determined by no menstrual period for at least one year or serum follicle stimulating hormone > 23 mIU/ml (as the lower limit of normal for postmenopausal women set by the Quest Diagnostics, IL).
[b] Determined from participant's annual screening mammogram.

Table 5. Scheduled data collection part of the Minnesota Green Tea Trial clinic visits, Minnesota, 2009-2014

	Screen	Baseline	month 1	month 2	month 3	month 4	month 5	month 6	month 7	month 8	month 9	month10	month11	month12
	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10	Visit 11	Visit 12	Visit 13	Visit 14
Weight/vital signs	X	X			X			X			X			X
Height	X													X
Measure waist and hip circumferences		X												X
Distribute supplement		X			X			X			X			
Pill count for compliance					X			X			X			X
Collect 24-hr urine		X						X						X
Collect spot urine					X						X			
Blood draw	X	X	X	X	X	X	X	X	X[a]	X[a]	X	X[a]	X[a]	X
Medical history	X													
HHQ		X												
DHQ		X												X
MENQOL questionnaire		X						X						X
Buffy coat collection	X													X[b]

Abbreviations: DHQ, diet history questionnaire; HHQ, health history questionnaire; MENQOL, menopause-specific quality of life questionnaire.

[a] Mammograms were performed up to 3.5 months before the baseline clinic visit.

[b] Mammograms were performed within 5 days of month 12 clinic visit.

[c] Done for 225 (24.0%) of the participants.

[d] Available for 625 (66.7%) of the participants.

Table 6. Catechin and caffeine contents of Corban Complex GTB used in the MGTT, Minnesota, 2009-2014.

Component	Quantity per Capsule[a,b] (mg)	Dose per Day[a] (mg)	Quantity per Capsule (%)
Total catechins	328.8 ± 28.9	1315.3	80.7
- Epigallocatechin (EGC)	26.7 ± 29.7	106.8	6.6
- Catechin	3.8 ± 2.1	15.2	0.9
- Epicatechin (EC)	26.8 ± 5.9	107.2	6.6
- Epigallocatechin Gallate (EGCG)	210.7 ± 11.0	842.8	51.7
- Gallocatechin Gallate (GCG)	8.4 ± 1.8	33.6	2.1
- Epicatechin Gallate (ECG)	50.6 ± 18.5	202.4	12.4
- Catechin Gallate (CG)	1.1 ± 0.5	4.2	0.3
- Gallocatechin (GC)	1.3 ± 1.4	5.1	0.3
Caffeine	3.9	15.8	1.0

Abbreviations: GTB, green tea blend; MGTT, Minnesota Green Tea Trial.

[a] Values are presented as means (SD) from eight batches. Catechin analyses were conducted by Covance Laboratories (Madison, WI).

[b] Each capsule's entity fill weight equals to 407.3 mg.

Table 7. Baseline demographic and clinical characteristics of randomized completer participants; ITT model (n= 937).

	GTE-Completers (n= 463)	Placebo-Completers (n= 474)	*p* value[1]
Age at baseline, y	60.0 (4.9)	59.7 (5.0)	0.26
Race			0.44
- White	453 (97.8%)	458 (96.6%)	
- Asian	3 (0.7%)	7 (1.5%)	
- Black	2 (0.4%)	5 (1.1%)	
- Others	4 (0.9%)	2 (0.4%)	
- Unknown	1 (0.2%)	2 (0.4%)	
Ethnicity			0.90
- Hispanic	5 (1.1%)	4 (0.8%)	
- Non-Hispanic	453 (97.8%)	464 (97.9%)	
- Unknown	5 (1.1%)	6 (1.3%)	
COMT genotype			0.32
- High	126 (27.2%)	122 (25.7%)	
- Intermediate	181 (39.1%)	208 (43.9%)	
- Low	156 (33.7%)	144 (30.4%)	
Level of education			0.14
- High school or less	27 (5.8%)	31 (6.5%)	
- Some college	313 (67.6%)	287 (60.6%)	
- Postgraduate or professional degree	121 (26.1%)	152 (32.1%)	
- Unknown	2 (0.4%)	4 (0.8%)	
Family history of breast cancer[a]			
- No	332 (71.7%)	355 (74.9%)	0.22
- Yes	120 (25.9%)	114 (24.1%)	
- Unknown	11 (2.4%)	5 (1.1%)	
Age at menarche, y[b]	13.0 (1.4)	12.9 (1.4)	0.78

Table 7, continued.

	GTE-Completers (n= 463)	Placebo-Completers (n= 474)	*p* value[1]
Parity Status			0.27
- Nulliparous	108 (23.3%)	110 (23.2%)	
- Parous	354 (76.5%)	359 (75.7%)	
- Unknown	1 (0.2%)	5 (1.1%)	
Age at first live birth, y[c,d]	27.9 (5.4)	28.6 (5.4)	0.07
Breastfeeding, total months[c,e]	13.3 (17.4)	13.5 (16.2)	0.87
Type of menopause			0.18
- Natural	397 (85.8%)	409 (86.3%)	
- Surgical: bilateral oophorectomy	35 (7.6%)	45 (9.5%)	
- Surgical: ovarian status unknown	27 (5.8%)	15 (3.2%)	
- Unknown	4 (0.9%)	5 (1.1%)	
Age at menopause, y[f]	49.0 (5.7)	49.3 (5.2)	0.47
Years since menopause[g]	11.1 (7.6)	10.3 (7.2)	0.13
Past use of oral contraceptives			0.85
- No	65 (14.0%)	70 (14.8%)	
- Yes	395 (85.3%)	402 (84.8%)	
- Unknown	3 (0.7%)	2 (0.4%)	
Past use of hormone therapy			0.95
- No	270 (58.3%)	278 (58.7%)	
- Yes	189 (40.8%)	191 (40.3%)	
- Unknown	4 (0.9%)	5 (1.1%)	

Table 7, continued.

	GTE-Completers (n= 463)	Placebo-Completers (n= 474)	*p* value[1]
Height, cm	163.6 (6.3)	164.3 (6.1)	0.10
Weight, kg	67.3 (10.7)	67.4 (10.3)	0.94
BMI, kg/m^2	25.2 (3.7)	25.0 (3.7)	0.52
Waist-to-hip ratio	0.84[h] (0.1)	0.83 (0.1)	0.21
Use of vitamin supplements			
- No	42 (9.1%)	66 (13.9%)	0.04
- Yes	420 (90.7%)	408 (86.1%)	
- Unknown	1 (0.2%)	0 (0.0%)	
Smoking status			0.84
- Never	316 (68.3%)	324 (68.4%)	
- Former	145 (31.3%)	149 (31.4%)	
- Unknown	2 (0.4%)	1 (0.2%)	
Alcohol drinks/week (drinkers only)[i]	3.4 (3.0)	3.4 (3.0)	0.81
Self-reported physical activity, MET-h/week[j]	45.7 (54.0)	51.0 (106.9)	0.34

Abbreviations: ITT, intention-to-treat; GTE, green tea extract; BMI, body mass index; COMT, catechol-O-methyltransferase; MET, metabolic equivalent.

NOTE: Data presented as the means (SD) for continuous variables and as frequencies (%) for categorical variables.

[1] *p* value for difference between the GTE-completers with the Placebo-completers.

[a] Only in first degree relatives.

[b] GTE (n): completers= 457; Placebo (n): completers= 460.

[c] Among parous women only.

[d] GTE (n): completers= 354; Placebo (n): completers= 359.

[e] GTE (n): completers= 348; Placebo (n): completers= 353.

[f] GTE (n): completers= 442; Placebo (n): completers= 454.

[g] GTE (n): completers= 440; Placebo (n): completers= 454.

[h] n= 461.

[i] GTE (n): completers= 368; Placebo (n): completers= 396.

[j] GTE (n): completers= 463; Placebo (n): completers= 473.

Table 8. Baseline demographics and clinical characteristics of all randomized participants; ITT Model, Minnesota, 2009-2014 (n= 1075).

	GTE (n= 538)	Placebo (n= 537)	*p* value[a]
Age at baseline, y	59.9 (5.0)	59.6 (5.1)	0.35
Race			0.44
- White	515 (95.7)	505 (94.0)	
- Asian	3 (0.6)	7 (1.3)	
- Black	4 (0.7)	7 (1.3)	
- Others	4 (0.7)	2 (0.4)	
- Unknown	12 (2.2)	16 (3.0)	
Ethnicity			0.76
- Hispanic	5 (0.9)	6 (1.1)	
- Non-Hispanic	516 (95.9)	510 (95.0)	
- Unknown	17 (3.2)	21 (3.9)	
COMT genotype			0.51
- High	144 (26.8)	141 (26.3)	
- Intermediate	216 (40.2)	233 (43.4)	
- Low	178 (33.1)	163 (30.3)	
Level of education			0.19
- High school or less	33 (6.1)	39 (7.3)	
- Some college	348 (64.7)	313 (58.3)	
- Postgraduate or professional degree	143 (26.6)	166 (30.9)	
- Unknown	14 (2.6)	19 (3.5)	
Family history of breast cancer[b]			0.71
- No	384 (71.4)	394 (73.4)	
- Yes	130 (24.2)	123 (22.9)	
- Unknown	24 (4.5)	20 (3.7)	
Age at menarche, y[c]	13.0 (1.4)	13.0 (1.4)	0.97

Table 8, continued.

	GTE (n= 538)	Placebo (n= 537)	*p* value[a]
Parity Status			0.46
- Nulliparous	118 (21.9)	125 (23.3)	
- Parous	407 (75.7)	393 (73.2)	
- Unknown	13 (2.4)	19 (3.5)	
Age at first live birth, y[d,e]	27.8 (5.4)	28.4 (5.5)	0.16
Breastfeeding, total months[d,f]	13.0 (16.7)	12.9 (15.8)	0.96
Type of menopause			0.11
- Natural	448 (83.3)	445 (82.9)	
- Surgical: bilateral oophorectomy	41 (7.6)	54 (10.1)	
- Surgical: ovarian status unknown	32 (6.0)	18 (3.4)	
- Unknown	17 (3.2)	20 (3.7)	
Age at menopause, y[g]	49.0 (5.8)	49.2 (5.3)	0.53
Years since menopause[h]	10.9 (7.8)	10.3 (7.2)	0.20
Past use of oral contraceptives			0.70
- No	68 (12.6)	77 (14.3)	
- Yes	455 (84.6)	444 (82.7)	
- Unknown	15 (2.8)	16 (3.0)	
Past use of hormone therapy			0.87
- No	306 (56.9)	302 (56.2)	
- Yes	216 (40.2)	216 (40.2)	
- Unknown	16 (3.0)	19 (3.5)	
Height, cm[i]	163.5 (6.3)	164.3 (6.2)	0.04

Table 8, continued.

	GTE (n= 538)	Placebo (n= 537)	*p* value[a]
Weight, kg[i]	67.4 (10.6)	67.9 (10.5)	0.41
BMI, kg/m^2 [i]	25.2 (3.7)	25.2 (3.9)	0.91
Waist-to-hip ratio[j]	0.84 (0.1)	0.83 (0.1)	0.26
Use of vitamin supplements			0.03
- No	48 (8.9)	76 (14.2)	
- Yes	477 (88.7)	447 (83.2)	
- Unknown	13 (2.4)	14 (2.6)	
Smoking status			0.98
- Never	357 (66.4)	356 (66.3)	
- Former	167 (31.0)	166 (30.9)	
- Unknown	14 (2.6)	15 (2.8)	
Alcohol drinks/week (drinkers only)[k]	3.3 (3.0)	3.4 (3.0)	0.77
Self-reported physical activity, MET-h/week[l]	45.3 (53.1)	51.4 (104.0)	0.23

Abbreviations: ITT, intention-to-treat; GTE, green tea extract; BMI, body mass index; COMT, catechol-O-methyltransferase; MET, metabolic equivalent.

NOTE: Data presented as the means (SD) for continuous variables and as frequencies (%) for categorical variables.

[a] *p* value for difference between the randomized GTE with the randomized Placebo participants.

[b] Only in first degree relatives.

[c] GTE (n)= 519; Placebo (n)= 509.

[d] Among parous women only.

[e] GTE (n)= 407; Placebo (n)= 393.

[f] GTE (n)= 401; Placebo (n)= 386.

[g] GTE (n)= 500; Placebo (n)= 501.

[h] GTE (n)= 498; Placebo (n)= 501.

[i] GTE (n)= 526; Placebo (n)= 523.

[j] GTE (n)= 524; Placebo (n)= 523.

[k] Only among alcohol drinkers; GTE (n)= 418; Placebo (n)= 434.

[l] GTE (n)= 519; Placebo (n)= 515.

Table 9. Daily food, nutrient and energy intake of completer participants by treatment group at baseline; ITT model (n= 937).*

Food or Nutrient	GTE-Completers (n= 463)	Placebo-Completers (n= 474)	p value[1]
Black or green tea intake/week[a]			0.51
- No/never	187 (40.5%)	180 (38.4%)	
- Yes	275 (59.5%)	289 (61.6%)	
times/week[b]	4.0 (3.8)	4.3 (4.4)	0.44
Soy intake/week[c]			0.09
- No	327 (71.4%)	308 (66.2%)	
- Yes	131 (28.6%)	157 (33.8%)	
times/week[d]	2.4 (2.7)	2.0 (2.5)	0.21
Total red meat intake, oz[e]	1.2 (0.9)	1.1 (0.8)	0.26
Total vegetable intake, servings	3.5 (2.3)	3.4 (2.1)	0.38
Total fruit intake, servings	2.3 (1.7)	2.4 (1.6)	0.34
Energy, Kcal	1438.3 (538.0)	1447.5 (524.7)	0.79
Carbohydrate, g	179.3 (67.9)	181.9 (69.5)	0.55
Protein, g	58.9 (24.2)	57.6 (22.2)	0.42
Total fat, g	53.3 (25.0)	54.1 (25.3)	0.63
- Total trans fat, g	2.7 (1.4)	2.7 (1.3)	0.79
- Saturated fat, g	16.3 (7.6)	16.4 (7.6)	0.83
- Monounsaturated fat, g	20.7 (10.5)	21.0 (10.4)	0.67
- Polyunsaturated fat, g	12.3 (6.5)	12.7 (6.9)	0.38
- Fatty acid 20:5 (EPA), g	0.02 (0.03)	0.02 (0.02)	0.52
- Fatty acid 22:6 (DHA), g	0.05 (0.04)	0.05 (0.04)	0.44

Table 9, continued.

Food or Nutrient	GTE-Completers (n= 463)	Placebo-Completers (n= 474)	p value[1]
Cholesterol, mg	146.0 (72.3)	142.6 (72.3)	0.47
Total Dietary Fiber, g	17.5 (8.2)	17.7 (8.0)	0.72
Beta-Carotene, mcg	3941.4 (3471.2)	3905.4 (3399.2)	0.87
Beta-Cryptoxanthin, mcg	133.3 (110.6)	136.7 (115.9)	0.65
Vitamin C, mg	109.2 (71.1)	112.3 (69.5)	0.51
Vitamin E, Total Alpha-tocopherol, mg	7.8 (4.6)	8.1 (4.7)	0.30
Vitamin D (calciferol), mcg	3.4 (2.4)	3.3 (2.2)	0.31
Caffeine, mg	353.6 (312.1)	379.1 (322.7)	0.22
Calcium, mg	767.7 (363.5)	761.3 (341.6)	0.78
Iron, mg	12.1 (4.9)	12.1 (4.9)	0.97
Selenium, mcg	72.7 (29.8)	72.1 (28.8)	0.73
Zinc, mg	9.2 (3.9)	9.0 (3.8)	0.51

Abbreviations: GTE, green tea extract; EPA, Eicosapentaenoic acid; DHA, Docosahexaenoic acid.

NOTE: Data presented as the means (SD) for continuous variables and as frequencies (%) for categorical variables.

* From the diet history questionnaire except for the soy and black or green tea data which come from the health history questionnaire.

[1] p value for difference between the GTE-completers with the Placebo-completers.

[a] GTE (n): completers= 462; Placebo (n): completers= 469.

[b] GTE (n): completers= 222; Placebo (n): completers= 226.

[c] GTE (n): completers= 458; Placebo (n): completers= 465.

[d] GTE (n): completers= 119; Placebo (n): completers= 143.

[e] Total red meat includes beef, pork, lamb, organ, franks and luncheon meats.

Table 10. Urinary concentrations of tea catechins (nmol/mg creatinine) by treatment group for compliance assessment, MGTT, Minnesota, 2009-2014 (n= 90).

Catechins	GTE (n= 43)		Placebo (n= 47)		*P* value[c]
	Mean[a,b]	95% CI	Mean[a]	95% CI	
EGC					
Month 0	0.2	0.04, 0.4	0.3	0.1, 0.5	0.6
Month 3	1.4	0.9, 2.0	0.2	-0.04, 0.4	<0.0001
Month 6	2.0	1.2, 3.1	0.3	-0.06, 0.7	0.0001
Month 9	3.5	2.3, 5.3	0.3	-0.06, 0.7	<0.0001
Month 12	6.8	4.4, 10.2	0.4	-0.02, 1.0	<0.0001
EC					
Month 0	0.8	0.5, 1.2	0.9	0.6, 1.3	0.7
Month 3	11.1	8.5, 14.5	0.6	0.3, 1.0	<0.0001
Month 6	11.5	8.9, 14.8	0.7	0.4, 1.2	<0.0001
Month 9	9.4	6.8, 12.7	0.6	0.2, 1.1	<0.0001
Month 12	15.5	12.4, 19.4	1.0	0.6, 1.4	<0.0001

Abbreviations: CI, confidence interval; EC, epicatechin; EGC, epigallocatechin; GTE, green tea extract; MGTT, Minnesota Green Tea Trial.

[a] Geometric means.

[b] *p* value for the effect of time in the GTE group for both EGC and EC metbolites was <0.0001.

[c] *p* value for difference between the GTE and the Placebo groups at each time point.

Table 11. Demographic and clinical characteristics of randomized dropout participants at baseline (n= 138).

Variables	GTE-dropouts (n= 75)	Placebo-dropouts (n= 63)	p value[1]
Age at baseline, y	59.4 (5.5)	59.5 (5.3)	0.86
Race			0.50
- White	62 (82.7%)	47 (74.6%)	
- Asian	0 (0.0%)	0 (0.0%)	
- Black	2 (2.7%)	2 (3.2%)	
- Others	0 (0.0%)	0 (0.0%)	
- Unknown	11 (14.7%)	14 (22.2%)	
Ethnicity			0.14
- Hispanic	0 (0.0%)	2 (3.2%)	
- Non-Hispanic	63 (84.0%)	46 (73.0%)	
- Unknown	12 (16.0%)	15 (23.8%)	
COMT genotype			0.65
- High	18 (24.0%)	19 (30.2%)	
- Intermediate	35 (46.7%)	25 (39.7%)	
- Low	22 (29.3%)	19 (30.2%)	
Level of education			0.44
- High school or less	6 (8.0%)	8 (12.7%)	
- Some college	35 (46.7%)	26 (41.3%)	
- Postgraduate or professional degree	22 (29.3%)	14 (22.2%)	
- Unknown	12 (16.0%)	15 (23.8%)	
Family history of breast cancer[a]			0.60
- No	52 (69.3%)	39 (61.9%)	
- Yes	10 (13.3%)	9 (14.3%)	
- Unknown	13 (17.3%)	15 (23.8%)	
Age at menarche, y[b]	12.9 (1.5)	13.1 (1.6)	0.51

Table 11, continued.

Variables	GTE-dropouts (n= 75)	Placebo-dropouts (n= 63)	*p* value[1]
Parity Status			0.12
- Nulliparous	10 (13.3%)	15 (23.8%)	
- Parous	53 (70.7%)	34 (54.0%)	
- Unknown	12 (16.0%)	14 (22.2%)	
Age at first live birth, y[c,d]	27.6 (5.8)	25.9 (5.6)	0.20
Breastfeeding, total months[c,e]	11.1 (10.6)	6.9 (8.0)	0.06
Type of menopause			0.42
- Natural	51 (68.0%)	36 (57.1%)	
- Surgical: bilateral oophorectomy	6 (8.0%)	9 (14.3%)	
- Surgical: ovarian status unknown	5 (6.7%)	3 (4.8%)	
- Unknown	13 (17.3%)	15 (23.8%)	
Age at menopause, y[f]	49.1 (6.2)	48.9 (6.2)	0.91
Years since menopause[f]	10 (8.6)	10.5 (7.4)	0.75
Past use of oral contraceptives			0.14
- No	3 (4.0%)	7 (11.1%)	
- Yes	60 (80.0%)	42 (66.7%)	
- Unknown	12 (16.0%)	14 (22.2%)	
Past use of hormone therapy			0.45
- No	36 (48.0%)	24 (38.1%)	
- Yes	27 (36.0%)	25 (39.7%)	
- Unknown	12 (16.0%)	14 (22.2%)	

Table 11, continued.

Variables	GTE-dropouts (n= 75)	Placebo-dropouts (n= 63)	p value[1]
Height, cm[g]	163.2 (6.1)	165.1 (6.5)	0.11
Weight, kg[g]	67.7 (10.2)	72.9 (10.7)	0.01
BMI, kg/m^2 [g]	25.4 (3.9)	26.8 (3.7)	0.07
Waist-to-hip ratio[g]	0.84 (0.1)	0.85 (0.1)	0.74
Use of vitamin supplements			0.17
- No	6 (8.0%)	10 (15.9%)	
- Yes	57 (76.0%)	39 (61.9%)	
- Unknown	12 (16.0%)	14 (22.2%)	
Smoking status			0.65
- Never	41 (54.7%)	32 (50.8%)	
- Former	22 (29.3%)	17 (27.0%)	
- Unknown	12 (16.0%)	14 (22.2%)	
Alcohol drinks/week (drinkers only)[h]	3.1 (3.1)	3.1 (3.1)	0.97
Self-reported physical activity, MET-h/week[i]	41.6 (45.7)	56.1 (63.2)	0.19

Abbreviations: ITT, intention-to-treat; GTE, green tea extract; BMI, body mass index; COMT, catechol-O-methyltransferase; MET, metabolic equivalent.

NOTE: Data presented as the means (SD) for continuous variables and as frequencies (%) for categorical variables.

[1] p value for difference between the GTE-dropouts with the Placebo-dropouts.

[a] Only in first degree relatives.

[b] GTE (n): dropouts= 62; Placebo (n): dropouts= 49.

[c] Among parous women only.

[d] GTE (n): dropouts= 53; Placebo (n): dropouts= 34.

[e] GTE (n): dropouts= 53; Placebo (n): dropouts= 33.

[f] GTE (n): dropouts= 58; Placebo (n): dropouts= 47.
[g] GTE (n): dropouts= 63; Placebo (n): dropouts= 49.
[h] GTE (n): dropouts= 50; Placebo (n): dropouts= 38.
[i] GTE (n): dropouts= 56; Placebo (n): dropouts= 42.

Table 12. Daily food, nutrient and energy intake of dropout participants by treatment group at baseline (n= 113).[1,2]

Food or Nutrient	GTE-dropouts (n= 65)	Placebo-dropouts (n= 48)	p value[3]
Black or green tea intake/week[a]			0.21
- No/never	32 (52.5)	19 (40.4)	
- Yes	29 (47.5)	28 (59.6)	
times/week[b]	4.5 (3.9)	4.6 (3.7)	0.90
Soy intake/week[c]			0.02
- No	57 (90.5)	35 (74.5)	
- Yes	6 (9.5)	12 (25.5)	
times/week[d]	0.6 (0.2)	2.4 (2.8)	0.18
Total red meat intake, oz[e]	1.4 (1.5)	1.3 (1.0)	0.70
Total vegetable intake, servings	3.4 (2.0)	3.8 (2.4)	0.38
Total fruit intake, servings	2.1 (1.1)	2.6 (1.7)	0.08
Energy, Kcal	1368.8 (672.6)	1519.9 (580.0)	0.21
Carbohydrate, g	170.7 (82.2)	195.7 (93.2)	0.13
Protein, g	56.6 (30.1)	61.1 (23.5)	0.39
Total fat, g	50.2 (30.4)	54.9 (19.8)	0.35
- Total trans fat, g	2.7 (1.9)	3.0 (1.7)	0.35
- Saturated fat, g	15.8 (10.2)	17.2 (7.0)	0.38
- Monounsaturated fat, g	19.3 (11.9)	21.0 (8.0)	0.42
- Polyunsaturated fat, g	11.3 (7.0)	12.4 (4.7)	0.35
- Fatty acid 20:5 (EPA), g	0.02 (0.03)	0.03 (0.04)	0.44
- Fatty acid 22:6 (DHA), g	0.05 (0.05)	0.06 (0.06)	0.57

Table 12, continued.

Food or Nutrient	GTE-dropouts (n= 65)	Placebo-dropouts (n= 48)	*p* value[3]
Cholesterol, mg	144.5 (88.8)	155.1 (62.6)	0.48
Total Dietary Fiber, g	16.4 (7.4)	18.2 (8.5)	0.24
Beta-Carotene, mcg	3726.5 (2400.9)	4037.4 (4680.9)	0.65
Beta-Cryptoxanthin, mcg	121.9 (76.4)	153.2 (119.8)	0.09
Vitamin C, mg	106.1 (52.2)	130.6 (86.1)	0.06
Vitamin E, Total Alpha-tocopherol, mg	7.6 (4.3)	8.3 (3.9)	0.35
Vitamin D (calciferol), mcg	30.0 (2.1)	3.6 (2.4)	0.11
Caffeine, mg	352.2 (330.3)	374.9 (323.9)	0.72
Calcium, mg	693.8 (344.0)	811.1 (379.3)	0.09
Iron, mg	11.7 (5.9)	12.9 (5.6)	0.29
Selenium, mcg	71.5 (40.7)	75.2 (30.6)	0.60
Zinc, mg	8.8 (4.8)	9.7 (4.5)	0.30

Abbreviations: GTE, green tea extract; EPA, Eicosapentaenoic acid; DHA, Docosahexaenoic acid.

NOTE: Data presented as the means (SD) for continuous variables and as frequencies (%) for categorical variables.

[1] From the diet history questionnaire except for the soy and black or green tea data which come from the health history questionnaire.

[2] Missing data for 25 dropout participants who did not complete the dietary history questionnaire at baseline.

[3] p value for difference between the GTE-dropouts with the Placebo-dropouts.

[a] GTE (n): dropouts= 61; Placebo (n): dropouts= 47.

[b] GTE (n): dropouts= 23; Placebo (n): dropouts= 23.

[c] GTE (n): dropouts= 63; Placebo (n): dropouts= 47.

[d] GTE (n): dropouts= 5; Placebo (n): dropouts= 9.

[e] Total red meat includes beef, pork, lamb, organ, franks and luncheon meats.

Table 13. Reason for withdrawal by the treatment group (n= 138).

Reason	GTE (n= 75)	Placebo (n= 63)	p value[1]
Participant Request	46 (61.3)	47 (74.6)	
Adverse Event	18 (24.0)	4 (6.4)	
Lost to Follow-Up	4 (5.3)	6 (9.5)	0.047
Investigator Judgment	2 (2.7)	1 (1.6)	
Other[2]	5 (6.7)	5 (7.9)	

Abbreviation: GTE, green tea extract.

NOTE: Values are frequencies (%).

[1] *p* value is derived from Fisher's exact test.

[2] Includes protocol violation cases such as being noncompliant in clinic visit attendance, start smoking, going on hormone replacement therapy, or one case of death.

Chapter 3: MGTT- Results

Circulating Reproductive Hormones and SHBG

As shown in **Figure 8**, 1,075 were randomized into either GTE (n= 463) or placebo (n= 474) groups among which 937 women in total completed the trial. Participants were further stratified by *COMT* genotype groups into one of low, intermediate, or high *COMT* genotype activity groups within GTE or placebo groups. Selected baseline demographic characteristics are displayed in **Table 14**, and as shown, there was no significant difference between the randomized GTE and placebo women who completed the study. In general, participants were white, non-Hispanic (not shown in the table), never smokers, past user of oral contraceptives, with some college education. Women did not differ with regard to BMI, and reproductive history factors such as age at menarche or menopause, parity status, type of menopause, years since menopause, or past use of HRT between two groups. Participants in the GTE group were slightly younger at their first live birth versus those in the placebo group. Baseline demographic data were also similar between the GTE and placebo participants who dropped out of the trial except for weight, which was higher in dropped out placebo women (see **Table 11**).

Energy and nutrient intake did not differ between two groups at baseline (See **Table 9**), and at month 12, only total vegetable intake was significantly higher in the GTE participants who competed the study compared with those in the placebo group (*P*<0.03; see **Table 15**).

Estrone and estradiol were significantly positively associated with BMI ($r= 0.24$ for E_1; $r= 0.26$ for E_2), weight ($r= 0.23$ for E_1; $r= 0.26$ for E_2), androstenedione ($r= 0.47$ for E_1; $r= 0.21$ for E_2), and testosterone ($r= 0.38$ for E_1; $r= 0.13$ for E_2), and negatively associated with SHBG ($r= -0.12$ for E_1; $r= -0.2$ for E_2). All *P* values were <0.0001 except for SHBG association with E1, which was <0.0002. Androstenedione was negatively associated with age ($r= -0.11$; $P= 0.0005$), years since menopause ($r= -0.09$; $P= 0.009$),

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and SHBG (r= -0.07; P= 0.04) while testosterone was positively associated with age (r= 0.09; P= 0.05) and SHBG (r= 0.11; P= 0.0008).

Table 16 presents results of the effects of GTE on levels of sex steroid hormones. Baseline concentrations of estrogens including estrone and estradiol (total, free, and bioavailable); androgens including androstenedione and testosterone (total, free, and bioavailable) as well as SHBG were not significantly different between the study groups. Similarly, ratios of total testosterone to total estradiol, and estrone to androstenedione were identical at baseline between two groups. However, significant differences were observed in serum concentrations of estradiol (total, free, and bioavailable), testosterone (total, free, and bioavailable), and the testosterone to estradiol ratio at month 12 between the study groups. In order to compare changes in the levels of sex hormones following 12- month of supplementation with GTE, the ratio of month 12 to baseline (hereafter will be referred to as "ratio of change") were computed and compared for all endpoint levels in both GTE and placebo groups based on one-way ANOVA test.

Within treatment group comparisons of hormones revealed that circulating levels of estrone, estradiol (total, free, and bioavailable); androstenedione and total testosterone to total estradiol ratio were significantly reduced in the placebo group, whereas the blood levels of total and bioavailable levels of testosterone significantly increased in the GTE group.

Contrary to the trial hypothesis, consumption of GTE for one year prevented a decrease in the ratio of changes of total, free, and bioavailable levels of estradiol and testosterone compared to placebo. Unexpectedly, there was a significant reduction in the total testosterone to total estradiol ratio in the GTE group compared to the placebo group.

Data were also analyzed for reproductive hormones and SHBG at month 6 for a subset samples of 371 participants (GTE= 184; Placebo= 187). As demonstrated in **Table 17**, results did not differ between the GTE and placebo groups at baseline, month 6, month 12, and ratios of changes for this subgroup. For within treatment group

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comparisons of this subset, there were only significant increases in the levels of total, free, and bioavailable estradiol in the GTE group.

When sex hormone results were stratified by *COMT* genotype, no significant differences were observed across H/H, H/L, and L/L *COMT* genotypes at baseline, month 12, and for ratio of change in the GTE group (**Table 18**). Similar null findings were noted in the placebo group (data not shown). When within *COMT* genotype group comparisons were conducted, total estradiol was significantly increased in the H/H *COMT* genotype, and androstenedione was significantly decreased in the H/L *COMT* genotype among women taking GTE.

In exploratory analysis of baseline sex hormone concentrations, regardless of treatment group, *COMT* stratification did not results in any significant changes (**Table 19**). These findings suggest that different *COMT* genotype activities do not affect circulating sex hormone levels.

Circulating Insulin-like Growth Factors

In total, out of 1075 randomized participants, 937 women completed the study. Among those who completed the trial, 59 participants did not finish the intervention due to experiencing adverse events; however, they were included in the final data analysis to comply with the ITT principle. Circulating IGF1 and IGFBP-3 were analyzed in 462 out of the 464 GTE participants as we were not able to collect blood samples at month 12 for 2 participants. **Table 7** shows the baseline demographic and clinical characteristics of the study participants who completed the trial. Participants were mostly white, non-Hispanic, and had some college educations. Except higher intake of vitamins use in the GTE group (*P*<0.04), participants were similar in other aspects between two groups. Energy and dietary intakes were also similar between two groups at baseline, but total vegetable intake was slightly higher in the GTE versus the placebo group at the end of study (*P*<0.03; see **Table 15**).

Table 20 summarizes findings from IGF-1, IGFBP-3 and IGF-1: IGFBP-3 ratio. As mentioned earlier, endpoint analytes were analyzed for 462 of the GTE women and all participants in the placebo group at baseline, months 6 and 12. In general, no significant differences were observed between two groups at baseline, month 6, month 12, or for ratio of changes. However, when comparisons were conducted within each treatment group, there were significant reductions in the IGF-1 levels between baseline and month 12 measures in both of the GTE and placebo groups. No significant within comparison changes were seen for IGFBP-3, but IGF-1: IGFBP-3 decreased in both groups.

When results were stratified by *COMT* genotype in the GTE group, IGF-1 levels were significantly different among three *COMT* genotypes at month 6 (see **Table 21**). Post hoc test revealed that IGF-1 values at month 6 were significantly different between low and intermediate *COMT* genotype groups, with higher levels detected in the low *COMT* genotype. Within *COMT* genotype comparisons of the IGF-1 concentrations were all significant and IGF-1 diminished in all three *COMT* genotypes after 12 month.

Additionally, one-way ANOVA analysis showed that baseline IGFBP-3 concentrations were significantly different across three *COMT* genotypes ($P<0.03$) in women randomized to the GTE. Further analysis revealed that IGFBP-3 levels were significantly different between the low *COMT* and intermediate *COMT* genotype groups. Comparisons between the *COMT* genotypes at month 6, month 12, and ratio of changes, or within the *COMT* genotype comparisons of IGFBP-3 did not differ in the GTE group.

No changes were observed among *COMT* genotypes at baseline, months 6, and 12, and ratio of changes for the IGF-1: IGFBP-3 in the GTE group. However, within comparisons of *COMT* genotypes for IGF-1: IGFBP-3 were all statistically significant in the direction consistent with the reduction from baseline at month 12. Similar analyses were conducted in the placebo group but no significant differences were found among three *COMT* genotypes at any time point or for the ratio of change. (data not shown).

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In exploratory analyses of IGF hormones stratified by *COMT* genotype for all participants, baseline IGF hormones did not differ significantly across high, low, and intermediate *COMT* genotypes (see **Table 22**).

Urinary Estrogens and Estrogen Metabolites

The flow of participants through screening, enrollment, and randomization are depicted in Figure 8. In total, 1075 were randomized into the study (538 in the GTE group, and 537 in the placebo group), and 937 participants completed the trial. Fifty-nine women chose to stop taking study pills due to adverse events but completed all other study protocols. Data from these participants were analyzed according to the ITT principle. Baseline demographic and clinical characteristics of study participants are shown in **Table 7**. Other than marginally higher intake of vitamin supplements by the GTE group ($P<0.04$), participants' characteristics did not vary significantly between two groups.

When baseline characteristics were compared between two groups for all women randomized into the study, placebo women were significantly and marginally taller than those in the GTE women (see **Table 8**). Also, use of vitamin intake was sill significantly higher among the GTE participants.

The *COMT* genotype distribution among all randomized participants (n= 1,075) was as follows: G/G or high *COMT* genotype, 26.5%; A/G or intermediate *COMT* genotype, 41.8%; and A/A or low *COMT* genotype, 31.7%. The allele frequency of A was 0.526, which is similar to previously reported data in normal Caucasians [315].

There were significant positive correlations between urinary and blood levels of primary estrogens at baseline and month 12. Urinary and blood estrone concentrations were significantly correlated at baseline (r = 0.64) and month 12 (r = 0.56). Positive but weaker correlations were observed for estradiol. Urinary and blood levels of estradiol were significantly correlated at baseline (r = 0.39) and month 12 (r = 0.37). All *P* values for reported Spearman correlation coefficients were smaller than 0.0001.

As demonstrated in **Table 9**, baseline dietary intakes were identical between two groups other than the total vegetable intake which was higher among the GTE women ($P<0.03$) at month 12 (see **Table 15**).

Urinary creatinine levels were measured for all urine samples and analysis was conducted with both urinary estrogens and estrogen metabolites (EM) per mg creatinine and per day units. Since no differences were observed between two units, results are expressed as nmol per day. **Table 23** presents the main findings regarding the effects of GTE on estrogens and estrogen metabolites. Twelve month supplementation with a highly concentrated GTE resulted in significant reductions in estriol ($P<0.02$) and slightly yet statistically significant increase in 2-hydroxyestrone levels compared to the placebo group ($P<0.03$). Moreover, 16α-hydroxyestrone was decreased significantly in both GTE and placebo groups ($P<0.03$), though reduction was more pronounced in the placebo group. On the other hand, 4-methoxyestrone concentrations were both significantly lower at baseline and month 12 in GTE versus placebo participants, but the ratio of change did not differ significantly.

We defined and assessed different estrogen metabolic pathways and ratios based on parent estrogens, i.e. estrone and estradiol, and metbolites derived from hydroxylation at positions C2, C4 and C16 of the steroid rings, and methylation of the 2- and 4-pathway catechol estrogens. **Table 24** summarizes findings of selected estrogen metabolic pathways and ratios for those with the P values of either statistically significant or at borderline. Total 2-hydroxy catechols, 2-pathway: 4, 16-hydroxylation pathway, and total 2-hydroxy catechols: total estrogens and metabolites were significantly lower in the placebo group compared to the green tea ($P<0.03$; $P<0.03$; and $P<0.02$, respectively). In contrast, total 2-methyl catechols: total 2-hydroxy catechols was significantly lower in the GTE group versus the placebo ($P<0.01$).

Compared with placebo, 2-hydroxyestrone: 16α-hydroxyestrone ratio, the most studied metabolic pathway ratio of estrogens, did not change by consuming green tea catechin extract.

When primary estrogens and estrogen analytes along with their metabolic pathways and ratios were stratified by *COMT* genotype, no significant differences were observed among *COMT* genotypes in the GTE group (**Table 25**). Similar data analysis was performed for the placebo group, and results were in agreement with null findings from the green tea group (**Table 26**).

As demonstrated in **Table 27**, we further examined baseline concentrations of estrogens and estrogen metabolites, metabolic pathways and ratios in all participants according to *COMT* genotypes for the exploratory purposes. Interestingly, significant results were noted in the majority of individual or metabolic pathways or ratios related to the methylated catechols. Other than 2-methoxyestradiol, women in the high *COMT* genotype had generally significantly higher levels of methylated catechols. Those in the low *COMT* genotype had also lower concentrations of individual or metabolic pathways and ratios of methylated catechols.

Table 14. Selected baseline characteristics of randomized completers participants; ITT model (n= 937).

Variables	GTE (n= 463)	Placebo (n= 474)	p value[a]
Age, yrs	60.0 (4.9)	59.7 (5.0)	0.26
Race			0.52
- White	453 (97.8)	458 (96.6)	
- Others	9 (1.9)	14 (3.0)	
- Unknown	1 (0.2)	2 (0.4)	
Level of education			0.14
- High school or less	27 (5.8%)	31 (6.5%)	
- Some college	313 (67.6%)	287 (60.6%)	
- Postgraduate or professional degree	121 (26.1%)	152 (32.1%)	
- Unknown	2 (0.4%)	4 (0.8%)	
Family history of breast cancer[b]			
- No	332 (71.7%)	355 (74.9%)	0.22
- Yes	120 (25.9%)	114 (24.1%)	
- Unknown	11 (2.4%)	5 (1.1%)	
Age at menarche, y[c]	13.0 (1.4)	12.9 (1.4)	0.78
Parity Status			0.27
- Nulliparous	108 (23.3%)	110 (23.2%)	
- Parous	354 (76.5%)	359 (75.7%)	
- Unknown	1 (0.2%)	5 (1.1%)	
Age at first live birth, y[d,e]	27.9 (5.4)	28.6 (5.4)	0.07
Number of live birth[d, f]	2.2 (0.9)	2.1 (0.8)	0.6
Breastfeeding, total months[d,g]	13.3 (17.4)	13.5 (16.2)	0.87

Table 14, continued

Variables	GTE (n= 463)	Placebo (n= 474)	p value[a]
Type of menopause			0.18
- Natural	397 (85.8%)	409 (86.3%)	
- Surgical: bilateral oophorectomy	35 (7.6%)	45 (9.5%)	
- Surgical: ovarian status unknown	27 (5.8%)	15 (3.2%)	
- Unknown	4 (0.9%)	5 (1.1%)	
Prior hysterectomy			0.26
- No	374 (80.8)	376 (79.3)	
- Yes	88 (19.0)	93 (19.6)	
- Unknown	1 (0.2)	5 (1.0)	
Age at menopause, y[h]	49.0 (5.7)	49.3 (5.2)	0.47
Years since menopause[i]	11.1 (7.6)	10.3 (7.2)	0.13
Past use of oral contraceptives			0.85
- No	65 (14.0%)	70 (14.8%)	
- Yes	395 (85.3%)	402 (84.8%)	
- Unknown	3 (0.7%)	2 (0.4%)	
Past use of HRT			0.76
- No	270 (58.3)	278 (58.7)	
- Yes			
6-12 months ago	35 (7.6)	27 (5.7)	
1-5 years ago	93 (20.1)	93 (19.6)	
≥6 years ago	61 (13.2)	71 (15.0)	
- Unknown	4 (0.9)	5 (1.1)	
BMI, kg/m^2			0.26
<20	25 (5.4)	26 (5.5)	
20-24.9	231 (49.9)	239 (50.4)	
25-29.9	147 (31.8)	166 (35.0)	
≥30	60 (13.0)	43(9.1)	

Table 14, continued

Variables	GTE (n= 463)	Placebo (n= 474)	*p* value[a]
Smoking status			0.84
- Never	316 (68.3%)	324 (68.4%)	
- Former	145 (31.3%)	149 (31.4%)	
- Unknown	2 (0.4%)	1 (0.2%)	
Aspirin use			0.77
- No	339 (73.2)	343 (72.4)	
- Yes	124 (26.8)	131 (27.6)	
Non-aspirin NSAIDs use			0.21
- No	134 (28.9)	155 (32.7)	
- Yes	329 (71.1)	319 (67.3)	
Acetaminophen use			0.27
- No	325 (70.2)	348 (73.4)	
- Yes	138 (29.8)	126 (26.6)	

NOTE: Data presented as the means (SD) for continuous variables and as frequencies (%) for categorical variables.

Abbreviations: ITT, intention-to-treat; GTE, green tea extract; BMI, body mass index; MET, metabolic equivalent; HRT, hormone replacement therapy; NSAID, non-steroidal anti-inflammatory drug.

[a] *P* value for difference between the GTE-completers with the Placebo-completers.
[b] Only in first degree relatives.
[c] GTE (n): completers= 457; Placebo (n): completers= 460.
[d] Among parous women only.
[e] GTE (n): completers= 354; Placebo (n): completers= 359.
[f] GTE (n): completers= 352; Placebo (n): completers= 359.
[g] GTE (n): completers= 348; Placebo (n): completers= 353.
[h] GTE (n): completers= 442; Placebo (n): completers= 454.
[i] GTE (n): completers= 440; Placebo (n): completers= 454.

Table 15. Energy and nutrient intake at month 12 by treatment group (n= 931).

Nutrient	GTE (n=461)	Placebo (n=470)	P value[1]
Total energy, kcal	1370.91 (523.00)	1364.70 (468.74)	0.85
Carbohydrate, g	169.07 (67.08)	168.01 (62.05)	0.8
Protein, g	56.21 (23.60)	54.71 (20.61)	0.30
Total fat, g	52.26 (24.51)	52.19 (22.30)	0.96
Saturated fat, g	16.04 (7.39)	15.85 (6.86)	0.68
MUFA, g	20.36 (10.19)	20.50 (9.36)	0.83
PUFA, g	12.03 (6.48)	12.04 (5.94)	0.97
Omega-3 FAs, g			
EPA (20:5)	0.02 (0.03)	0.02 (0.02)	0.53
DHA (22:6)	0.05 (0.05)	0.05 (0.04)	0.56
ALA (18:3)	0.99 (0.52)	0.98 (0.45)	0.78
Trans-FA, g	2.62 (1.39)	2.53 (1.26)	0.31
Cholesterol, mg	141.94 (73.35)	140.23 (68.79)	0.71
Total dietary fiber, g	16.83 (7.95)	16.46 (6.80)	0.45
Soluble fiber, g	5.56 (2.69)	5.45 (2.25)	0.49
Caffeine, mg	336.59 (296.57)	361.54 (306.87)	0.21
Alcohol, g	5.76 (8.00)	6.36 (8.88)	0.28

Table 15, Continued

Nutrient	GTE (n=461)	Placebo (n=470)	*P* value[1]
Total red meat intake, oz[2]	1.11 (0.80)	1.05 (0.71)	0.22
Total vegetable intake, servings	3.41 (2.23)	3.12 (1.78)	0.03

Abbreviations: ALA, alpha-linolenic acid; GTE, green tea extract; EPA, Eicosapentaenoic acid; DHA, Docosahexaenoic acid; MUFA, Monounsaturated fatty acids; PUFA, Polyunsaturated fatty acids.

NOTE: Data presented as the means (SD) for continuous variables and as frequencies (%) for categorical variables.

[1] *P* value for difference between the treatment groups at month 12.

[2] Total red meat includes beef, pork, lamb, organ, franks and luncheon meats

Table 16. Geometric means (95% CI) of circulating concentrations of sex steroid hormones at baseline, month 12, and month 12/baseline ratios by treatment group (n= 937).

Sex hormone	GTE (n= 463)	Placebo (n= 474)	p value[a]
Estrone (E₁), pg/mL			
- Baseline	22.8 (21.6, 24.0)	23.6 (22.4, 24.9)	0.33
- Month 12	22.5 (21.3, 23.7)	22.4 (21.3, 23.6)	0.98
- Raito	0.99 (0.94, 1.04)	0.95 (0.90, 1.00)	0.31
- P value [b]	0.58	0.046	
Estradiol[c] (E₂), pg/mL			
- Baseline	3.4 (3.1, 3.8)	3.7 (3.3, 4.1)	0.27
- Month 12	3.7 (3.4, 4.1)	3.1 (2.8, 3.5)	0.01
- Raito	1.11 (0.99, 1.24)	0.85 (0.76, 0.95)	0.001
- P value [b]	0.07	0.004	
Free estradiol[d] (E₂), pg/mL			
- Baseline	0.051 (0.046, 0.057)	0.055 (0.050, 0.062)	0.32
- Month 12	0.056 (0.050, 0.062)	0.0047 (0.042, 0.052)	0.02
- Raito	1.10 (0.98, 1.22)	0.85 (0.76, 0.95)	0.002
- P value [b]	0.11	0.004	
Bioavailable estradiol[e] (E₂), pg/mL			
- Baseline	1.5 (1.4, 1.7)	1.7 (1.5, 1.9)	0.24
- Month 12	1.7 (1.5, 1.9)	1.4 (1.3, 1.6)	0.02
- Raito	1.12 (1.00, 1.25)	0.85 (0.76, 0.95)	0.001
- P value [b]	0.05	0.006	

Table 16, continued

Sex hormone or ratio	GTE (n= 463)	Placebo (n= 474)	p value[a]
Androstenedione, pg/mL			
- Baseline	479.8 (459.4, 501.2)	507.2 (485.9, 529.5)	0.08
- Month 12	468.8 (450.2, 488.3)	479.5 (460.6, 499.1)	0.44
- Raito	0.98 (0.94, 1.01)	0.95 (0.91, 0.98)	0.19
- P value [b]	0.19	0.001	
Testosterone[f], pg/mL			
- Baseline	151.0 (142.7, 159.7)	161.6 (152.8, 170.8)	0.09
- Month 12	158.1 (149.9, 166.9)	155.1 (147.1, 163.6)	0.62
- Raito	1.05 (1.00, 1.09)	0.96 (0.92, 1.00)	0.006
- P value [b]	0.04	0.07	
Free testosterone[g], pg/mL			
- Baseline	16.0 (15.1, 17.1)	17.1 (16.0, 18.1)	0.16
- Month 12	16.6 (15.7, 17.7)	16.4 (15.4, 17.4)	0.71
- Raito	1.04 (0.99, 1.09)	0.96 (0.92, 1.00)	0.02
- P value [b]	0.10	0.08	
Bioavailable testosterone[h], pg/mL			
- Baseline	38.6 (36.2, 41.1)	41.6 (39.1, 44.3)	0.1
- Month 12	40.8 (38.4, 43.4)	40.2 (37.8, 42.7)	0.71
- Raito	1.06 (1.01, 1.11)	0.97 (0.92, 1.01)	0.007
- P value [b]	0.02	0.14	

Table 16, continued

Sex hormone or ratio	GTE (n= 463)	Placebo (n= 474)	p value[a]
SHBG, nmol/L			
- Baseline	68.1 (65.0, 71.3)	69.1 (66.0, 72.3)	0.66
- Month 12	68.7 (65.5, 72.0)	68.7 (65.5, 72.0)	0.99
- Raito	1.01 (0.98, 1.04)	0.99 (0.97, 1.02)	0.46
- *P* value [b]	0.54	0.67	
Testosterone: Estradiol[i]			
- Baseline	44.6 (39.9, 49.9)	43.9 (39.3, 49.1)	0.85
- Month 12	42.3 (38.1, 46.9)	49.5 (44.7, 54.9)	0.03
- Raito	0.95 (0.84, 1.06)	1.13 (1.01, 1.26)	0.04
- *P* value [b]	0.36	0.04	
Androstenedione: Estrone			
- Baseline	21.1 (20.0, 22.2)	21.5 (20.4, 22.6)	0.60
- Month 12	20.9 (19.9, 21.9)	21.4 (20.4, 22.4)	0.50
- Raito	0.99 (0.94, 1.05)	0.99 (0.94, 1.05)	0.92
- *P* value [b]	0.75	0.86	

Abbreviations: CI, confidence intervals; GTE, green tea extract; SHBG, sex hormone binding globulin.

[a] *P* value for difference between GTE and placebo groups at baseline, month 12, and ratio.

[b] *P* value for difference between baseline and month 12 within GTE or placebo group based on repeated measures ANOVA.

[c] $P= 0.001$ for the interaction between treatment and time.

[d] $P= 0.002$ for the interaction between treatment and time.

[e] $P= 0.0009$ for the interaction between treatment and time.

[f] $P= 0.006$ for the interaction between treatment and time.

[g] $P= 0.02$ for the interaction between treatment and time.

[h] $P= 0.007$ for the interaction between treatment and time.

[i] $P= 0.04$ for the interaction between treatment and time.

Table 17. Geometric means (95% CI) of circulating concentrations of sex steroid hormones at baseline, months 6 and 12, and month 12/baseline ratios by treatment group (n= 371).

Sex hormone	GTE (n= 184)	Placebo (n= 187)	p value[a]
Estrone (E_1), pg/mL			
- Baseline	22.8 (21.0, 24.8)	24.2 (22.3, 26.3)	0.34
- Month 6	23.2 (21.3, 25.2)	22.6 (20.9, 24.6)	0.69
- Month 12	22.7 (20.9, 24.7)	23.3 (21.5, 25.3)	0.66
- Month 12/Baseline	1.00 (0.94, 1.06)	0.98 (0.93, 1.04)	0.70
- P value [b]	0.89	0.38	
Estradiol[c] (E_2), pg/mL			
- Baseline	3.0 (2.6, 3.6)	3.4 (2.8, 3.9)	0.37
- Month 6	2.9 (2.5, 3.4)	3.2 (2.7, 3.7)	0.41
- Month 12	3.7 (3.1, 4.3)	3.0 (2.5, 3.5)	0.06
- Month 12/Baseline	1.11 (0.97, 1.27)	0.94 (0.82, 1.07)	0.09
- P value [b]	0.04	0.18	
Free estradiol[d] (E_2), pg/mL			
- Baseline	0.04 (0.04, 0.05)	0.05 (0.04, 0.06)	0.43
- Month 6	0.04 (0.04, 0.05)	0.05 (0.04, 0.06)	0.53
- Month 12	0.05 (0.05, 0.06)	0.04 (0.04, 0.05)	0.05
- Month 12/Baseline	1.11 (0.97, 1.27)	0.94 (0.82, 1.07)	0.09
- P value [b]	0.04	0.16	

Table 17, continued

Sex hormone	GTE (n= 184)	Placebo (n= 187)	p value[a]
Bioavailable estradiol[e] (E$_2$), pg/mL			
- Baseline	1.3 (1.1, 1.6)	1.5 (1.2, 1.7)	0.42
- Month 6	1.3 (1.1, 1.6)	1.4 (1.2, 1.7)	0.52
- Month 12	1.6 (1.4, 1.9)	1.3 (1.1, 1.5)	0.06
- Month 12/Baseline	1.11 (0.97, 1.28)	0.94 (0.82, 1.08)	0.09
- P value [b]	0.03	0.23	
Androstenedione, pg/mL			
- Baseline	473.9 (443.7, 506.1)	483.7 (453.1, 516.4)	0.66
- Month 6	470.0 (439.3, 502.9)	455.8 (426.2, 487.4)	0.53
- Month 12	465.8 (438.2, 495.0)	463.9 (436.7, 492.8)	0.93
- Month 12/Baseline	0.99 (0.95, 1.03)	0.98 (0.94, 1.02)	0.68
- P value [b]	0.55	0.14	
Testosterone, pg/mL			
- Baseline	150.4 (138.2, 163.6)	152.2 (140.0, 165.5)	0.84
- Month 6	147.2 (134.4, 161.3)	140.0 (127.9, 153.2)	0.44
- Month 12	157.4 (144.6, 171.4)	143.4 (131.8, 155.9)	0.13
- Month 12/Baseline	1.02 (0.97, 1.08)	0.97 (0.92, 1.02)	0.16
- P value [b]	0.20	0.10	

Table 17, continued

Sex hormone	GTE (n= 184)	Placebo (n= 187)	p value[a]
Free testosterone, pg/mL			
- Baseline	15.3 (13.9, 16.9)	15.4 (14.0, 16.9)	0.97
- Month 6	15.5 (14.0, 17.2)	14.4 (13.0, 16.0)	0.34
- Month 12	16.2 (14.7, 17.9)	14.4 (13.1, 15.9)	0.10
- Month 12/Baseline	1.03 (0.97, 1.09)	0.97 (0.91, 1.03)	0.17
- P value [b]	0.17	0.11	
Bioavailable testosterone, pg/mL			
- Baseline	37.1 (33.7, 40.8)	37.3 (33.9, 40.9)	0.95
- Month 6	37.8 (34.0, 42.0)	35.2 (31.7, 39.1)	0.35
- Month 12	39.7 (36.0, 43.8)	35.6 (32.3, 39.3)	0.13
- Month 12/Baseline	1.03 (0.97, 1.10)	0.98 (0.92, 1.04)	0.20
- P value [b]	0.09	0.25	
SHBG, nmol/L			
- Baseline	71.6 (66.2, 77.4)	73.2 (67.7, 79.1)	0.69
- Month 6	68.3 (62.8, 74.2)	70.6 (65.0, 76.7)	0.58
- Month 12	70.6 (65.2, 76.4)	72.9 (67.4, 78.9)	0.57
- Month 12/Baseline	0.99 (0.96, 1.03)	1.00 (0.96, 1.04)	0.85
- P value [b]	0.56	0.88	

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Table 17, continued

Sex hormone or ratio	GTE (n= 184)	Placebo (n= 187)	p value[a]
Testosterone: Estradiol			
- Baseline	50.0 (41.9, 59.7)	45.5 (38.1, 54.3)	0.46
- Month 6	50.5 (42.4, 60.3)	43.8 (36.7, 52.1)	0.26
- Month 12	42.8 (36.1, 50.8)	48.6 (41.0, 57.6)	0.31
- Month 12/Baseline	0.93 (0.8, 1.07)	1.03 (0.9, 1.19)	0.28
- P value [b]	0.12	0.50	
Androstenedione: Estrone			
- Baseline	20.8 (19.1, 22.6)	20.0 (18.4, 21.8)	0.54
- Month 6	20.3 (18.6, 22.1)	20.1 (18.5, 21.9)	0.91
- Month 12	20.5 (18.9, 22.3)	19.9 (18.4, 21.6)	0.60
- Month 12/Baseline	0.99 (0.93, 1.06)	1.00 (0.93, 1.07)	0.94
- P value [b]	0.81	0.94	

Abbreviation: CI, confidence intervals; GTE, green tea extract; SHBG, sex hormone binding globulin.

[a] P value for difference between GTE and placebo groups at baseline, month 6, month 12, and ratio.

[b] P value for difference between baseline and month 12 means within GTE or placebo group based on repeated measures ANOVA.

[c] P= 0.03 for the interaction between treatment and time.

[d] P= 0.03 for the interaction between treatment and time.

[e] P= 0.03 for the interaction between treatment and time.

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Table 18. Geometric means (95% CI) circulating concentrations of sex steroid hormones at baseline, month 12, and month 12/baseline ratios by *COMT* genotype activity in green tea extract group.

Estrogen or estrogen metabolites	H/H *COMT* Genotype (n = 126)	L/L *COMT* Genotype (n= 156)	H/L *COMT* Genotype (n= 181)	*p* value[a]
Estrone (E$_1$), pg/mL				
- Baseline	23.5 (21.1, 26.0)	22.3 (20.3, 24.5)	22.8 (20.9, 24.9)	0.77
- Month 12	24.1 (21.8, 26.7)	22.5 (20.5, 24.6)	21.4 (19.6, 23.3)	0.20
- Ratio	1.03 (0.93, 1.14)	1.01 (0.92, 1.11)	0.94 (0.86, 1.02)	0.34
- *P* value [b]	0.59	0.84	0.15	
Estradiol (E$_2$), pg/mL				
- Baseline	3.3 (2.7, 4.0)	3.6 (3.0, 4.3)	3.3 (2.8, 3.9)	0.75
- Month 12	4.1 (3.4, 4.9)	3.4 (2.9, 4.1)	3.8 (3.2, 4.5)	0.43
- Ratio	1.24 (1.00, 1.54)	0.96 (0.79, 1.16)	1.15 (0.96, 1.38)	0.18
- *P* value [b]	0.05	0.66	0.12	
Androstenedione, pg/mL				
- Baseline	478.8 (438.2, 523.0)	485.5 (448.4, 525.7)	475.8 (441.9, 512.2)	0.93
- Month 12	493.1 (456.9, 532.2)	454.8 (424.7, 487.1)	464.7 (436.0, 495.2)	0.29
- Ratio	1.03 (0.96, 1.10)	0.94 (0.88, 1.00)	0.98 (0.92, 1.03)	0.13
- *P* value [b]	0.40	0.04	0.42	

Table 18, continued

Estrogen or estrogen metabolites	H/H *COMT* Genotype (n = 126)	L/L *COMT* Genotype (n= 156)	H/L *COMT* Genotype (n= 181)	*p* value[a]
Testosterone, pg/mL				
- Baseline	157.8 (140.4, 177.3)	146.8 (132.2, 163.0)	150.1 (136.1, 165.3)	0.66
- Month 12	165.0 (149.1, 182.5)	152.1 (138.9, 166.5)	158.8 (146.0, 172.8)	0.50
- Ratio	1.05 (0.96, 1.14)	1.04 (0.96, 1.12)	1.06 (0.98, 1.14)	0.93
- *P* value [b]	0.33	0.40	0.14	
SHBG, nmol/L				
- Baseline	70.2 (63.9, 77.1)	68.3 (62.8, 74.3)	66.4 (61.4, 71.8)	0.67
- Month 12	68.0 (61.8, 74.7)	70.9 (65.2, 77.2)	67.3 (62.2, 72.8)	0.65
- Ratio	0.97 (0.92, 1.02)	1.04 (0.99, 1.09)	1.01 (0.97, 1.06)	0.17
- *P* value [b]	0.24	0.13	0.57	
Testosterone: Estradiol				
- Baseline	48.1 (39.0, 59.3)	41.0 (34.0, 49.5)	45.5 (38.2, 54.2)	0.51
- Month 12	40.5 (33.3, 49.4)	44.3 (37.1, 52.9)	41.8 (35.4, 49.2)	0.79
- Ratio	0.84 (0.67, 1.05)	1.08 (0.88, 1.32)	0.92 (0.76, 1.10)	0.24
- *P* value [b]	0.13	0.45	0.36	

Table 18, continued

Estrogen or estrogen metabolites	H/H *COMT* Genotype (n = 126)	L/L *COMT* Genotype (n= 156)	H/L *COMT* Genotype (n= 181)	*p* value[a]
Androstenedione: Estrone				
- Baseline	20.4 (18.4, 22.6)	21.8 (19.9, 23.9)	20.9 (19.2, 22.8)	0.63
- Month 12	20.4 (18.6, 22.5)	20.2 (18.6, 22.0)	21.8 (20.1, 23.5)	0.42
- Ratio	1.00 (0.89, 1.12)	0.93 (0.84, 1.03)	1.04 (0.95, 1.14)	0.26
- *P* value [b]	0.98	0.15	0.40	

Abbreviations: CI, confidence interval; COMT, catechol-O-methyltransferase; SHBG, sex hormone binding globulin.

[a] *P* value for difference across H/H vs. L/L vs. H/L *COMT* Genotype groups based on one-way ANOVA test.

[b] P value for difference between baseline and month 12 means within GTE or placebo group based on repeated measures ANOVA.

Table 19. Baseline geometric means (95% CI) of circulating concentrations of sex steroid hormones by *COMT* genotype activity regardless of treatment group.

Estrogen or estrogen metabolites	H/H *COMT* Genotype (n = 248)	L/L *COMT* Genotype (n= 300)	H/L *COMT* Genotype (n= 389)	*p* value[a]
Estrone (E_1), pg/mL	23.8 (22.2, 25.5)	22.3 (20.9, 23.7)	23.6 (22.3, 25.0)	0.29
Estradiol (E_2), pg/mL	3.3 (2.8, 3.8)	3.6 (3.2, 4.1)	3.7 (3.3, 4.1)	0.41
Androstenedione, pg/mL	484.6 (456.6, 514.4)	491.7 (465.8, 519.1)	500.6 (477.4, 525.0)	0.70
Testosterone, pg/mL	164.9 (152.6, 178.1)	150.8 (140.6, 161.8)	155.1 (145.9, 165.0)	0.24
SHBG, nmol/L	69.8 (65.5, 74.3)	70.1 (66.2, 74.3)	66.7 (63.4, 70.1)	0.35
Testosterone: Estradiol	50.8 (43.6, 59.2)	41.7 (36.3, 47.9)	42.5 (37.6, 48.0)	0.12
Androstenedione: Estrone	20.4 (19.0, 21.8)	22.1 (20.8, 23.6)	21.2 (20.1, 22.4)	0.23

Abbreviations: CI, confidence interval; COMT, catechol-O-methyltransferase; SHBG, sex hormone binding globulin.

[a] *P* value for difference across H/L vs. H/L vs. L/L *COMT* Genotype groups based on one-way ANOVA test.

Table 20. Geometric means (95% CI) circulating levels of IGF-I, IGFBP-3, and IGF-I/IGFBP-3 at baseline, months 6 and 12, and month 12/month0 ratios by treatment group.

	GTE (n= 462)	Placebo (n= 473)	p value[a]
IGF-I, ng/mL			
- Baseline	87.5 (85.3, 89.8)	88.3 (86.1, 90.5)	0.64
- Month 6 [b]	83.6 (81.6, 85.7)	83.4 (81.4, 85.5)	0.92
- Month 12	83.7 (81.6, 85.8)	83.1 (81.0, 85.2)	0.68
- Raito	0.98 (0.97, 0.99)	0.97 (0.96, 0.98)	0.29
- P value [c]	<0.0001	<0.0001	
IGFBP-3, ng/mL			
- Baseline	2048.0 (2008.9, 2088.0)	2047.6 (2008.9, 2087.1)	0.99
- Month 6 [b]	2021.2 (1981.5, 2061.6)	2029.7 (1990.4, 2069.8)	0.75
- Month 12	2048.1 (2008.0, 2089.1)	2040.7 (2001.1, 2081.0)	0.80
- Raito	1.00 (0.99, 1.01)	1.00 (0.99, 1.00)	0.70
- P value [c]	0.99	0.48	
IGF-1/IGFBP-3			
- Baseline	0.043 (0.042, 0.044)	0.043 (0.042, 0.044)	0.61
- Month 6 [b]	0.041 (0.040, 0.042)	0.041 (0.040, 0.042)	0.70
- Month 12	0.041 (0.040, 0.042)	0.041 (0.040, 0.042)	0.81
- Raito	0.978 (0.969, 0.987)	0.972 (0.963, 0.981)	0.36
- P value [c]	<0.0001	<0.0001	

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Abbreviations: CI, confidence intervals; GTE, green tea extract; IGF-I, insulin-like growth factor; IGFBP-3, IGF binding protein.

[a] *P* value for difference between GTE and placebo groups at baseline, month 6, month 12, and ratio.

[b] Placebo (n)= 471.

[c] *P* value for difference between baseline and month 12 means within GTE or placebo group based on repeated measures ANOVA pairwise test.

Table 21. Geometric means (95% CI) circulating concentrations of IGF-I, IGFBP-3, and IGF-I/IGFBP-3 by *COMT* genotype activity in green tea extract group.

	H/H *COMT* Genotype (n = 126)	L/L *COMT* Genotype (n= 156)	H/L *COMT* Genotype (n= 181)	*p* value[a]
IGF-I, ng/mL				
- Baseline	87.0 (82.7, 91.5)	90.8 (86.7, 95.0)	85.2 (81.7, 88.9)	0.13
- Month 6	83.1[bc] (79.1, 87.2)	87.5[b] (83.7, 91.4)	80.8[c] (77.6, 84.2)	0.04
- Month 12	83.1 (79.1, 87.3)	86.9 (83.2, 90.9)	81.4 (78.1, 84.8)	0.10
- Raito	0.978 (0.958, 0.998)	0.979 (0.961, 0.997)	0.977 (0.961, 0.994)	0.99
- *P* value [b]	0.006	0.004	0.0009	
IGFBP-3, ng/mL				
- Baseline	2073.8[bc] (1997.0, 2153.5)	2106.8[b] (2036.3, 2179.8)	1981.7[c] (1920.3, 2045.1)	0.03
- Month 6	2041.4 (1962.7, 2123.2)	2078.0 (2005.7, 2152.9)	1960.1 (1896.8, 2025.4)	0.052
- Month 12	2052.6 (1975.0, 2133.3)	2110.6 (2038.5, 2185.3)	1993.1 (1929.9, 2058.2)	0.06
- Raito	0.995 (0.982, 1.008)	1.001 (0.989, 1.013)	1.003 (0.992, 1.014)	0.64
- *P* value [b]	0.28	0.84	0.47	

Table 21. Continued.

	H/H *COMT* Genotype (n = 126)	L/L *COMT* Genotype (n= 156)	H/L *COMT* Genotype (n= 181)	*p* value[a]
IGF-1/IGFBP-3				
- Baseline	0.042 (0.040, 0.044)	0.043 (0.041, 0.045)	0.043 (0.041, 0.045)	0.63
- Month 6	0.041 (0.039, 0.043)	0.042 (0.040, 0.044)	0.041 (0.040, 0.043)	0.52
- Month 12	0.040 (0.039, 0.042)	0.041 (0.040, 0.043)	0.041 (0.039, 0.042)	0.85
- Raito	0.983 (0.965, 1.000)	0.978 (0.962, 0.994)	0.975 (0.960, 0.989)	0.79
- *P* value [b]	0.01	0.0004	<0.0001	

Abbreviations: CI, confidence intervals; COMT, catechol-O-methyltransferase; IGF-I, insulin-like growth factor; IGFBP-3, IGF binding protein.

Means with different letters within each row indicate statistically significant differences (P<0.05), and are based on Tukey's test.

[a] *P* value for difference across H/H vs. L/L vs. H/L *COMT* genotype groups based on one-way ANOVA test.

[b] *P* value for difference between baseline and month 12 means within GTE or placebo group based on repeated measures ANOVA.

Table 22. Baseline geometric means (95% CI) circulating concentrations of IGF-I, IGFBP-3, and IGF-I/IGFBP-3 by *COMT* genotype regardless of treatment group.

	H/H *COMT* Genotype (n = 248)	L/L *COMT* Genotype (n = 300)	H/L *COMT* Genotype (n= 389)	*p* value[a]
IGF-I, ng/mL	88.4 (85.4, 91.5)	88.2 (85.4, 91.0)	87.4 (85.0, 89.8)	0.86
IGFBP-3, ng/mL	2057.2 (2003.6, 2112.2)	2075.2 (2026.0, 2125.6)	2021.1 (1979.1, 2064.0)	0.25
IGF-1/IGFBP-3	0.043 (0.042, 0.044)	0.042 (0.041, 0.044)	0.043 (0.042, 0.044)	0.67

Abbreviations: CI, confidence intervals; COMT, catechol-O-methyltransferase; IGF-I, insulin-like growth factor; IGFBP-3, IGF binding protein.

[a] *P* value for difference across H/H vs. L/L vs. H/L *COMT* genotype groups based on one-way ANOVA test.

Table 23. Geometric mean (95% CI) of urinary excretion concentrations of primary estrogens and estrogen metabolites (nmol/day) at baseline, month 12, and ratio (month 12/month 0) by treatment group.

Estrogen or estrogen metabolites	GTE (n= 463)	Placebo (n= 474)	p value[a]
Estrone (E$_1$)[b]			
- Baseline	6.39 (5.96, 6.84)	6.74 (6.29, 7.21)	0.28
- Month 12	6.25 (5.84, 6.68)	6.30 (5.90, 6.74)	0.85
- Ratio	0.98 (0.93, 1.03)	0.94 (0.89, 0.99)	0.26
- P value [c]	0.43	0.02	
Estradiol (E$_2$)			
- Baseline	1.47 (1.37, 1.59)	1.52 (1.42, 1.64)	0.53
- Month 12	1.47 (1.37, 1.58)	1.48 (1.38, 1.59)	0.94
- Ratio	1.00 (0.94, 1.07)	0.97 (0.91, 1.04)	0.53
- P value [c]	0.99	0.37	
Estriol (E$_3$)[d]			
- Baseline	6.16 (5.39, 7.04)	7.19 (6.30, 8.20)	0.11
- Month 12	5.04 (4.38, 5.79)	7.30 (6.36, 8.39)	0.0002
- Ratio	0.82 (0.71, 0.93)	1.02 (0.89, 1.16)	0.02
- P value [c]	0.003	0.81	
2-Hydroxyestrone (2OH-E$_1$)[e]			
- Baseline	5.69 (5.19, 6.24)	5.68 (5.18, 6.22)	0.98
- Month 12	5.77 (5.27, 6.32)	5.23 (4.78, 5.72)	0.13
- Ratio	1.01 (0.95, 1.08)	0.92 (0.87, 0.98)	0.03
- P value [c]	0.65	0.009	
4-Hydroxyestrone (4OH-E$_1$)[f]			
- Baseline	0.90 (0.84, 0.96)	0.89 (0.83, 0.95)	0.81
- Month 12	1.01 (0.94, 1.08)	1.00 (0.93, 1.07)	0.85
- Ratio	1.12 (1.05, 1.2)	1.12 (1.05, 1.2)	0.95
- P value [c]	0.001	0.0007	

Table 23, continued

Estrogen or estrogen metabolites	GTE (n= 463)	Placebo (n= 474)	*p* value[a]
2-Hydroxyestradiol (2OH-E$_2$)			
- Baseline	0.26 (0.23, 0.29)	0.28 (0.25, 0.32)	0.43
- Month 12	0.22 (0.2, 0.25)	0.22 (0.20, 0.25)	0.99
- Ratio	0.86 (0.75, 0.98)	0.80 (0.70, 0.91)	0.46
- *P* value [c]	0.03	0.001	
4-Hydroxyestradiol (4OH-E$_2$)			
- Baseline	0.09 (0.08, 0.1)	0.09 (0.08, 0.1)	0.83
- Month 12	0.07 (0.06, 0.08)	0.08 (0.07, 0.09)	0.16
- Ratio	0.74 (0.62, 0.87)	0.86 (0.73, 1.02)	0.18
- *P* value [c]	0.0003	0.09	
2-Methoxyestrone (2Me-E$_1$)			
- Baseline	0.39 (0.33, 0.45)	0.37 (0.32, 0.42)	0.63
- Month 12	0.31 (0.27, 0.36)	0.34 (0.30, 0.40)	0.32
- Ratio	0.80 (0.70, 0.92)	0.94 (0.82, 1.07)	0.12
- *P* value [c]	0.002	0.34	
2-Methoxyestradiol (2Me-E$_2$)			
- Baseline	0.09 (0.08, 0.1)	0.08 (0.08, 0.1)	0.92
- Month 12	0.07 (0.06, 0.08)	0.07 (0.07, 0.08)	0.37
- Ratio	0.79 (0.69, 0.91)	0.87 (0.75, 0.99)	0.39
- *P* value [c]	0.001	0.04	
4-Methoxyestrone (4Me-E$_1$)			
- Baseline	0.071 (0.06, 0.08)	0.084 (0.08, 0.09)	0.03
- Month 12	0.063 (0.06, 0.07)	0.078 (0.07, 0.09)	0.01
- Ratio	0.89 (0.78, 1.03)	0.92 (0.80, 1.05)	0.78
- *P* value [c]	0.11	0.22	

Table 23, continued

Estrogen or estrogen metabolites	GTE (n= 463)	Placebo (n= 474)	p value[a]
4-Methoxyestradiol (4Me-E$_2$)			
- Baseline	0.074 (0.07, 0.08)	0.070 (0.06, 0.08)	0.58
- Month 12	0.068 (0.06, 0.08)	0.066 (0.06, 0.07)	0.66
- Ratio	0.93 (0.81, 1.06)	0.94 (0.82, 1.07)	0.91
- P value [c]	0.28	0.34	
16α-Hydroxyestrone (16αOH-E$_1$)[g]			
- Baseline	0.54 (0.48, 0.61)	0.62 (0.55, 0.70)	0.10
- Month 12	0.51 (0.45, 0.58)	0.48 (0.42, 0.55)	0.55
- Ratio	0.94 (0.83, 1.07)	0.77 (0.68, 0.88)	0.03
- P value [c]	0.38	<0.0001	

Abbreviations: CI, confidence intervals; GTE, green tea extract;

[a] P value for difference between GTE and placebo groups at baseline, month 12, and ratio.

[b] GTE (n) = 455; Placebo (n) = 468 for baseline; GTE (n) = 462; Placebo (n) = 471 for month 12; GTE (n) = 455; Placebo (n) = 466 for ratios.

[c] P value for difference between baseline and month 12 within GTE or placebo group based on repeated measures ANOVA.

[d] P= 0.02 for the interaction between treatment and time.

[e] P= 0.03 for the interaction between treatment and time.

[f] GTE (n) = 456; Placebo (n) = 469 for baseline; GTE (n) = 462; Placebo (n) = 472 for month 12; GTE (n) = 456; Placebo (n) = 468 for ratios.

[g] P= 0.03 for the interaction between treatment and time.

Table 24. Geometric mean (95% CI) of urinary estrogen pathways or ratios at baseline, month 12, and ratio (month 12/month 0) by treatment group.

	GTE (n= 463)	Placebo (n= 474)	*p* value[a]
All estrogen metabolites: Parent estrogens			
- Baseline	2.48 (2.36, 2.61)	2.45 (2.33, 2.58)	0.76
- Month 12	2.37 (2.25, 2.50)	2.50 (2.37, 2.63)	0.17
- Ratio	0.96 (0.91, 1.00)	1.02 (0.97, 1.07)	0.07
- *P* value [b]	0.23	0.63	
2-Hydroxylation pathway			
- Baseline	7.18 (6.64, 7.78)	7.12 (6.58, 7.70)	0.87
- Month 12	7.07 (6.54, 7.65)	6.56 (6.07, 7.08)	0.18
- Ratio	0.98 (0.93, 1.04)	0.92 (0.87, 0.97)	0.08
- *P* value [b]	0.56	0.002	
Total 2-hydroxy catechols			
- Baseline	6.22 (5.71, 6.77)	6.17 (5.68, 6.71)	0.90
- Month 12	6.20 (5.69, 6.76)	5.64 (5.18, 6.14)	0.12
- Ratio	1.00 (0.94, 1.06)	0.91 (0.86, 0.97)	0.03
- *P* value [b]	0.94	0.002	
Total 2-hydroxy catechols:16-Hydroxylation pathway			
- Baseline	0.81 (0.72, 0.90)	0.69 (0.62, 0.77)	0.06
- Month 12	0.86 (0.77, 0.97)	0.66 (0.59, 0.75)	0.002
- Ratio	1.07 (0.98, 1.17)	0.96 (0.88, 1.05)	0.09
- *P* value [b]	0.13	0.36	

Table 24, continued

	GTE (n= 463)	Placebo (n= 474)	*p* value[a]
2-Pathway:4,16-Hydroxylation pathway			
- Baseline	0.70 (0.64, 0.77)	0.64 (0.59, 0.70)	0.13
- Month 12	0.72 (0.66, 0.79)	0.59 (0.54, 0.65)	0.003
- Ratio	1.03 (0.96, 1.10)	0.93 (0.87, 0.99)	0.03
- *P* value [b]	0.39	0.03	
Total 2-methyl catechols: Total 2-hydroxy catechols			
- Baseline	0.10 (0.09, 0.11)	0.09 (0.09, 0.11)	0.89
- Month 12	0.08 (0.07, 0.09)	0.09 (0.09, 0.11)	0.02
- Ratio	0.82 (0.74, 0.92)	1.00 (0.90, 1.11)	0.01
- *P* value [b]	0.01	0.98	
Methylated catechol EM: Catechol EMs			
- Baseline	0.12 (0.11, 0.13)	0.12 (0.11, 0.13)	0.77
- Month 12	0.10 (0.09, 0.11)	0.12 (0.11, 0.13)	0.03
- Ratio	0.86 (0.79, 0.94)	0.96 (0.89, 1.05)	0.058
- *P* value [b]	0.01	0.55	
Total 2-hydroxy catechols: total estrogens and metabolites			
- Baseline	0.23 (0.21, 0.24)	0.21 (0.20, 0.23)	0.17
- Month 12	0.23 (0.21, 0.24)	0.20 (0.19, 0.21)	0.008
- Ratio	1.01 (0.97, 1.05)	0.95 (0.91, 0.98)	0.02
- *P* value [b]	0.58	0.006	

Table 24, continued

	GTE (n= 463)	Placebo (n= 474)	*p* value[a]
Total 2-methyl catechols: total estrogens and metabolites			
- Baseline	0.02 (0.02, 0.02)	0.02 (0.02, 0.02)	0.32
- Month 12	0.02 (0.02, 0.02)	0.02 (0.02, 0.02)	0.45
- Ratio	0.83 (0.86, 1.05)	0.95 (0.86, 1.05)	0.08
- *P* value [b]	0.0005	0.3	

Abbreviations: CI, confidence intervals; GTE, green tea extract.

NOTE:
2-pathway catechols: 2-Hydroxyestrone and 2-Hydroxyestradiol.
4-pathway catechols: 4-Hydroxyestrone and 4-Hydroxyestradiol.
2-Hydroxylation pathway: 2-hydroxyestrone, 2-methoxyestrone, 2-hydroxyestradiol, and 2-methoxyestradiol.
4-Hydroxylation pathway: 4-hydroxyestrone, 4-methoxyestrone, 4-hydroxyestradiol, and 4-methoxyestradiol.
16-Hydroxylation pathway: 16α-hydroxyestrone and estriol.
2-pathway methylated catechols: 2-Methoxyestrone and 2-Methoxyestradiol.
4-pathway methylated catechols: 4-Methoxyestrone and 4-Methoxyestradiol.
Catechol EMs: 2-Hydroxyestrone, 2-Hydroxyestradiol, 4-Hydroxyestrone, and 4-Hydroxyestradiol.
Methylated catechol EMs: 2-Methoxyestrone, 2-Methoxyestradiol, 4-Methoxyestrone, and 4-Methoxyestradiol.
Parent estrogens: estrone and estradiol.
All estrogen metabolites: estriol as well as metabolites from 2-pathway catechols, 4-pathway catechols, 2-pathway methylated catechols and 4-pathway methylated catechols.
Total estrogens and metabolites: primary estrogens and all estrogen metabolites.
[a] *P* value for difference between GTE and placebo groups at baseline, month 12, and ratio.

[b] *P* value for difference between baseline and month 12 within GTE or placebo group based on repeated measures ANOVA.

Table 25. Geometric means (95% CI) of urinary estrogens, estrogen metabolites, and selected estrogen pathways or ratios at baseline, month 12, and ratio (month 12/month 0) by COMT genotype activity in green tea extract group.

	H/H COMT Genotype (n = 126)	L/L COMT Genotype (n= 156)	H/L COMT Genotype (n= 181)	*p* value[a]
Estrone (E$_1$)				
- Baseline	6.52 (5.67, 7.49)	6.31 (5.57, 7.15)	6.36 (5.67, 7.14)	0.91
- Month 12	6.96 (6.07, 7.98)	6.14 (5.43, 6.94)	5.88 (5.25, 6.59)	0.17
- Ratio	1.07 (0.96, 1.19)	0.97 (0.88, 1.07)	0.92 (0.84, 1.01)	0.12
- *P* value [b]	0.24	0.59	0.09	
Estradiol (E$_2$)				
- Baseline	1.56 (1.35, 1.80)	1.40 (1.23, 1.59)	1.48 (1.31, 1.67)	0.56
- Month 12	1.63 (1.40, 1.89)	1.41 (1.23, 1.61)	1.43 (1.26, 1.62)	0.29
- Ratio	1.05 (0.93, 1.18)	1.00 (0.90, 1.12)	0.97 (0.87, 1.07)	0.62
- *P* value [b]	0.47	0.97	0.51	
Estriol (E$_3$)				
- Baseline	5.65 (4.32, 7.39)	6.76 (5.31, 8.61)	6.05 (4.83, 7.57)	0.61
- Month 12	5.74 (4.22, 7.81)	5.75 (4.36, 7.57)	4.10 (3.17, 5.30)	0.13
- Ratio	1.02 (0.78, 1.32)	0.85 (0.67, 1.08)	0.68 (0.54, 0.85)	0.07
- *P* value [b]	0.90	0.18	0.0006	

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Table 25, continued

	H/H COMT Genotype (n = 126)	L/L COMT Genotype (n= 156)	H/L COMT Genotype (n= 181)	p value[a]
2-Hydroxyestrone (2OH-E$_1$)				
- Baseline	6.04 (5.03, 7.24)	5.96 (5.06, 7.02)	5.24 (4.51, 6.10)	0.40
- Month 12	6.45 (5.48, 7.61)	5.85 (5.05, 6.78)	5.28 (4.60, 6.06)	0.18
- Ratio	1.07 (0.95, 1.20)	0.98 (0.88, 1.09)	1.01 (0.91, 1.11)	0.54
- P value [b]	0.26	0.72	0.89	
4-Hydroxyestrone (4OH-E$_1$)				
- Baseline	1.00 (0.87, 1.15)	0.86 (0.76, 0.98)	0.87 (0.77, 0.97)	0.20
- Month 12	1.06 (0.93, 1.21)	1.07 (0.95, 1.20)	0.92 (0.83, 1.03)	0.13
- Ratio	1.06 (0.94, 1.21)	1.24 (1.11, 1.38)	1.06 (0.96, 1.18)	0.10
- P value [b]	0.34	0.0002	0.23	
2-Hydroxyestradiol (2OH-E$_2$)				
- Baseline	0.27 (0.21, 0.35)	0.26 (0.21, 0.33)	0.25 (0.20, 0.30)	0.82
- Month 12	0.22 (0.17, 0.29)	0.24 (0.19, 0.30)	0.21 (0.17, 0.26)	0.75
- Ratio	0.82 (0.63, 1.07)	0.90 (0.71, 1.14)	0.86 (0.68, 1.07)	0.87
- P value [b]	0.14	0.39	0.17	

Table 25, continued

	H/H COMT Genotype (n = 126)	L/L COMT Genotype (n= 156)	H/L COMT Genotype (n= 181)	p value[a]
2-Methoxyestrone (2Me-E$_1$)				
- Baseline	0.51 (0.39, 0.68)	0.35 (0.28, 0.46)	0.34 (0.27, 0.43)	0.07
- Month 12	0.34 (0.25, 0.45)	0.31 (0.24, 0.41)	0.29 (0.23, 0.36)	0.67
- Ratio	0.66 (0.51, 0.86)	0.89 (0.70, 1.12)	0.84 (0.67, 1.04)	0.22
- P value [b]	0.002	0.32	0.10	
2-Methoxyestradiol (2Me-E$_2$)				
- Baseline	0.09 (0.07, 0.11)	0.08 (0.06, 0.10)	0.09 (0.07, 0.11)	0.56
- Month 12	0.07 (0.06, 0.09)	0.07 (0.06, 0.09)	0.06 (0.05, 0.07)	0.35
- Ratio	0.84 (0.65, 1.09)	0.93 (0.74, 1.18)	0.66 (0.54, 0.83)	0.10
- P value [b]	0.18	0.55	0.0002	
4-Methoxyestrone (4Me-E$_1$)				
- Baseline	0.08 (0.06, 0.10)	0.07 (0.06, 0.08)	0.07 (0.06, 0.08)	0.54
- Month 12	0.06 (0.05, 0.08)	0.06 (0.05, 0.08)	0.06 (0.05, 0.07)	0.97
- Ratio	0.80 (0.61, 1.04)	0.93 (0.74, 1.18)	0.93 (0.75, 1.16)	0.61
- P value [b]	0.09	0.55	0.54	

Table 25, continued

	H/H COMT Genotype (n = 126)	L/L COMT Genotype (n= 156)	H/L COMT Genotype (n= 181)	*p* value[a]
4-Methoxyestradiol (4Me-E$_2$)				
- Baseline	0.07 (0.06, 0.09)	0.07 (0.06, 0.08)	0.08 (0.06, 0.09)	0.67
- Month 12	0.07 (0.05, 0.09)	0.07 (0.06, 0.09)	0.07 (0.05, 0.08)	0.88
- Ratio	0.91 (0.70, 1.19)	1.04 (0.82, 1.32)	0.85 (0.68, 1.07)	0.49
- *P* value [b]	0.50	0.75	0.16	
16α-Hydroxyestrone (16αOH-E$_1$)				
- Baseline	0.56 (0.44, 0.72)	0.56 (0.45, 0.69)	0.50 (0.41, 0.62)	0.72
- Month 12	0.58 (0.45, 0.75)	0.52 (0.42, 0.65)	0.45 (0.37, 0.56)	0.31
- Ratio	1.03 (0.81, 1.31)	0.93 (0.75, 1.15)	0.90 (0.74, 1.10)	0.67
- *P* value [b]	0.79	0.51	0.29	
Total estrogens and metabolites				
- Baseline	28.06 (25.03, 31.46)	28.23 (25.47, 31.28)	26.77 (24.34, 29.45)	0.72
- Month 12	29.06 (25.99, 32.49)	27.72 (25.07, 30.65)	25.63 (23.35, 28.13)	0.22
- Ratio	1.04 (0.94, 1.14)	0.98 (0.90, 1.07)	0.96 (0.89, 1.03)	0.44
- *P* value [b]	0.46	0.67	0.27	

Table 25, continued

	H/H COMT Genotype (n = 126)	L/L COMT Genotype (n= 156)	H/L COMT Genotype (n= 181)	*p* value[a]
Parent estrogens				
- Baseline	7.89 (6.85, 9.10)	7.48 (6.58, 8.50)	7.75 (6.89, 8.73)	0.85
- Month 12	8.74 (7.66, 9.99)	7.58 (6.73, 8.55)	7.45 (6.67, 8.32)	0.16
- Ratio	1.11 (0.98, 1.25)	1.01 (0.90, 1.14)	0.96 (0.87, 1.06)	0.20
- *P* value [b]	0.09	0.80	0.43	
All estrogen metabolites				
- Baseline	19.38 (17.30, 21.72)	19.92 (17.98, 22.07)	18.23 (16.57, 20.04)	0.44
- Month 12	19.50 (17.40, 21.85)	19.26 (17.39, 21.33)	17.39 (15.81, 19.12)	0.22
- Ratio	1.01 (0.92, 1.11)	0.97 (0.89, 1.05)	0.95 (0.88, 1.03)	0.69
- *P* value [b]	0.90	0.44	0.24	
All estrogen metabolites: Parent estrogens				
- Baseline	2.46 (2.22, 2.71)	2.66 (2.44, 2.91)	2.35 (2.16, 2.55)	0.13
- Month 12	2.23 (2.02, 2.46)	2.54 (2.32, 2.77)	2.33 (2.15, 2.53)	0.14
- Ratio	0.91 (0.82, 1.00)	0.95 (0.87, 1.04)	0.99 (0.91, 1.08)	0.39
- *P* value [b]	0.054	0.29	0.86	

Table 25, continued

	H/H COMT Genotype (n = 126)	L/L COMT Genotype (n= 156)	H/L COMT Genotype (n= 181)	p value[a]
2-Hydroxylation pathway				
- Baseline	7.59 (6.52, 8.83)	7.37 (6.43, 8.45)	6.76 (5.96, 7.68)	0.47
- Month 12	7.78 (6.73, 8.99)	7.11 (6.24, 8.09)	6.58 (5.84, 7.43)	0.22
- Ratio	1.03 (0.93, 1.13)	0.96 (0.88, 1.05)	0.97 (0.90, 1.05)	0.61
- P value [b]	0.61	0.41	0.51	
4-Hydroxylation pathway				
- Baseline	1.49 (1.30, 1.70)	1.32 (1.17, 1.49)	1.39 (1.24, 1.56)	0.46
- Month 12	1.58 (1.40, 1.78)	1.52 (1.37, 1.70)	1.39 (1.26, 1.54)	0.27
- Ratio	1.06 (0.93, 1.21)	1.15 (1.03, 1.30)	1.00 (0.90, 1.12)	0.23
- P value [b]	0.38	0.02	0.98	
16-Hydroxylation pathway				
- Baseline	7.43 (6.06, 9.11)	8.24 (6.86, 9.90)	7.48 (6.31, 8.86)	0.68
- Month 12	7.88 (6.45, 9.62)	7.90 (6.60, 9.46)	6.20 (5.24, 7.32)	0.09
- Ratio	1.06 (0.89, 1.27)	0.96 (0.82, 1.12)	0.83 (0.72, 0.96)	0.10
- P value [b]	0.52	0.60	0.01	

Table 25, continued

	H/H COMT Genotype (n = 126)	L/L COMT Genotype (n= 156)	H/L COMT Genotype (n= 181)	*p* value[a]
Catechol EM				
- Baseline	7.92 (6.87, 9.14)	7.97 (7.02, 9.06)	7.24 (6.43, 8.16)	0.48
- Month 12	8.53 (7.48, 9.74)	7.94 (7.05, 8.94)	7.16 (6.41, 7.99)	0.12
- Ratio	1.08 (0.98, 1.18)	1.00 (0.92, 1.08)	0.99 (0.91, 1.07)	0.33
- *P* value [b]	0.12	0.92	0.76	
Methylated catechol EM				
- Baseline	1.07 (0.89, 1.28)	0.84 (0.71, 0.98)	0.90 (0.77, 1.05)	0.14
- Month 12	0.82 (0.68, 0.98)	0.81 (0.69, 0.96)	0.78 (0.67, 0.91)	0.90
- Ratio	0.76 (0.65, 0.90)	0.97 (0.84, 1.13)	0.87 (0.76, 0.99)	0.10
- *P* value [b]	0.001	0.74	0.04	
2-Hydroxylation pathway: 16-Hydroxylation pathway				
- Baseline	1.02 (0.82, 1.27)	0.89 (0.73, 1.09)	0.90 (0.75, 1.09)	0.63
- Month 12	0.99 (0.79, 1.23)	0.90 (0.74, 1.09)	1.06 (0.89, 1.27)	0.48
- Ratio	0.97 (0.82, 1.14)	1.01 (0.87, 1.17)	1.17 (1.02, 1.35)	0.15
- *P* value [b]	0.69	0.94	0.02	

Table 25, continued

	H/H COMT Genotype (n = 126)	L/L COMT Genotype (n= 156)	H/L COMT Genotype (n= 181)	*p* value[a]
2-OHE1: 16α-OHE1				
- Baseline	10.71 (8.29, 13.84)	10.68 (8.48, 13.44)	10.39 (8.39, 12.87)	0.98
- Month 12	11.09 (8.51, 14.43)	11.26 (8.88, 14.28)	11.64 (9.34, 14.51)	0.96
- Ratio	1.03 (0.82, 1.31)	1.05 (0.85, 1.30)	1.12 (0.92, 1.36)	0.86
- *P* value [b]	0.77	0.62	0.25	
2-Pathway: 4, 16-Pathway				
- Baseline	0.75 (0.63, 0.88)	0.68 (0.59, 0.79)	0.69 (0.60, 0.79)	0.68
- Month 12	0.76 (0.65, 0.90)	0.68 (0.58, 0.79)	0.73 (0.64, 0.85)	0.56
- Ratio	1.02 (0.91, 1.15)	1.00 (0.90, 1.10)	1.06 (0.96, 1.17)	0.66
- *P* value [b]	0.73	0.93	0.22	
2-Hydroxylation pathway: 4-Hydroxylation pathway				
- Baseline	5.11 (4.50, 5.80)	5.58 (4.97, 6.25)	4.85 (4.37, 5.40)	0.22
- Month 12	4.94 (4.36, 5.60)	4.66 (4.17, 5.22)	4.72 (4.25, 5.24)	0.78
- Ratio	0.97 (0.85, 1.10)	0.84 (0.75, 0.94)	0.97 (0.87, 1.08)	0.12
- *P* value [b]	0.60	0.002	0.60	

Table 25, continued

Abbreviations: COMT, catechol-O-methyltransferase; CI, confidence intervals.

NOTE:
2-pathway catechols: 2-Hydroxyestrone and 2-Hydroxyestradiol.
4-pathway catechols: 4-Hydroxyestrone and 4-Hydroxyestradiol.
2-Hydroxylation pathway: 2-hydroxyestrone, 2-methoxyestrone, 2-hydroxyestradiol, and 2-methoxyestradiol.
4-Hydroxylation pathway: 4-hydroxyestrone, 4-methoxyestrone, 4-hydroxyestradiol, and 4-methoxyestradiol.
16-Hydroxylation pathway: 16α-hydroxyestrone and estriol.
2-pathway methylated catechols: 2-Methoxyestrone and 2-Methoxyestradiol.
4-pathway methylated catechols: 4-Methoxyestrone and 4-Methoxyestradiol.
Catechol EMs: 2-Hydroxyestrone, 2-Hydroxyestradiol, 4-Hydroxyestrone, and 4-Hydroxyestradiol.
Methylated catechol EMs: 2-Methoxyestrone, 2-Methoxyestradiol, 4-Methoxyestrone, and 4-Methoxyestradiol.
Parent estrogens: estrone and estradiol.
All estrogen metabolites: estriol as well as metabolites from 2-pathway catechols, 4-pathway catechols, 2-pathway methylated catechols and 4-pathway methylated catechols
Total estrogens and metabolites: primary estrogens and all estrogen metabolites.

[a] *P* value for difference across H/H vs. L/L vs. H/L COMT Genotype groups based on one-way ANOVA test.

[b] *P* value for difference between baseline and month 12 means within GTE or placebo group based on repeated measures ANOVA.

Table 26. Geometric means (95% CI) of urinary estrogens and its metabolites at baseline, month 12, and ratio (month 12/month 0) by COMT genotype activity in placebo group.

Estrogen or estrogen metabolites	H/H COMT Genotype (n = 122)	L/L COMT Genotype (n= 144)	H/L COMT Genotype (n= 208)	*p* value[a]
Estrone (E$_1$)				
- Baseline	6.62 (5.82, 7.52)	6.29 (5.59, 7.08)	7.15 (6.47, 7.88)	0.30
- Month 12	6.51 (5.77, 7.35)	5.66 (5.06, 6.33)	6.67 (6.08, 7.31)	0.08
- Ratio	0.99 (0.89, 1.09)	0.90 (0.82, 0.99)	0.93 (0.86, 1.01)	0.43
- *P* value [b]	0.76	0.03	0.08	
Estradiol (E$_2$)				
- Baseline	1.48 (1.28, 1.72)	1.38 (1.20, 1.58)	1.66 (1.49, 1.86)	0.1
- Month 12	1.56 (1.37, 1.78)	1.30 (1.15, 1.47)	1.57 (1.42, 1.73)	0.04
- Ratio	1.05 (0.92, 1.21)	0.94 (0.83, 1.08)	0.94 (0.85, 1.04)	0.37
- *P* value [b]	0.44	0.36	0.25	
Estriol (E$_3$)				
- Baseline	7.71 (6.03, 9.88)	6.94 (5.53, 8.72)	7.07 (5.85, 8.54)	0.80
- Month 12	8.50 (6.78, 10.65)	7.67 (6.23, 9.45)	6.46 (5.43, 7.68)	0.14
- Ratio	1.10 (0.88, 1.38)	1.11 (0.90, 1.36)	0.91 (0.75, 1.11)	0.36
- *P* value [b]	0.45	0.40	0.36	

Table 26, continued

Estrogen or estrogen metabolites	H/H COMT Genotype (n = 122)	L/L COMT Genotype (n= 144)	H/L COMT Genotype (n= 208)	p value[a]
2-Hydroxyestrone (2OH-E$_1$)				
- Baseline	5.82 (4.88, 6.93)	5.21 (4.43, 6.11)	5.95 (5.20, 6.80)	0.44
- Month 12	5.65 (4.69, 6.80)	4.54 (3.83, 5.38)	5.51 (4.78, 6.35)	0.15
- Ratio	0.97 (0.86, 1.10)	0.87 (0.78, 0.98)	0.93 (0.84, 1.02)	0.46
- P value [b]	0.65	0.02	0.13	
4-Hydroxyestrone (4OH-E$_1$)				
- Baseline	0.94 (0.82, 1.07)	0.91 (0.80, 1.03)	0.84 (0.76, 0.94)	0.42
- Month 12	1.10 (0.95, 1.27)	0.90 (0.79, 1.03)	1.01 (0.90, 1.12)	0.14
- Ratio	1.18 (1.02, 1.36)	0.99 (0.87, 1.13)	1.19 (1.07, 1.32)	0.07
- P value [b]	0.02	0.95	0.001	
2-Hydroxyestradiol (2OH-E$_2$)				
- Baseline	0.26 (0.21, 0.34)	0.29 (0.23, 0.36)	0.28 (0.23, 0.34)	0.87
- Month 12	0.21 (0.16, 0.27)	0.22 (0.18, 0.28)	0.23 (0.19, 0.28)	0.75
- Ratio	0.78 (0.61, 1.00)	0.77 (0.61, 0.96)	0.84 (0.69, 1.01)	0.82
- P value [b]	0.054	0.02	0.07	

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Table 26, continued

Estrogen or estrogen metabolites	H/H COMT Genotype (n = 122)	L/L COMT Genotype (n= 144)	H/L COMT Genotype (n= 208)	p value[a]
4-Hydroxyestradiol (4OH-E$_2$)				
- Baseline	0.09 (0.07, 0.12)	0.08 (0.06, 0.10)	0.09 (0.08, 0.12)	0.50
- Month 12	0.06 (0.05, 0.08)	0.08 (0.06, 0.10)	0.09 (0.07, 0.11)	0.17
- Ratio	0.67 (0.50, 0.90)	0.98 (0.74, 1.29)	0.92 (0.72, 1.18)	0.18
- P value [b]	0.01	0.88	0.53	
2-Methoxyestrone (2Me-E$_1$)				
- Baseline	0.40 (0.30, 0.53)	0.29 (0.22, 0.37)	0.41 (0.33, 0.51)	0.09
- Month 12	0.45 (0.34, 0.59)	0.25 (0.20, 0.32)	0.37 (0.30, 0.45)	0.01
- Ratio	1.12 (0.85, 1.49)	0.87 (0.67, 1.13)	0.88 (0.71, 1.09)	0.33
- P value [b]	0.41	0.29	0.25	
2-Methoxyestradiol (2Me-E$_2$)				
- Baseline	0.07 (0.06, 0.09)	0.07 (0.06, 0.09)	0.10 (0.09, 0.12)	0.02
- Month 12	0.07 (0.05, 0.08)	0.07 (0.05, 0.08)	0.08 (0.07, 0.10)	0.20
- Ratio	0.91 (0.69, 1.19)	0.93 (0.72, 1.19)	0.80 (0.65, 0.99)	0.63
- P value [b]	0.51	0.56	0.04	

Table 26, continued

Estrogen or estrogen metabolites	H/H COMT Genotype (n = 122)	L/L COMT Genotype (n= 144)	H/L COMT Genotype (n= 208)	*p* value[a]
4-Methoxyestrone (4Me-E$_1$)				
- Baseline	0.09 (0.07, 0.11)	0.08 (0.06, 0.09)	0.09 (0.07, 0.10)	0.59
- Month 12	0.09 (0.07, 0.11)	0.07 (0.06, 0.09)	0.08 (0.07, 0.09)	0.33
- Ratio	1.01 (0.78, 1.31)	0.91 (0.72, 1.15)	0.87 (0.71, 1.08)	0.69
- *P* value [b]	0.92	0.45	0.21	
4-Methoxyestradiol (4Me-E$_2$)				
- Baseline	0.07 (0.06, 0.09)	0.06 (0.05, 0.08)	0.07 (0.06, 0.09)	0.40
- Month 12	0.07 (0.05, 0.08)	0.06 (0.05, 0.08)	0.07 (0.06, 0.08)	0.95
- Ratio	0.92 (0.72, 1.18)	1.03 (0.82, 1.29)	0.89 (0.73, 1.08)	0.64
- *P* value [b]	0.53	0.82	0.24	
16α-Hydroxyestrone (16αOH-E$_1$)				
- Baseline	0.61 (0.48, 0.77)	0.73 (0.59, 0.91)	0.56 (0.47, 0.67)	0.16
- Month 12	0.51 (0.40, 0.66)	0.50 (0.40, 0.63)	0.45 (0.37, 0.54)	0.65
- Ratio	0.84 (0.66, 1.07)	0.69 (0.55, 0.86)	0.80 (0.66, 0.98)	0.47
- *P* value [b]	0.18	0.002	0.03	

Abbreviations: COMT, catechol-O-methyltransferase; CI, confidence intervals.

[a] *P* value for difference across H/H vs. L/L vs. H/L COMT Genotype groups based on one-way ANOVA test.

[b] *P* value for difference between baseline and month 12 means within GTE or placebo group based on repeated measures ANOVA.

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Table 27. Baseline geometric means (95% CI) of urinary estrogens and estrogen metabolites (nmol/day), estrogen pathways and ratios by different COMT genotypes in all participants (n= 937)*

Estrogen or estrogen metabolites	H/H COMT Genotype (n = 248)	L/L COMT Genotype (n = 300)	H/L COMT Genotype (n= 389)	p value[**]
Estrone (E$_1$)	6.61 (6.01, 7.26)	6.31 (5.79, 6.87)	6.75 (6.27, 7.28)	0.50
Estradiol (E$_2$)	1.52 (1.37, 1.69)	1.39 (1.27, 1.53)	1.58 (1.45, 1.71)	0.14
Estriol (E$_3$)	6.59 (5.49, 7.90)	6.85 (5.80, 8.08)	6.57 (5.68, 7.60)	0.93
2-Hydroxyestrone (2OH-E$_1$)	5.93 (5.23, 6.72)	5.59 (4.98, 6.26)	5.61 (5.07, 6.20)	0.75
4-Hydroxyestrone (4OH-E$_1$)	0.97 (0.88, 1.07)	0.88 (0.80, 0.96)	0.85 (0.79, 0.92)	0.12
2-Hydroxyestradiol (2OH-E$_2$)	0.27 (0.23, 0.32)	0.28 (0.24, 0.32)	0.26 (0.23, 0.30)	0.90
4-Hydroxyestradiol (4OH-E$_2$)	0.09 (0.07, 0.10)	0.09 (0.07, 0.11)	0.09 (0.08, 0.11)	0.85
2-Methoxyestrone (2Me-E$_1$)	0.45[b] (0.37, 0.55)	0.32[c] (0.27, 0.38)	0.38[bc] (0.32, 0.44)	0.046
2-Methoxyestradiol (2Me-E$_2$)	0.080[bc] (0.07, 0.09)	0.075[b] (0.06, 0.09)	0.097[c] (0.09, 0.11)	0.03

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Table 27, continued

Estrogen or estrogen metabolites	H/H COMT Genotype (n = 248)	L/L COMT Genotype (n = 300)	H/L COMT Genotype (n= 389)	p value[**]
4-Methoxyestrone (4Me-E$_1$)	0.08 (0.07, 0.10)	0.07 (0.06, 0.08)	0.08 (0.07, 0.09)	0.53
4-Methoxyestradiol (4Me-E$_2$)	0.07 (0.06, 0.09)	0.07 (0.06, 0.08)	0.08 (0.07, 0.09)	0.30
16α-Hydroxyestrone (16αOH-E$_1$)	0.59 (0.50, 0.69)	0.64 (0.55, 0.74)	0.53 (0.47, 0.61)	0.23
Methylated catechol EM	1.01[b] (0.89, 1.15)	0.80[c] (0.71, 0.89)	0.98[b] (0.89, 1.09)	0.008
Total 2-methyl catechols	0.67[b] (0.57, 0.78)	0.49[c] (0.43, 0.57)	0.63[b] (0.55, 0.71)	0.01
Total 2-methyl catechols: total estrogens and metabolites	0.023[b] (0.02, 0.03)	0.018[c] (0.02, 0.02)	0.022[b] (0.02, 0.02)	0.004
Total 2-methyl catechols: Total 2-hydroxy catechols	0.10[b] (0.09, 0.12)	0.08[c] (0.07, 0.09)	0.10[b] (0.09, 0.11)	0.01
Methylated catechol EM: Catechols EM	0.13[b] (0.12, 0.15)	0.10[c] (0.09, 0.12)	0.13[b] (0.12, 0.14)	0.003

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Table 27, continued

Estrogen or estrogen metabolites	H/H COMT Genotype (n = 248)	L/L COMT Genotype (n = 300)	H/L COMT Genotype (n= 389)	*p* value[**]
All estrogen metabolites: Parent estrogens	2.46[bc] (2.29, 2.64)	2.65[b] (2.48, 2.83)	2.34[c] (2.21, 2.48)	0.02

Abbreviations: CI, confidence intervals; COMT, catechol-O-methyltransferase.

* Means with different letters within each row indicate statistically significant differences (*P*<0.05) based on Tukey's test.

** *P* value for difference across H/H vs. L/L vs. H/L COMT genotype groups based on one-way ANOVA test.

NOTE:
2-pathway catechols: 2-Hydroxyestrone and 2-Hydroxyestradiol.
2-pathway methylated catechols: 2-Methoxyestrone and 2-Methoxyestradiol.
Catechol EMs: 2-Hydroxyestrone, 2-Hydroxyestradiol, 4-Hydroxyestrone, and 4-Hydroxyestradiol.
Methylated catechol EMs: 2-Methoxyestrone, 2-Methoxyestradiol, 4-Methoxyestrone, and 4-Methoxyestradiol.
Parent estrogens: estrone and estradiol.
All estrogen metabolites: estriol as well as metabolites from 2-pathway catechols, 4-pathway catechols, 2-pathway methylated catechols and 4-pathway methylated catechols
Total estrogens and metabolites: primary estrogens and all estrogen metabolites.

Chapter 4: MGTT- Discussion

Circulating Reproductive Hormones, SHBG, and IGF Axis Proteins

Higher incidence of breast cancer among Western countries than the Asian populations has been partly explained by the differences in lifestyle factors and environmental exposures. According to the GLOBOCAN 2012 report [322] , incidence of breast cancer (age-standardized rate) has been more than threefold higher in the Northern America and Western Europe compared with the Eastern Asia where green tea intake is very common.

Previous epidemiological studies have provided conflicting evidence regarding the role of green tea intake in prevention of breast cancer. Cohort studies [70-72, 74] have primarily reported null findings, whereas case-control studies [66-69, 73] have suggested a protective role for consumption of green tea against risk of breast cancer. There are currently very limited number of human intervention studies exploring the effect of green tea on risk of breast cancer.

We conducted a randomized double-blind placebo-controlled trial in 1,075 healthy postmenopausal women at high risk of breast cancer. We assessed the effects of a highly concentrated green tea catechin extract with EGCG doses equal to 843 mg daily for 12-month on circulating sex hormones and IGF axis proteins.

The results of this human intervention study indicated that green tea intake for relatively long period of time does not reduce the circulating levels of reproductive hormones as well as IGF-1 and IGFBP-3 compared with placebo. Surprisingly, we observed moderate but significant increases in the total, free, and bioavailable fractions of estradiol and testosterone in the green tea extract group with corresponding small reductions in the placebo group. In addition, testosterone: estradiol (T/E2) ratio was significantly higher in the placebo compared to the modest decrease in the green tea group. As discussed in more detail in the result section, we also observed several significant reductions in the trial endpoints within the placebo group and increases within the green tea group.

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Previous observational studies, mostly in Asian populations, have shown that drinking green tea may influence circulating sex hormones. Nagata *et al* [97] reported that after adjustment for age and BMI, green tea intake was inversely correlated with estradiol on day 11 of menstrual cycle in 50 premenopausal Japanese women. In addition, among healthy postmenopausal Chinese women, regular green tea drinkers had 13% lower levels of plasma estrone compared to irregular or non-green tea drinkers [80]. Circulating estradiol and androstenedione concentrations were also lower in the regular green tea drinkers, although the differences were not significant. More recently [100], it has been demonstrated that among premenopausal Japanese-American women daily green tea intake was associated with significantly lower urinary luteal levels of 16-pathway estrogen metabolites and total estrogens and estrogen metabolites. In postmenopausal women of the same study, green tea intake was linked with smaller urinary levels of parent estrogens and estrone.

Few prior epidemiological studies have examined the association of green tea with circulating IGF-1 and IGFBP-3. Maruyama *et al* [137] reported direct association between high intake of green tea and IGF-1 and no association with IGFBP-3 in Japanese women. In another study, green tea was unrelated to blood levels of IGF-1 and IGFBP-3 among Asian-American women [65].

Consistent with the findings from our clinical trial, two other shorter and smaller intervention studies did not observe significant reductions in sex hormones and IGF axis proteins following consumption of green tea vs. placebo. Wu *et al* [88] have demonstrated that supplementation with 400 mg and 800 mg of EGCG for two month in the form of Polyphenon E (PPE) capsules did not lower serum levels of estrone, estradiol, testosterone, androstenedione, and SHBG compared to those who took placebo. Interestingly, change in SHBG levels between the two study EGCG doses was significant ($P= 0.008$) with the opposite directions (reduction in the 400 mg EGCG and increase in the 800 mg EGCG group). Within group comparisons of this study only revealed a significant reduction in SHBG levels in the PPE group taking 400 mg of EGCG ($P= 0.002$) while we observed no changes in the serum levels of SHBG for either between or

within comparisons. Although not statistically significant, there was a borderline decrease in the blood levels of estradiol in the placebo (P= 0.084) in the Wu's study. This direction of change is in agreement with the decrease of estradiol in the placebo group of our trial.

The same patterns of null results were observed for both IGF-1 and IGFBP-3, except there was a borderline significant reduction in blood levels of IGFBP-3 between the placebo and both PPE groups (P= 0.078). Furthermore, there were borderline significant reduction in IGFBP-3 levels within each PPE group (P= 0.068 for 400 mg EGCG, and P= 0.053 for 800 mg EGCG regimen). In contrast, our participants in both of GTE and placebo groups experienced a small yet statistically different reduction in IGF-1 levels and IGF1:IGFBP3 ratio. However, consistent with the Wu's study the difference between the study groups did not reach statistical significance level.

There are some considerable differences between the MGTT and the Wu's study in terms of design, population, assay methods, and so forth. First, study population in Wu's study were healthy overweight women in which white participants were in the minority while we completed the study for women who were predominantly white and at high risk of breast cancer, and had BMI within the normal range. Second, the MGTT was longer in duration (one year versus 2 month) and took advantage of a novel liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay, whereas the former study used conventional radioimmunoassay method for the measurements of sex hormones. According to Houghton *et al[323]*, levels of IGF proteins are higher in serum than plasma, and this may explain lower levels of these hormones in our study. Lastly, *COMT* genotyping was not performed in the Wu's trial and IGF1:IGFBP3 ratio was not reported either.

Daily PPE intake with different doses of 800 mg, 1200 mg, and 1600 mg EGCG compared to placebo in participants with a history of breast cancer for six month did not result in reduction of sex hormones or IGF axis proteins [89]. These findings are similar to our study results. Participant in this study were 40 overweight, mostly white postmenopausal women and 10-170 months post diagnosis of an estrogen and

progesterone receptor negative breast carcinoma. As a secondary endpoint analysis, serum sex hormones including estradiol and testosterone were quantified by radioimmunoassay, and serum SHBG along with IGF-1 and IGFBP-3 were measured by enzyme immunoassay. IGF-1:IGFBP3 ratio was correspondingly calculated as well. Following six month of the PPE supplementation, there were no significant differences in any of the measured hormones between the PPE (combined) and placebo groups. With regard to within group comparisons, there were significant reductions in estradiol and SHBG levels, and increase in IGFBP-3 concentrations within the PPE group. Consistent with our study, there were decrease in estradiol and testosterone levels in the placebo group, although these reductions were not significant. It should be noted that this study was originally designed as a phase IB RCT to identify the maximum tolerable dose of PPE, and investigating the effects of green tea extract on biomarkers of breast cancer risk was its secondary objective. This study was not well powered; the sample size was very small; and patients were those with a history of breast carcinoma, therefore, its findings should be interpreted with caution in the context of disease free or high risk populations.

By turning into menopause stage, biosynthesis of estrogens shifts dominantly from ovaries to peripheral tissues such as adipose and breast [159, 324]. Aromatase enzyme catalyzes the conversion of androgens into estrogens by which testosterone is converted to estradiol, and androstenedione is turned into estrone. In postmenopausal women, the majority of estrogens are produced from their corresponding androgens by the action of aromatase. As discussed earlier, animal studies [92, 93] have suggested that green tea catechin constituents, in particular, EGCG inhibits the activity of aromatase. Thus, it was intriguing to speculate that EGCG intake may modulate the pathways of aromatization by which less testosterone and androstenedione will be converted to estradiol and estrone, respectively. We correspondingly calculated the ratios of T/E2, and androstenedione: estrone (A/E1). In contrast to what was expected, these ratios slightly decreased in the GTE group, and the T/E2 ratio significantly increased in the placebo group ($P = 0.04$). Similar patterns were seen in the Wu's study in which the T/E2 ratio

increased in the placebo group and decreased in the 400 mg EGCG as the PPE group, although none of these changes were statistically significant.

Placebo capsule composition in this study was maltodextrin and cellulose as bulk agent and magnesium stearate as flow agent. This composition has been widely used as placebo in the previous RCTs including the two clinical trials conducted on this subject. To the best of our knowledge, pharmaceutical excipients of our placebo capsules have no effects on urinary estrogens, and circulating sex hormones and IGF axis proteins.

Polymorphisms in the genes that encode enzymes involved in the metabolism of estrogens, catechins, and folate have been proposed to modify the effects of green tea on risk of breast cancer. One of these genes is *COMT*. Couple of decades ago, it was shown that polymorphism in the *COMT* gene results in a low activity variant of *COMT*, which has 3-4 fold lower enzymatic activity than its wild-type variant [19, 325]. It has been reported that Asian-American women tea drinkers with at least one low activity allele of *COMT* gene had lower risk of breast cancer compared to non-tea drinkers [22]. The rationale for this effect modification was the slower metabolism of catechins and subsequently longer exposure to the green tea catechins in those with the low activity *COMT* alleles. Neither Wu nor Crew's clinical trials examined the modifying effect of *COMT* genotype on the studied breast cancer risk biomarkers. We stratified our findings by *COMT* genotype, but did not find any modifying effects of this gene. Similar null results were obtained for the IGF-1 and IGFBP-3 measures. Along the same lines, baselines sex hormones and IGF axis proteins, regardless of treatment group, were not affected by *COMT* genotype activity. These data are in accordance with our findings in the urinary primary estrogens in which urinary levels of estrone and estradiol were not altered by different *COMT* genotype activity.

As suggested by a few studies [69, 73], polymorphisms in the aromatase (*CYP19A1*), *ACE*, methylenetetrahydrofolate reductase (*MTHFR*)/thymidylate synthase (*TYMS*) genes, or dietary folate intake level may modify the effect of green tea on breast cancer. We did not genotype for SNPs in the *CYP19A1*, *MTHFR*, and *TYMS* genes, but

since folate intake was similar between the GTE and placebo groups at baseline we do not anticipate dietary folate intake influences our findings.

Study participants were highly compliant to the intervention protocol, and on average took 96.5% of the prescribed doses. We also measured urinary catechins in a random 10% of the participants, and as expected, levels were significantly different between two study groups. Compared to the baseline values, urinary and plasma catechins were also significantly increased following 12 month supplementation with the GTE indicating a large boost in the systemic exposure to catechins (data not shown in this dissertation). This is of great importance because tea polyphenols have commonly poor systemic bioavailability.

Strengths of our study include large sample size, double-blind, randomized controlled design, low rate of dropout, and rather long intervention. Participants exhibited excellent compliance with dietary intervention and following study guidelines. We employed the gold standard method of LC/MS/MS for analysis of circulating hormones, and were also well powered to detect a statistically meaningful difference in estrone and IGF-1 concentrations. This study has also some limitations. We were not successful in recruitment of racial and ethnic minorities. Moreover, sex hormone measurements were based on single sample collection, and we had close to 35 % non-detectable values for serum estradiol. Additionally, we did not collect data regarding past exposure to tea. Finally, we used one isolate dose of green tea extract, so we cannot rule out the possibility that green tea beverage may show somewhat different responses.

In conclusion, the MGTT is the largest and longest intervention study conducted to date investigating the effects of high dose of green tea catechin extract on biomarkers of breast cancer risk. Findings of this clinical trial indicate that potential protective effects of green tea on breast cancer are not mediated through circulating sex hormones and IGF axis proteins. Furthermore, our results suggest that green tea intake may slightly increase circulating levels of estradiol and testosterone in healthy postmenopausal women. These findings may have some applications in the management of menopause symptoms and worth further investigations. Finally, we found no evidence that genetic polymorphisms

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in the *COMT* gene modifies the effect of green tea on sex hormones and IGF axis proteins.

Urinary Estrogens and Estrogen Metabolites

Consistent results from observation studies strongly support the notion that estrogens play an important role in the pathogenesis of breast cancer [316]. Green tea intake has been suggested as a chemopreventive agent by putative mechanisms such as modulation of estrogen metabolism. The findings from this randomized controlled trial in healthy postmenopausal women at high risk of breast cancer showed that 12 month supplementation with a highly concentrated green tea extract resulted in significant reduction in the urinary concentrations of estriol compared to increased levels of estriol in placebo. The urinary levels of 2-hydroxyestrone were almost unchanged in the GTE group, but decreased in the placebo. In addition, there was also less of a reduction in the urinary levels of 16α-hydroxyestrone in the GTE versus placebo group. Of note was the statistically significant difference in the changes between two groups for estriol, 2-hydroxyestrone as well as 16α-hydroxyestrone. Other than 4-hydroxyestrone, the majority of urinary estrogens or estrogen metabolites demonstrated a tendency towards reduction in both of the GTE and placebo groups while the magnitude of change was mixed in two groups.

Very few studies have examined the association of green tea intake with urinary estrogen profiles in women. In the only observational study carried out to date, Fuhrman *et al* [100] reported significantly lower urinary levels of parent estrogens (sum of estrone and estradiol) and estrone in 72 healthy postmenopausal Japanese-American women drinking green tea 7 times or more weekly versus those drinking once or less per week. These statistically significant findings were not observed in our study, where we only noticed non-significant reductions of estrone levels in both treatment groups, and reduction in parent estrogens only in the placebo group. In contrast to the significant reduction of urinary estriol levels in the GTE group compared to the placebo in our study, concentration of estriol was non-significantly higher in women drinking tea daily compared to those drinking less than once per week in the Fuhrman's study. With regard

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to different estrogen metabolic pathways and ratios, findings of these two studies were not in agreement either. Parent estrogens: estrogen metabolites ratio was significantly lower while 4-pathway methylated catechols: 4-pathway catechol ratio was significantly higher in daily green tea drinkers versus those drinking <1 time/week in the Fuhrman's study. However, we found opposite results in which none of these two metabolic ratios were statistically significant. It should be noted that opposite to the abovementioned cross-sectional study which had adjusted for potential confounding factors, our results are completely unadjusted. This is primarily due to the inherent design of our study as a randomized controlled trial and the fact that a wide range of breast cancer risk factors were equally distributed between two groups at baseline. Interestingly, the results of Fuhrman's study differed by menopausal status in which 16-hydroxylation pathway and its individual estrogen metabolites including 16α-hydroxyestrone, estriol, 16-ketoestradiol, 16-epiestriol, and 17-epiestriol were all significantly lower in premenopausal women consuming higher amounts of green tea. These findings are in sharp contrast to our results of lower levels of estriol in GTE versus placebo. Nonetheless, it is important to mention that direction or magnitude of the results of studies looking into the urinary or blood levels of estrogens are not unanimously consistent between pre- and postmenopausal populations. This can be partly explained by different concentrations or sources of hormones, and higher intra- and interindividual variabilities of hormones due to menstrual cycle in premenopausal women.

Discrepancies in the results of these two studies can be attributed to several factors such as different study design, population, and sample size; residual confounding, and different exposure assessment with recall bias inherent in the case-control studies. Additionally, we used a highly concentrated green tea extract with approximately 52% of the dietary regimen weight as EGCG; however, green tea beverage may contain other ingredients responsible for the observed effects. Of note is the difference in concentrations of urinary estrogens and estrogen metabolites among various ethnic populations. Our study along with two other ones [317, 318] that were primarily conducted among non-Hispanic white women show generally higher levels of urinary

estrogens than the concentrations observed in Japanese-American women of the Fuhrman's study. It is intriguing to speculate that lower incidence rate of breast cancer in Asian women is partly attributed to the lower levels of urinary estrogens. Variations in urinary estrogen profiles between white and Asian populations could be due to several possible reasons including differences in diet, lifestyle, environment, or genetic factors, but the extent of the contribution of these factors remains to be fully elucidated. Another potential avenue for future research is to perform studies with similar designs and populations but different doses of catechins than our trial's supplement or even other polyphenols or ingredients which normally exist in the green tea beverage.

There are a few observational studies that have investigated the association of green tea intake with circulating primary estrogens. Although circulating and urinary estrogens in pre- and postmenopausal women may show different pattern of results in response to different diets and lifestyle exposures, and extrapolating their results can be challenging, they will be briefly discussed here. Nagata *et al* [97] reported inverse correlation between follicular estradiol levels and green tea intake in a cross-sectional studies of 50 healthy college premenopausal Japanese women. Luteal serum levels of estradiol was not correlated with green tea intake in this study and circulating estrone was not measured. In a larger cross-sectional study by Wu *et al* [80], regular compared to non- or irregular green intake was associated with statistically significant lower concentrations of plasma estrone in postmenopausal subjects. A similar pattern of results, yet statistically borderline, was observed for plasma estradiol. Participants were 130 postmenopausal women taking part in the Singapore Chinese Health Study, which is a population-based prospective study of diet and cancer risk. In addition to these two studies, two human intervention studies have examined the effects of green tea intake, with high doses of EGCG regimen, on circulating estrogens. In a randomized controlled trial of healthy postmenopausal women mostly Hispanic American (n=103) [88], different doses of EGCG in amounts equal to 400 mg EGCG and 800 mg EGCG per day were consumed for two months. Study active treatment capsules were Polyphenon E (PPE), which is a highly pure green tea extract with catechin contents similar to our

trial's GTE capsules. Participants in the control group also took placebo pills daily for two months. Circulating sex hormones including estrone, estradiol, testosterone, and androstenedione were measured in fasting blood with radioimmunoassay. Results from this study did not show a significant reduction in any of the hormones following two month supplementation with two different doses of EGCG. Moreover, when changes in hormone levels were compared between two doses or with the placebo group, null results persisted. Crew *et al* [89] have investigated the effects of daily doses of EGCG in amounts up to 1600 mg for 6 month in 40 women with a history of stage I-III hormone receptor-negative breast cancer. Of the 40 women who were randomized, 34 completed the study (26 in the active treatment group of the PPE, and 8 in the placebo group) and their data was used for the sex hormone analyses. Among estrogens, only estradiol was measured, and that was quantified with the radioimmunoassay. Within comparison results showed significant reduction in estradiol level in the PPE group, however, when it was compared to the placebo, this change was not significant. Circulating estrogen results of the intervention studies are consistent with our null findings of the urinary estrone and estradiol, but extrapolation of the circulating hormone results to the urinary estrogens should be done with caution.

Catechol-*O*-methyltransferase (COMT) enzyme is involved in both estrogens and tea catechin metabolism. *COMT* genotype appears to have opposite effects on risk of breast cancer with regard to estrogens and tea catechins. Methylation of catechol estrogens is considered as a detoxification pathway because it decreases circulating levels of catechol estrogens and as a result less quinones and ROS are generated. In addition, it has been suggested that 2-methoxyestradiol possess anticancer activity [319, 320]. By contrast, earlier studies have shown that the variant isoform of *COMT* genotype with the lower activity may metabolize catechins slower and modify the effect of green tea intake on breast cancer risk. In a case-control study [22] of Asian-American women including 589 breast cancer cases, it was reported that tea drinker women with at least one low activity allele of *COMT* gene (i.e. G/A or A/A alleles) had 52 percent lower risk of breast cancer compared with non-tea drinkers (adjusted OR= 0.48; 95% CI, 0.29- 0.77). On the

other hand, women with the wild-type *COMT* genotype (G/G) did not show lower risk of breast cancer when tea drinkers were compared to non-tea drinkers (adjusted OR= 1.02; 95% CI, 0.66- 1.60). Investigator from the Shanghai cohort study have also demonstrated that participants with the homozygous low activity *COMT* genotype (A/A) have significantly lower urinary concentrations of individual or metabolites of tea catechins compared with those with at least one high activity allele of *COMT* gene (G/A or G/G) [23]. In our study, C*OMT* genotype did not modify the effects of green tea on estrogen metabolism. This finding is in agreement with the result of a cross-sectional study conducted among postmenopausal Chinese women in Singapore [80]. *COMT* genotype effect on estrogens or tea catechin metabolism was not examined in other epidemiological [97, 100] or intervention studies conducted so far [88, 89]. Furthermore, the most recent case-control study from Japan showed that polymorphisms in the *COMT* gene was unrelated to the risk of breast cancer in women drinking less than 120 ml of green tea daily relative to those who drank more than 600 ml daily [69].

In exploratory analyses, we examined the effect of *COMT* genotype on baseline estrogen metabolism in all participants regardless of their treatment assignments. As discussed previously here, and given the role of COMT enzyme on methylation of catechol estrogens, it was anticipated that COMT activity level to be correlated with methoxyestrogen levels. To the best of our knowledge, no other studies have reported the association between *COMT* genotype and urinary methoxyestrogens in postmenopausal women. In our study, we observed higher levels of 2-methoxyestrone, methylated catechol EM, total 2-methyl catechols, and a few more respective methylated estrogen ratios in the *COMT*-GG genotype compared with the *COMT*-AA genotype. Tworoger *et al* [321] have previously reported higher urinary levels of 2-hydroxyestrone ($P= 0.08$) and 16α-hydroxyestrone ($P= 0.02$) levels in women with the *COMT*-AA versus those possessing the *COMT*-GG genotype. We observed non-significant higher levels of 16α-hydroxyestrone in the *COMT*-AA compared with the *COMT*-GG or *COMT*-GA genotypes, and no significant result for 2-hydroxyestrone analyte. Our findings support

the hypothesis that polymorphism in the *COMT* gene is involved in determining the urinary levels of methoxyestrogens.

Study limitations are that we used one dose of green tea catechins, therefore, it is hard to extrapolate our findings to other forms of green tea extract, supplement, or green tea beverages. Another drawback is that we did not collect data related to the past exposure to green tea from our participants, although it might be safe to assume that randomization has already taken care of distributing these data equally between two groups. Additionally, we did not conducted power calculations to ascertain the appropriate sample size for the effects of green tea intake on urinary estrogens, thus we may not have had adequate power to detect meaningful changes. Lastly, we had relatively large number of samples with levels lower than detectable limit for the following estrogen metabolites: 4-methoxyestrone, 2-methoxyestrone, 2-methoxyestradiol, 4-methoxyestradiol, and 4-hydroxyestradiol. This study has several strengths as well. Randomized double-blind controlled design along with the large sample size and long trial period are among the main strengths of this study. Moreover, participants' compliance with taking study pills was great, and drop rate was very low. We were able to show for the first time how different *COMT* genotypes influence metabolites in the methylated estrogen pathway. Lastly, we took advantage of the state-of-the-art technology in quantifying urinary estrogens and estrogen metabolites, and were constantly controlling the stability of study supplement contents throughout the trial.

Future research should focus more on non-hormonal mechanisms of tea polyphenols on breast cancer. These studies can take advantage of animal model studies in earlier time points, and well-designed human intervention studies using polyphenol biomarker endpoints. Also, it is important that future work evaluates multiple doses and examines the combination of green tea with other food components considering the bioavailability issues. The role of biological active metabolites of green tea constituents formed by microbial metabolism on breast cancer also needs further attention. In addition, designing large cohort studies in which tea polyphenol exposures are more accurately determined by specific biomarkers cannot be emphasized further. Finally,

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further research is warranted to assess the hormonal effects of green tea extract on menopause symptoms including hot flashes, night sweats, and sleep problems.

In summary, our study provides modest evidence that green tea intake influences estrogen metabolism in postmenopausal women. We further showed that genetic variations in the *COMT* gene does not modify the effects of green tea intake on estrogen metabolism. Nonetheless, the *COMT* genotype modulates metabolism of methylated catechol estrogens. Ingestion of green tea extract with 843 mg of EGCG was safe and tolerated well by women in this study, but the extent to which green tea may alter the risk of breast cancer remains to be further elucidated through other mechanisms.

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Appendices:

Appendix 1. Letter of invitation mailed to the potential study participants.

June 30, 2011

Dear Ms. «Last_name»,

Congratulations on your normal screening mammogram.

Are you interested in contributing to research on breast cancer prevention? Up to one in eight American women are diagnosed with breast cancer during their lifetimes, and we are studying ways to prevent this disease. Our most recent study is investigating the possible breast cancer preventive effects of green tea consumption.

We're looking for healthy postmenopausal women whose normal mammograms show that they have relatively dense breasts. Your recent normal mammogram showed that you have relatively dense breasts, making you eligible to participate in medical research at the University of Minnesota. Reducing breast density has been shown to decrease the risk of breast cancer, and we are conducting research to find out if green tea will lower breast density.

If you are willing to participate in this 1-year study, you will be compensated up to $450. You would need to take 4 capsules daily, either decaffeinated green tea capsules or placebo (inactive) capsules, provide a urine and/or blood sample once a month (takes about 20 minutes), and have another mammogram in 1 year. 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.

This is a wonderful opportunity for you to contribute to research on women's health and breast cancer prevention! If you are interested in participating, we need to hear from you within two weeks of your receiving this letter. Please call **(612) 624-3412** or visit www.greenteastudy.umn.edu to complete a brief screening questionnaire. You may also

email the study staff at greentea@umn.edu. We look forward to speaking with you! It is your decision whether or not to participate. For your convenience, if we don't hear from you in the next few weeks we may call you.

If you have any questions about breast density and cancer risk, please contact Carolyn Torkelson, MD, a breast specialist and research physician with the University of Minnesota Breast Center, at (612) 625-8718.

Sincerely yours,

Tim Emory, MD, Meghan McKeon, MD, and Jessica Kuehn-Hajder, MD
University of Minnesota Medical Center, Fairview Southdale Medical Center, and Maple Grove Medical Center Breast Center Lead Interpreting Radiologists

Appendix 2. Phone interview questionnaire administered by the study staff.

Name of caller _____ Subject ID# _____

Phone (h) _____ (w) _____Email _____

Interviewer _____ Date _____/_____/_____

Phone Interview Questions: Green Tea Study

1. To verify your identity, what is your date of birth? _____/_____/_____

2. Is this your first time contacting the Green Tea Study? _____

3. What is your age? _____ *(50-70)*

4. Do you use tobacco products? _____ **Includes nicotine cessation products containing nicotine**

5. When was your last menstrual period? _____/_____/_____ *(must be at least 1 yr ago)*

6. What was the location and date of your last mammogram? _____/_____/_____

   _____

7. Have you ever had breast cancer or proliferative breast disease? _____

Proliferative breast disease, also called hyperplasia, is an overgrowth of breast cells diagnosed with a needle or surgical biopsy. It is not a cancerous condition but usually requires more frequent breast exams to examine the breast tissue.

8. Have you ever had ovarian cancer? _____

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

10. Have you been on any of the following medications at any time during the past 6 months?
    The individual qualifies for the study if NONE of these drugs have been taken in the past 6 months.

	Y/N
• **Hormone Replacement Therapy** *(For menopause symptoms)*	_____
• **Tamoxifen** *(For breast cancer treatment or risk reduction)*	_____
• **Raloxifene** *(Osteoporosis treatment or risk reduction, or breast cancer risk reduction)*	_____
• **Aromatase Inhibitors** Such as Arimidex, Aromasin, or Femara *(For breast cancer treatment)*	_____
• **Methotrexate or Enbrel** (*For rheumatoid arthritis*)	_____

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

201

_____

_____

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

_____

_____

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

   • Diabetes?   _____

   • Hyperthyroidism?   _____

   • Uncontrolled high blood pressure (hypertension)?   _____

   • Crohn's or Ulcerative Colitis: _____

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

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

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

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

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

    spirits)

16. Do you consume tea? _____ If so, what kind? _____

    If you consume green tea, how often? _____ *(Less than one cup/wk)*

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

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

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

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

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

~OR~

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

_____

_____

Thank you again, and have a great day.

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

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

several biological factors from your blood and urine that can help indicate your risk for breast cancer.

Would you like to hear more information?   _____

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

Would you like to hear more information?   _____

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

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

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

Circle one of each of the following:

Status: **Preferred contact:**

Orientation session:

ELIGIBLE	**EMAIL**
_____/_____/_____	
INELIGIBLE	**PHONE**
_____	

Appendix 3. Participant orientation session PowerPoint slides.

University of Minnesota — Department of Food Science & Nutrition
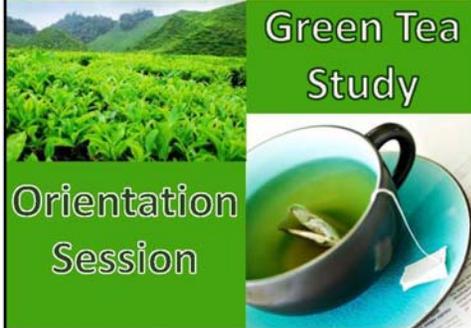
Green Tea Study
Orientation Session

University of Minnesota — Department of Food Science & Nutrition

Green tea and reduction of breast cancer risk

Orientation Objectives
- To gain a better understanding of:
  - Study background and purpose
  - Study requirements
    - Timeline of participation
    - Data collection
- To help you decide if you are willing and able to assist us in this important research!

GTS Orientation 2

University of Minnesota — Department of Food Science & Nutrition

Green tea and reduction of breast cancer risk

Green Tea Study Staff

Mindy S. Kurzer, Ph.D.
- Principal Investigator
- Phone: (612) 624-9789
- Email: mkurzer@umn.edu

GTS Orientation 3

University of Minnesota — Department of Food Science & Nutrition

Green tea and reduction of breast cancer risk

Green Tea Study Staff

Hamed Samavat
- Ph.D. Student, Nutrition
- Study Coordinator

GTS Orientation 4

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Green Tea Study Staff — Ali Dostal — Ph.D. Student, Nutrition & Epidemiology — Study Coordinator

Green Tea Study Staff — Alyssa Perry — M.S. Student, Nutrition — Study Coordinator

Green Tea Study Staff — Tara M. Research Assistant — April R. Research Assistant

Green Tea Study Staff — Sarah B. (Study Coordinator), Jane (Registered Nurse for Green Tea Study), Nicole S. (Post-doctoral Research Associate), Ed S. (Laboratory Scientist), Erin L. (Research Assistant), Weston B. (Research Assistant)

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University of Minnesota — Department of Food Science & Nutrition
Green tea and reduction of breast cancer risk — Green Tea Study

Breast Cancer Statistics in the US

— 1 in 8 American women may develop breast cancer in her lifetime

— Estimated new breast cancer cases in the US in 2011: 288,130

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University of Minnesota — Department of Food Science & Nutrition
Green tea and reduction of breast cancer risk — Green Tea Study

Green Tea Study Purpose

— To research the effects of consuming green tea on breast cancer risk
— Measure of breast cancer biomarkers such as:
  - Breast density
  - Reproductive hormones (like estrogen)
  - Growth factors (like insulin-like growth factor)

GTS Orientation 10

University of Minnesota — Department of Food Science & Nutrition
Green tea and reduction of breast cancer risk — Green Tea Study

How Do We Measure These Biomarkers?

— Annual mammogram → breast density

— Urine and blood samples → reproductive hormones, growth factors, and distribution of green tea components

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University of Minnesota — Department of Food Science & Nutrition
Green tea and reduction of breast cancer risk — Green Tea Study

Hypotheses

Plant compounds in green tea (catechins) will:

— *Decrease* the risk of breast cancer as measured by these biomarkers
— Affect estrogen metabolism in a direction consistent with *lowered* breast cancer risk
— *Reduce* oxidative stress

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Oxidative Stress

= Damage to cells, tissues, and organs caused by oxygen molecules called free radicals

– Oxidative stress is involved in cancers, cardiovascular diseases, and aging

– We think green tea may also reduce breast cancer risk through reduction of oxidative stress

Recruitment

Total enrollment : 800 women

400 women in treatment group, taking green tea extract capsules

400 women in control group, taking placebo (inactive) capsules

Genetic Component

– All human beings have a unique genetic makeup

– We will test you for one gene (COMT) before you can enter the study

– We think this gene may influence how green tea is metabolized differently between individuals

Recruitment

Total enrollment : 800 women

400 women in treatment group, taking green tea extract capsules

400 women in control group, taking placebo (inactive) capsules

200 high-activity COMT

200 low-activity COMT

200 high-activity COMT

200 low-activity COMT

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Slide 17

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Green tea and reduction of breast cancer risk — Green Tea Study

Study Participation

– Time: 1 year

– 10 clinic visits at Human Nutrition Research Clinic and/or local Fairview Clinic

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Green tea and reduction of breast cancer risk — Green Tea Study

Participant Timeline

Screening ($) CV1 — Annual Mammogram

CVs 3-7 and 9:
Weight and non-fasted blood draw ONLY (CV5 & 9 spot urine AND CV1,2,5,8-10 Vitals)

CV2 | CV3 | CV4 | CV5 $ | CV6 | CV7 | CV8 $ | CV9 $ | CV10 $

CVs 2, 8, and 10:
•Bring 24-hour urine collection
•Fasted blood drawing
•Questionnaires

CV= Clinic Visit

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Green tea and reduction of breast cancer risk — Green Tea Study

Clinic Visits 2, 8, and 10

Measurement	2 (month 0)	8 (month 6)	10 (month 12)
Fasted Blood Drawing	X	X	X
Waist and Hip Circumferences	X		X
Weight		X	X
Vital Signs	X	X	X
Health Survey	X		
Dietary History Questionnaire	X		X
Menopause-specific Quality of Life Questionnaire	X	X	X
Bring 24-hour Urine Collection	X	X	X

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Required Time for Each Clinic Visit

Clinic Visit #	Time (min)
1	30
2	100
3	20
4	20
5	30
6	20
7	20
8	45
9	20
10	100

≈ 6.5 hours

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Green tea and reduction of breast cancer risk Green Tea Study

Required Time For Other Study Activities

– 24-hour urine collection X 3 (72 hours total)

– Daily supplement consumption and study log completion (<5 min. daily)

– Health surveys (3 hours total)

– Transportation to clinic (individual)

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Green tea and reduction of breast cancer risk Green Tea Study

Clinic Visits

– All clinic visits can occur on weekdays at the Human Nutrition Research Clinic (free meter parking will be available)

– Four of the ten visits can be completed at your closest Fairview Clinic

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Green tea and reduction of breast cancer risk Green Tea Study

Clinic Visit 1 (screening)

– Measurements include:
  • Blood drawing
  • Weight and height
  • Vital signs (blood pressure, heart rate, body temperature, etc.)

– Your eligibility status will be announced within 2 – 4 weeks

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Green tea and reduction of breast cancer risk Green Tea Study

The Other 6 Clinic Visits

– Do not need to fast

– Can be at HNRC (FScN 116) or 4 out of 10 visits can be at Fairview Clinic

– Measurements:
  • Blood drawing (all visits)
  • Weight (CVs 1, 2, 5, 8, 9, and 10)
  • Vital signs (CVs 1, 2, 5, 8, 9, and 10)
  • Small urine sample at clinic visits 5 and 9

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Slide 1

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Green tea and reduction of breast cancer risk

Green Tea Study

Satellite Green Tea Study Fairview Clinics

- Maple Grove
- Jonathan
- Crosstown/Southdale
- Oxboro
- Farmington

Slide 2

University of Minnesota — Department of Food Science & Nutrition

Green tea and reduction of breast cancer risk

Green Tea Study

Blood Drawing

Fasted state	Non-fasted state
Don't take anything except water for the last 10 hours before your clinic visit	No restriction on food
During clinic visits **2, 8** and **10**	The remaining clinic visits
7:00 am- 10:00 am	7:00 am- 2:00 pm 7:00 am- 5:00 pm select weeknights

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Slide 3

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Green tea and reduction of breast cancer risk

Green Tea Study

Mammogram

- Please schedule your annual mammogram within 1 week of clinic visit 10 - this means your mammogram will take place more than a year after your previous mammogram

- Inform the Green Tea staff of the date and location of your mammogram at clinic visit 9

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Slide 4

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Green tea and reduction of breast cancer risk

Green Tea Study

Supplement Distribution

- Green tea extract OR placebo (inactive) capsules
  - Consume 4 daily, 2 following breakfast and 2 following dinner, for 1 year
- Neither you nor study staff will know which capsule you are taking
- You will receive supplement supply during your clinic visits at the HNRC every 3 months

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Green tea and reduction of breast cancer risk — Green Tea Study

Supplement Distribution

- Keep track of taken pills with provided study log
- Bring back the previous 3 month's bottles (and capsules) to the HNRC at each clinic visit
- You will be provided with 15 extra capsules for each month
  - Do not worry if some capsules remain when you return your bottles

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Green tea and reduction of breast cancer risk — Green Tea Study

What if you miss a dose?

- If you miss your morning capsules:
  - Take the missed capsules as soon as you remember, preferably with food
- If you miss your afternoon/evening capsules:
  - Record missed dose in study log, and DO NOT take capsules
  - DO NOT take more than 4 capsules/day

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Green tea and reduction of breast cancer risk — Green Tea Study

5-minute Break for Individual Questions

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Green tea and reduction of breast cancer risk — Green Tea Study

24-hour Urine Collection

★ Urine collection is very important to the study! Estrogen metabolites and catechins (antioxidants in green tea) are measured through urine collection. ★

- The day before clinic visits 2, 8, and 10
- You will be provided with materials at the previous month's clinic visit:
  - 3-liter container
  - Travel jug
  - 2 collection hats
  - 1 packet of vitamin C
  - 2 ice packs
  - Cooler bag
- You should bring your collected urine containers to the HNRC on the following day

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Urine Collection Procedure

- Beginning 24 hours prior to clinic visits 2, 8, and 10, collect ALL urine in provided containers
- Discard the first urine of the morning but record the time as the "Start Time" on your label
  - Start the clock with an empty bladder

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Urine Collection Label

Complete this information →

24 HOURS COLLECTION   Clinic Visit 2
GREEN TEA STUDY Protocol # 10039
Study ID
VIT C ADDED
Baseline
Start DATE: 07.23.10
End DATE: 07.24.10
Start Time: 7:00 am End Time: 7:00 am

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Green tea and reduction of breast cancer risk — Green Tea Study

Urine Collection Rules

- Keep the urine collection jug in the refrigerator after each void (<u>not</u> in the freezer)

- Do not consume alcohol the days of the collections and 24 hours prior to collection

- Detailed instructions can be found in the participant handbook (pg. 23)

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Green tea and reduction of breast cancer risk — Green Tea Study

Questionnaires

	Health History	Dietary History	Menopause-Specific Quality of Life
What does it ask?	•Exercise Habits •Smoking History •Dietary Habits •Medical History	Foods, drinks, and supplements consumed over the past year	Menopause symptoms over the past week
How long does it take?	15 min.	1 hr.	15 min.
When?	CV 2	CVs 2 and 10	CVs 2, 8, and 10
What is the format?	Paper	Web	Web

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Green tea and reduction of breast cancer risk — Green Tea Study

Study Logs

- Will be provided at the second visit
- Use to keep track of:
  - Consumed capsules
  - Clinic visit dates
  - 24-hour urine collection dates
  - Date of mammogram at the end of the study
  - Any changes in health status (illnesses, hospitalizations, new medications, etc).

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Are your answers to all of the following questions yes? If not, please reconsider your participation.

1. Do you know your plans for the next year? Do you have the time to stay focused on the study for 1 year?
2. Are you able to take capsules twice daily (with breakfast and with dinner) for 1 year?
3. Are you able to respond to staff calls or emails within 2-3 days?
4. Are you free in the mornings between 7:00 and 10:00 am to go to the clinic?

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Green tea and reduction of breast cancer risk — Green Tea Study

Consent & HIPAA Forms Discussion

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Green tea and reduction of breast cancer risk — Green Tea Study

Helpful Hints

- Call (612) 624-3412 as soon as you have any questions or concerns
- Please drink no more than 1 cup of green tea per week while participating
- Please drink no more than 7 alcoholic beverages per week while participating
  - 12 oz. beer
  - 5 oz. wine
  - 1.5 oz. spirits
- Please let the study staff know of any changes in health or medications

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Appendix 4. Study consent form

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 6th and 12th 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 Questionnaire
- 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

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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

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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.

Appendix 5. Health history questionnaire

HEALTH HISTORY QUESTIONNAIRE

Green Tea Study

Name: _____   Date:

_____

Date of Birth (mm/dd/yyyy): _____

Level of Education, please circle one:

- Some high school (1)
- High school graduate (2)
- Some college (3)
- College degree (4)
- Masters/PhD/Professional (5)

Ethnicity (Please circle one):

• Hispanic (1)

• Non-Hispanic (2)

• Do not wish to provide (3)

Race (Please circle one)**:**

• American Indian or Alaska Native (1)

• Asian (2)

• Native Hawaiian or Other Pacific Islander (3)

• Black or African American (4)

• White (5)

• More than one race (6)

• Do not wish to provide (7)

Please answer the following questions as accurately as possible.

General Health

1. How would you describe your overall health?

    Poor (1)   Good (2)

    Very Good (3)   Excellent (4)

2. Has your weight changed in the past year?
    YES (1)   NO (2)

    A) If yes, how many pounds have you:
        Gained: _____
        Lost: _____

    B) Please explain the reason
        _____

3. Do you exercise regularly?
    YES (1)   NO (2)

    A) How many <u>days per week</u> do you exercise? _____

    B) How intense is your exercise:

     Mild (1)   Moderate (2)   Maximal (3)

4. Please indicate in the table below, how many hours per week were spent on
    the listed activities:

	# of Hours Per Week:
Sitting at work (1)	
Sitting or driving (e.g. car, bus) (2)	
Sitting or lying watching TV (3)	
Sitting at home reading (4)	
Sitting other (e.g. eating, computer) (5)	
Walking to work or for exercise (including golf) (6)	
Jogging (slower than 10 minutes/mile) (7)	
Running (10 minutes/mile or faster) (8)	
Bicycling (including stationary machine) (9)	
Lap swimming (10)	
Tennis (11)	
Squash or racquetball (12)	
Calisthenics (e.g. rowing, stair, elliptical machine, etc.) (13)	
Weightlifting or weight machine (14)	
Heavy outdoor work (e.g. digging, chopping) (15)	
Other (Please specify): (16)	

5. Do you have any allergies (other than foods)
   YES (1)   NO (2)

   A) If yes, please describe _____

   _____

   _____

6. Are you a regular blood donor?

   YES (1)   NO (2)

A) If yes, <u>how many times per year</u> do you donate?
_____/per year

7. Have you smoked at least 100 cigarettes in your lifetime?
YES (1)   NO (2)

8. Do you now smoke cigarettes?
YES (1)   NO (2)

9. If you've stopped smoking:
A) How long has it been since you quit? _____(years)

B) How frequently did you smoke? _____ (<u># of days/week</u>)

C) On average, how many cigarettes did you smoke per day?
_____

10. How many people living in your household regularly smoke at home (one cigarette or more on 4 days or more out of the week)?
_____

11. Do you use recreational drugs?
YES (1)   NO (2)

Food Consumption Patterns

12. Are you on a special diet now?
YES (1)   NO (2)

A) If yes, please circle one:

- Gluten-free (1)
- High Fiber (2)

- Lactose Intolerance (3)
- Low Fat (4)
- Low Carbohydrate (5)
- Low Sodium (6)
- Vegetarian (7)
- Other (8), please describe: _____

13. Have you significantly changed your eating habits in the past 5 years?

   YES (1)   NO (2)

   A) If yes, please describe when, why, and how eating habits changed

   _____
   _____

14. Do you have any food allergies?
   YES (1)   NO (2)

   A) If yes, what are they? _____

   _____

15. Are you a vegetarian?
   YES (1)   NO (2)

      A) If you are a vegetarian, do you eat eggs?
         YES (1)   NO (2)

      B) If you are a vegetarian, do you drink milk?
         YES (1)   NO (2)

16. Do you consume soy milk, foods, or supplements of any kind?
   YES (1)   NO (2)

      A) If yes, what type of soy products do you consume?

- Tofu (1)
- Soy milk (2)
- Soy burger (3)

- Soy nuts (4)
- Edamame (5)
- Soy protein bar (6)
- Others (7)

B) How often do you consume them (if you consume more than one product, please take an average and circle only one option below?

- Never /Hardly Ever (1)
- Once a Week (2)
- 2–3 Times a Week (3)
- 4–6 Times a Week (4)
- 1–3 Times a Month (5)
- Once a Day (6)
- 2+ Times a Day (7)

17. Do you consume Tea?
    YES (1)   NO (2)

If yes, how many cups of the following do you consume each week?
    A) Green Tea (1): _____/Per Week
    B) Black Tea (2): _____/Per Week
    C) Herbal Tea (3): _____/Per Week
    D) Other (4); Please Specify: _____/Per Week

18. Do you consume alcohol?
    YES (1)   NO (2)

    A) If yes, how many drinks* per week?
       _____

    *1 drink = 12oz beer, 4oz wine, 1oz hard liquor

Medical History

19. Please indicate if you or a family member has ever been diagnosed with any of the following. If it is a family member, please indicate if it is <u>first-</u> , or <u>second</u>-degree relative.
- Examples of first-degree relative: parent, offspring or sibling.
- Examples of second-degree relative: grandparent, grandchild, uncle, aunt, nephew, or niece.

Condition	You	First Degree	Second Degree
Breast cancer (1)	Y / N	Y / N	Y / N
Fibrocystic breast disease (2)	Y / N	Y / N	Y / N
Uterine cancer (3)	Y / N	Y / N	Y / N
Endometrial cancer (4)	Y / N	Y / N	Y / N
Cervical cancer (5)	Y / N	Y / N	Y / N
Colon cancer (6)	Y / N	Y / N	Y / N
Any other cancer (7)	Y / N	Y / N	Y / N
Endometriosis (8)	Y / N	Y / N	Y / N
Pelvic inflammatory disease (9)	Y / N	Y / N	Y / N
Frequent bladder infections (>1 per yr) (10)	Y / N	Y / N	Y / N
Frequent yeast infections (>1 per yr) (11)	Y / N	Y / N	Y / N
Diabetes (12)	Y / N	Y / N	Y / N
Overactive/underactive thyroid (13)	Y / N	Y / N	Y / N
Other hormone related diseases (E.g. pituitary, fertility, adrenal, etc.) (14)	Y / N	Y / N	Y / N
Eating Disorder (15)	Y / N	Y / N	Y / N
High cholesterol (16)	Y / N	Y / N	Y / N
High blood pressure (17)	Y / N	Y / N	Y / N
Stomach/intestinal ulcers (18)	Y / N	Y / N	Y / N

Diverticular disease (19)	Y / N	Y / N	Y / N
Hemorrhoids (20)	Y / N	Y / N	Y / N
Ulcerative colitis (21)	Y / N	Y / N	Y / N
Chronic constipation (22)	Y / N	Y / N	Y / N
Chronic diarrhea (23)	Y / N	Y / N	Y / N
Liver disorder (Hepatitis, abnormal liver enzymes level, etc.) (24)	Y / N	Y / N	Y / N

20. Are there any other mental or physical health issues we should be aware of?   YES (1)   NO (2)

    A) If yes, please list._____

_____

_____

Medication/Dietary Supplement/Treatment History

21. Are you currently taking any prescription medications?
YES (1)   NO (2)

    A) If yes, please list type, amount, and frequency _____

_____

_____

22. Have you taken any additional prescription medications in the past 6 months?

    YES (1)   NO (2)

  A) If yes, please list type, amount, and frequency. _____

_____

_____

23. Have you ever undergone chemotherapy?
     YES (1)   NO (2)

     If yes, at <u>what age</u> and for <u>how long</u> where you on chemotherapy?

          A) Age: _____B) Duration: _____
               (months)

     C) What was the reason for needing chemotherapy? _____

24. Have you ever taken Tamoxifen (1), Raloxifene (2) or aromatase inhibitors
     (3)?
     YES (1)   NO (2)
     A) If yes, which one(s)? _____
     B) <u>How long</u> did you take it (years)? _____
     C) <u>When</u> did you discontinue taking it?_____

25. Do you use any over-the-counter drugs, including pain relievers?

     YES (1)   NO (2)
   A) If yes, please list drugs and frequency of use _____
     _____
     _____

26. Do you take vitamin and/or mineral supplements?
     YES (1)   NO (2)

If yes, please list brands, amounts and frequency in the following table.

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Type	Brand	Amount	Frequency
Multi-vitamin (1)			
Vitamin A (2)			
Vitamin B (including any type of vitamin B) (3)			
Vitamin C (4)			
Vitamin D or Vitamin D plus Calcium (5)			
Vitamin E (6)			
Calcium or calcium plus others (not including vitamin D) (7)			
Fish oil (8)			
Minerals (any) (9)			
Others (10)			

27.   Do you take any herbal supplements?
       YES (1)   NO (2)

       If yes, please list types, brands, amounts and frequency

       _____

       _____

       _____

28.   Do you use any other complementary medicine therapies?
        (Homeopathy, acupuncture, meditation, massage, etc.)

         YES (1)   NO (2)

A) If yes, please describe.

_____

_____

_____

OB/GYN History

29. At approximately <u>what age</u> did you first get your period? _____

30. At approximately <u>what age</u> did your periods became regular (about once per month)? _____

31. Have you had your uterus removed?   YES (1)   NO (2)

32. Have you had any of your ovaries removed?   YES (1)   NO (2)

    A) If yes, please circle one: 1 Ovary   Both Ovaries

    B) <u>What age</u> did you have 1 or both ovaries removed: _____

33. When was your last menstrual period?

    Note: If you do not remember the exact date, please provide <u>an estimated</u>

    <u>*age*</u>. _____

34. If you are not currently, taking hormone replacement therapy, have you ever taken it in the past?
     YES (1)   NO (2)

    A) If yes, for <u>how long</u> (years)?_____

    B) At approximately <u>what age</u> did you begin? _____

    C) At approximately <u>what age</u> did you stop hormone replacement therapy?

D) What product(s) did you use? _____

_____

E) What dose(s)? _____

_____

35. Have you ever used any other treatments for menopause?
     YES (1)   NO (2)

     A) If yes, what product(s) did you use? _____

     _____

     B) How often? _____

     At what age did you use these treatments?_____

36. Have you ever taken birth control pills?
     YES (1)   NO (2)

     A) If so, for how many years total?_____

     B) At approximately what age did you start taking them? _____

     C) At approximately what age did you stop taking them? _____

     D) What was the name of the birth control pill that you used? _____

     _____

37. Please give your pregnancy history:

     A) Number of pregnancies _____
     B) Number of live births _____

38. What was the outcome of your first pregnancy? (Please circle one)

- Live birth (1)
- Still birth (2)
- Tubal pregnancy (3)
- Miscarriage (4)
- Induced abortion (5)

39.   <u>What year</u> was your first child born? _____

40.   <u>What year</u> was your youngest child born? _____

41.   Did you breast feed one or more of your children?

YES (1)   NO (2)

42.   If you have breast fed, <u>how many months</u> total? _____

Study Logistics

43.   Why do you want to be in this study?

- Compensation (1)
- Altruistic reasons (2)
- Bored (3)
- Referred by health care practitioner (4)
- Personal health benefit (5)
- Other (6): A) please, specify:

_____

_____

_____

Thank you for your time in answering this questionnaire.

When you have completed the questions, please take a moment and make sure all questions are answered to the best of your ability.

Appendix 6. Web-based dietary history questionnaire

This is a sample form. Do not use for scanning.

NATIONAL INSTITUTES OF HEALTH

Diet History Questionnaire

Today's date:

MONTH	DAY	YEAR
☐ Jan		☐ 2007
☐ Feb		☐ 2008
☐ Mar	☐0 ☐0	☐ 2009
☐ Apr	☐1 ☐1	☐ 2010
☐ May	☐2 ☐2	☐ 2011
☐ Jun	☐3 ☐3	
☐ Jul	☐4	
☐ Aug	☐5	
☐ Sep	☐6	
☐ Oct	☐7	
☐ Nov	☐8	
☐ Dec	☐9	

In what month were you born?

☐ Jan
☐ Feb
☐ Mar
☐ Apr
☐ May
☐ Jun
☐ Jul
☐ Aug
☐ Sep
☐ Oct
☐ Nov
☐ Dec

In what year were you born?

19 |_|_|
☐0 ☐0
☐1 ☐1
☐2 ☐2
☐3 ☐3
☐4 ☐4
☐5 ☐5
☐6 ☐6
☐7 ☐7
☐8 ☐8
☐9 ☐9

Are you male or female?

☐ Male
☐ Female

BAR CODE LABEL OR SUBJECT ID HERE

|_|_|_|_|_|_|_|_|_|

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1. Over the <u>past 12 months</u>, how often did you drink **tomato juice** or **vegetable juice**?

☐ NEVER (GO TO QUESTION 2)

☐ 1 time per month or less ☐ 1 time per day
☐ 2–3 times per month ☐ 2–3 times per day
☐ 1–2 times per week ☐ 4–5 times per day
☐ 3–4 times per week ☐ 6 or more times per day
☐ 5–6 times per week

1a. Each time you drank **tomato juice** or **vegetable juice**, how much did you usually drink?

☐ Less than ¾ cup (6 ounces)
☐ ¾ to 1¼ cups (6 to 10 ounces)
☐ More than 1¼ cups (10 ounces)

2. Over the <u>past 12 months</u>, how often did you drink **orange juice** or **grapefruit juice?**

☐ NEVER (GO TO QUESTION 3)

☐ 1 time per month or less ☐ 1 time per day
☐ 2–3 times per month ☐ 2–3 times per day
☐ 1–2 times per week ☐ 4–5 times per day
☐ 3–4 times per week ☐ 6 or more times per day
☐ 5–6 times per week

2a. Each time you drank **orange juice** or **grapefruit juice**, how much did you usually drink?

☐ Less than ¾ cup (6 ounces)
☐ ¾ to 1¼ cups (6 to 10 ounces)
☐ More than 1¼ cups (10 ounces)

3. Over the <u>past 12 months</u>, how often did you drink **other 100% fruit juice** or **100% fruit juice mixtures** (such as apple, grape, pineapple, or others)?

☐ NEVER (GO TO QUESTION 4)

☐ 1 time per month or less ☐ 1 time per day
☐ 2–3 times per month ☐ 2–3 times per day
☐ 1–2 times per week ☐ 4–5 times per day
☐ 3–4 times per week ☐ 6 or more times per day
☐ 5–6 times per week

3a. Each time you drank **other fruit juice** or **fruit juice mixtures**, how much did you usually drink?

☐ Less than ¾ cup (6 ounces)
☐ ¾ to 1½ cups (6 to 12 ounces)
☐ More than 1½ cups (12 ounces)

Question 4 appears in the next column

Over the <u>past 12 months</u>…

4. How often did you drink other **fruit drinks** (such as cranberry cocktail, Hi-C, lemonade, or Kool-Aid, diet or regular)?

☐ NEVER (GO TO QUESTION 5)

☐ 1 time per month or less ☐ 1 time per day
☐ 2–3 times per month ☐ 2–3 times per day
☐ 1–2 times per week ☐ 4–5 times per day
☐ 3–4 times per week ☐ 6 or more times per day
☐ 5–6 times per week

4a. Each time you drank **fruit drinks**, how much did you usually drink?

☐ Less than 1 cup (8 ounces)
☐ 1 to 2 cups (8 to 16 ounces)
☐ More than 2 cups (16 ounces)

4b. How often were your fruit drinks **diet** or **sugar-free drinks**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

5. How often did you drink **milk as a beverage** (NOT in coffee, NOT in cereal)? (Please include chocolate milk and hot chocolate.)

☐ NEVER (GO TO QUESTION 6)

☐ 1 time per month or less ☐ 1 time per day
☐ 2–3 times per month ☐ 2–3 times per day
☐ 1–2 times per week ☐ 4–5 times per day
☐ 3–4 times per week ☐ 6 or more times per day
☐ 5–6 times per week

5a. Each time you drank **milk as a beverage**, how much did you usually drink?

☐ Less than 1 cup (8 ounces)
☐ 1 to 1½ cups (8 to 12 ounces)
☐ More than 1½ cups (12 ounces)

5b. What kind of **milk** did you usually drink?

☐ Whole milk
☐ 2% fat milk
☐ 1 % fat milk
☐ Skim, nonfat, or ½% fat milk
☐ Soy milk
☐ Rice milk
☐ Other

Question 6 appears on the next page

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Over the past 12 months…

6. How often did you drink **meal replacement, energy, or high-protein beverages** such as Instant Breakfast, Ensure, Slimfast, Sustacal or others?

☐ NEVER (GO TO QUESTION 7)

☐ 1 time per month or less ☐ 1 time per day
☐ 2–3 times per month ☐ 2–3 times per day
☐ 1–2 times per week ☐ 4–5 times per day
☐ 3–4 times per week ☐ 6 or more times per day
☐ 5–6 times per week

6a. Each time you drank **meal replacement beverages**, how much did you usually drink?

☐ Less than 1 cup (8 ounces)
☐ 1 to 1½ cups (8 to 12 ounces)
☐ More than 1½ cups (12 ounces)

7. Over the past 12 months, did you drink **soft drinks, soda, or pop**?

☐ NO (GO TO QUESTION 8)

☐ YES

7a. How often did you drink **soft drinks, soda, or pop IN THE SUMMER**?

☐ NEVER

☐ 1 time per month or less ☐ 1 time per day
☐ 2–3 times per month ☐ 2–3 times per day
☐ 1–2 times per week ☐ 4–5 times per day
☐ 3–4 times per week ☐ 6 or more times
☐ 5–6 times per week per day

7b. How often did you drink **soft drinks, soda, or pop DURING THE REST OF THE YEAR**?

☐ NEVER

☐ 1 time per month or less ☐ 1 time per day
☐ 2–3 times per month ☐ 2–3 times per day
☐ 1–2 times per week ☐ 4–5 times per day
☐ 3–4 times per week ☐ 6 or more times
☐ 5–6 times per week per day

7c. Each time you drank **soft drinks, soda, or pop,** how much did you usually drink?

☐ Less than 12 ounces or less than 1 can or bottle
☐ 12 to 16 ounces or 1 can or bottle
☐ More than 16 ounces or more than 1 can or bottle

7d. How often were these soft drinks, soda, or pop **diet** or **sugar-free**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

7e. How often were these soft drinks, soda, or pop **caffeine-free**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

8. Over the past 12 months, did you drink **beer**?

☐ NO (GO TO QUESTION 9)

☐ YES

8a. How often did you drink **beer IN THE SUMMER**?

☐ NEVER

☐ 1 time per month or less ☐ 1 time per day
☐ 2–3 times per month ☐ 2–3 times per day
☐ 1–2 times per week ☐ 4–5 times per day
☐ 3–4 times per week ☐ 6 or more times
☐ 5–6 times per week per day

8b. How often did you drink **beer DURING THE REST OF THE YEAR**?

☐ NEVER

☐ 1 time per month or less ☐ 1 time per day
☐ 2–3 times per month ☐ 2–3 times per day
☐ 1–2 times per week ☐ 4–5 times per day
☐ 3–4 times per week ☐ 6 or more times
☐ 5–6 times per week per day

8c. Each time you drank **beer**, how much did you usually drink?

☐ Less than a 12-ounce can or bottle
☐ 1 to 3 12-ounce cans or bottles
☐ More than 3 12-ounce cans or bottles

Question 8 appears in the next column

Question 9 appears on the next page

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Over the past 12 months…

9. How often did you drink **wine** or **wine coolers**?

☐ NEVER (GO TO QUESTION 10)

☐ 1 time per month or less ☐ 1 time per day
☐ 2–3 times per month ☐ 2–3 times per day
☐ 1–2 times per week ☐ 4–5 times per day
☐ 3–4 times per week ☐ 6 or more times per day
☐ 5–6 times per week

9a. Each time you drank **wine** or **wine coolers**, how much did you usually drink?

☐ Less than 5 ounces or less than 1 glass
☐ 5 to 12 ounces or 1 to 2 glasses
☐ More than 12 ounces or more than 2 glasses

10. How often did you drink **liquor** or **mixed drinks**?

☐ NEVER (GO TO QUESTION 11)

☐ 1 time per month or less ☐ 1 time per day
☐ 2–3 times per month ☐ 2–3 times per day
☐ 1–2 times per week ☐ 4–5 times per day
☐ 3–4 times per week ☐ 6 or more times per day
☐ 5–6 times per week

10a. Each time you drank **liquor** or **mixed drinks**, how much did you usually drink?

☐ Less than 1 shot of liquor
☐ 1 to 3 shots of liquor
☐ More than 3 shots of liquor

11. Over the past 12 months, did you eat **oatmeal, grits,** or **other cooked cereal**?

☐ NO (GO TO QUESTION 12)

☐ YES

11a. How often did you eat **oatmeal, grits,** or **other cooked cereal IN THE WINTER**?

☐ NEVER

☐ 1–6 times per winter ☐ 2 times per week
☐ 7–11 times per winter ☐ 3–4 times per week
☐ 1 time per month ☐ 5–6 times per week
☐ 2–3 times per month ☐ 1 time per day
☐ 1 time per week ☐ 2 or more times per day

Question 12 appears in the next column

11b. How often did you eat **oatmeal, grits,** or **other cooked cereal DURING THE REST OF THE YEAR**?

☐ NEVER

☐ 1–6 times per year ☐ 2 times per week
☐ 7–11 times per year ☐ 3–4 times per week
☐ 1 time per month ☐ 5–6 times per week
☐ 2–3 times per month ☐ 1 time per day
☐ 1 time per week ☐ 2 or more times per day

11c. Each time you ate **oatmeal, grits,** or **other cooked cereal**, how much did you usually eat?

☐ Less than ¾ cup
☐ ¾ to 1¼ cups
☐ More than 1¼ cups

12. How often did you eat **cold cereal**?

☐ NEVER (GO TO QUESTION 13)

☐ 1–6 times per year ☐ 2 times per week
☐ 7–11 times per year ☐ 3–4 times per week
☐ 1 time per month ☐ 5–6 times per week
☐ 2–3 times per month ☐ 1 time per day
☐ 1 time per week ☐ 2 or more times per day

12a. Each time you ate **cold cereal**, how much did you usually eat?

☐ Less than 1 cup
☐ 1 to 2½ cups
☐ More than 2½ cups

12b. How often was the cold cereal you ate **Total, Product 19,** or **Right Start**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

12c. How often was the cold cereal you ate **All Bran, Fiber One, 100% Bran,** or **Bran Buds**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

Question 13 appears on the next page

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Over the past 12 months…

12d. How often was the cold cereal you ate **some other bran** or **fiber cereal** (such as Cheerios, Shredded Wheat, Raisin Bran, Bran Flakes, Grape-Nuts, Granola, Wheaties, or Healthy Choice)?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

12e. How often was the cold cereal you ate any **other type of cold cereal** (such as Corn Flakes, Rice Krispies, Frosted Flakes, Special K, Froot Loops, Cap'n Crunch, or others)?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

12f. Was **milk** added to your cold cereal?

☐ NO (GO TO QUESTION 13)
☐ YES

12g. What kind of **milk** was usually added?

☐ Whole milk
☐ 2% fat milk
☐ 1% fat milk
☐ Skim, nonfat, or ½% fat milk
☐ Soy milk
☐ Rice milk
☐ Other

12h. Each time **milk was added to your cold cereal**, how much was usually added?

☐ Less than ½ cup
☐ ½ to 1 cup
☐ More than 1 cup

13. How often did you eat **applesauce**?

☐ NEVER (GO TO QUESTION 14)

☐ 1–6 times per year ☐ 2 times per week
☐ 7–11 times per year ☐ 3–4 times per week
☐ 1 time per month ☐ 5–6 times per week
☐ 2–3 times per month ☐ 1 time per day
☐ 1 time per week ☐ 2 or more times per day

Question 14 appears in the next column

13a. Each time you ate **applesauce**, how much did you usually eat?

☐ Less than ½ cup
☐ ½ to 1 cup
☐ More than 1 cup

14. How often did you eat **apples**?

☐ NEVER (GO TO QUESTION 15)

☐ 1–6 times per year ☐ 2 times per week
☐ 7–11 times per year ☐ 3–4 times per week
☐ 1 time per month ☐ 5–6 times per week
☐ 2–3 times per month ☐ 1 time per day
☐ 1 time per week ☐ 2 or more times per day

14a. Each time you ate **apples**, how many did you usually eat?

☐ Less than 1 apple
☐ 1 apple
☐ More than 1 apple

15. How often did you eat **pears** (fresh, canned, or frozen)?

☐ NEVER (GO TO QUESTION 16)

☐ 1–6 times per year ☐ 2 times per week
☐ 7–11 times per year ☐ 3–4 times per week
☐ 1 time per month ☐ 5–6 times per week
☐ 2–3 times per month ☐ 1 time per day
☐ 1 time per week ☐ 2 or more times per day

15a. Each time you ate **pears**, how many did you usually eat?

☐ Less than 1 pear
☐ 1 pear
☐ More than 1 pear

16. How often did you eat **bananas?**

☐ NEVER (GO TO QUESTION 17)

☐ 1–6 times per year ☐ 2 times per week
☐ 7–11 times per year ☐ 3–4 times per week
☐ 1 time per month ☐ 5–6 times per week
☐ 2–3 times per month ☐ 1 time per day
☐ 1 time per week ☐ 2 or more times per day

Question 17 appears on the next page

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Over the past 12 months...

16a. Each time you ate **bananas**, how many did you usually eat?

☐ Less than 1 banana
☐ 1 banana
☐ More than 1 banana

17. How often did you eat **dried fruit**, such as prunes or raisins (not including dried apricots)?

☐ NEVER (GO TO QUESTION 18)

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times per day

17a. Each time you ate **dried fruit**, how much did you usually eat (not including dried apricots)?

☐ Less than 2 tablespoons
☐ 2 to 5 tablespoons
☐ More than 5 tablespoons

18. Over the past 12 months, did you eat **peaches, nectarines, or plums**?

☐ NO (GO TO QUESTION 19)

☐ YES

18a. How often did you eat **fresh peaches, nectarines**, or **plums WHEN IN SEASON**?

☐ NEVER

☐ 1–6 times per season   ☐ 2 times per week
☐ 7–11 times per season   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times
                            per day

18b. How often did you eat **peaches, nectarines, or plums** (fresh, canned, or frozen) **DURING THE REST OF THE YEAR**?

☐ NEVER

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times
                            per day

Question 19 appears in the next column

18c. Each time you ate **peaches, nectarines, or plums**, how much did you usually eat?

☐ Less than 1 fruit or less than ½ cup
☐ 1 to 2 fruits or ½ to ¾ cup
☐ More than 2 fruits or more than ¾ cup

19. How often did you eat **grapes**?

☐ NEVER (GO TO QUESTION 20)

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times per day

19a. Each time you ate **grapes**, how much did you usually eat?

☐ Less than ½ cup or less than 10 grapes
☐ ½ to 1 cup or 10 to 30 grapes
☐ More than 1 cup or more than 30 grapes

20. Over the past 12 months, did you eat **cantaloupe**?

☐ NO (GO TO QUESTION 21)

☐ YES

20a. How often did you eat **fresh cantaloupe WHEN IN SEASON**?

☐ NEVER

☐ 1–6 times per season   ☐ 2 times per week
☐ 7–11 times per season   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times
                            per day

20b. How often did you eat **fresh or frozen cantaloupe DURING THE REST OF THE YEAR**?

☐ NEVER

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times
                            per day

Question 21 appears on the next page

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Over the past 12 months...

20c. Each time you ate **cantaloupe**, how much did you usually eat?

☐ Less than ¼ melon or less than ½ cup
☐ ¼ melon or ½ to 1 cup
☐ More than ¼ melon or more than 1 cup

21. Over the past 12 months, did you eat **melon, other than cantaloupe** (such as watermelon or honeydew)?

☐ NO (GO TO QUESTION 22)
☐ YES

21a. How often did you eat **fresh melon, other than cantaloupe** (such as watermelon or honeydew) **WHEN IN SEASON**?

☐ NEVER

☐ 1–6 times per season   ☐ 2 times per week
☐ 7–11 times per season   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times
                     per day

21b. How often did you eat **fresh** or **frozen melon, other than cantaloupe** (such as watermelon or honeydew) **DURING THE REST OF THE YEAR**?

☐ NEVER

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times
                     per day

21c. Each time you ate **melon other than cantaloupe**, how much did you usually eat?

☐ Less than ½ cup or 1 small wedge
☐ ½ to 2 cups or 1 medium wedge
☐ More than 2 cups or 1 large wedge

Question 22 appears in the next column

22. Over the past 12 months, did you eat **strawberries**?

☐ NO (GO TO QUESTION 23)
☐ YES

22a. How often did you eat **fresh strawberries WHEN IN SEASON**?

☐ NEVER

☐ 1–6 times per season   ☐ 2 times per week
☐ 7–11 times per season   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times
                     per day

22b. How often did you eat **fresh** or **frozen strawberries DURING THE REST OF THE YEAR**?

☐ NEVER

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times
                     per day

22c. Each time you ate **strawberries**, how much did you usually eat?

☐ Less than ¼ cup or less than 3 berries
☐ ¼ to ¾ cup or 3 to 8 berries
☐ More than ¾ cup or more than 8 berries

23. Over the past 12 months, did you eat **oranges, tangerines, or tangelos**?

☐ NO (GO TO QUESTION 24)
☐ YES

23a. How often did you eat **fresh oranges, tangerines, or tangelos WHEN IN SEASON**?

☐ NEVER

☐ 1–6 times per season   ☐ 2 times per week
☐ 7–11 times per season   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times
                     per day

Question 24 appears on the next page

Over the past 12 months…

23b. How often did you eat **oranges, tangerines,** or **tangelos** (fresh or canned) **DURING THE REST OF THE YEAR?**

☐ NEVER

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week

☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

23c. Each time you ate **oranges, tangerines,** or **tangelos,** how many did you usually eat?

☐ Less than 1 fruit
☐ 1 fruit
☐ More than 1 fruit

24. Over the past 12 months, did you eat **grapefruit?**

☐ NO (GO TO QUESTION 25)

☐ YES

24a. How often did you eat **fresh grapefruit WHEN IN SEASON?**

☐ NEVER

☐ 1–6 times per season
☐ 7–11 times per season
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week

☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

24b. How often did you eat **grapefruit** (fresh or canned) **DURING THE REST OF THE YEAR?**

☐ NEVER

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week

☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

24c. Each time you ate **grapefruit,** how much did you usually eat?

☐ Less than ½ grapefruit
☐ ½ grapefruit
☐ More than ½ grapefruit

Question 25 appears in the next column

25. How often did you eat **other kinds of fruit?**

☐ NEVER (GO TO QUESTION 26)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week

☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

25a. Each time you ate **other kinds of fruit,** how much did you usually eat?

☐ Less than ¼ cup
☐ ¼ to ¾ cup
☐ More than ¾ cup

26. How often did you eat **COOKED greens** (such as spinach, turnip, collard, mustard, chard, or kale)?

☐ NEVER (GO TO QUESTION 27)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week

☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

26a. Each time you ate **COOKED greens,** how much did you usually eat?

☐ Less than ½ cup
☐ ½ to 1 cup
☐ More than 1 cup

27. How often did you eat **RAW greens** (such as spinach, turnip, collard, mustard, chard, or kale)? *(We will ask about lettuce later.)*

☐ NEVER (GO TO QUESTION 28)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week

☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

27a. Each time you ate **RAW greens,** how much did you usually eat?

☐ Less than ½ cup
☐ ½ to 1 cup
☐ More than 1 cup

Question 28 appears on the next page

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Over the past 12 months…

28. How often did you eat **coleslaw**?

☐ NEVER (GO TO QUESTION 29)

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times per day

28a. Each time you ate **coleslaw**, how much did you usually eat?

☐ Less than ¼ cup
☐ ¼ to ¾ cup
☐ More than ¾ cup

29. How often did you eat **sauerkraut** or **cabbage** (other than coleslaw)?

☐ NEVER (GO TO QUESTION 30)

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times per day

29a. Each time you ate **sauerkraut** or **cabbage**, how much did you usually eat?

☐ Less than ¼ cup
☐ ¼ to 1 cup
☐ More than 1 cup

30. How often did you eat **carrots** (fresh, canned, or frozen)?

☐ NEVER (GO TO QUESTION 31)

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times per day

30a. Each time you ate **carrots**, how much did you usually eat?

☐ Less than ¼ cup or less than 2 baby carrots
☐ ¼ to ½ cup or 2 to 5 baby carrots
☐ More than ½ cup or more than 5 baby carrots

Question 31 appears in the next column

31. How often did you eat **string beans** or **green beans** (fresh, canned, or frozen)?

☐ NEVER (GO TO QUESTION 32)

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times per day

31a. Each time you ate **string beans** or **green beans**, how much did you usually eat?

☐ Less than ½ cup
☐ ½ to 1 cup
☐ More than 1 cup

32. How often did you eat **peas** (fresh, canned, or frozen)?

☐ NEVER (GO TO QUESTION 33)

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times per day

32a. Each time you ate **peas**, how much did you usually eat?

☐ Less than ¼ cup
☐ ¼ to ¾ cup
☐ More than ¾ cup

33. Over the past 12 months, did you eat **corn**?

☐ NO (GO TO QUESTION 34)

☐ YES

33a. How often did you eat **fresh corn WHEN IN SEASON**?

☐ NEVER

☐ 1–6 times per season   ☐ 2 times per week
☐ 7–11 times per season   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times per day

Question 34 appears on the next page

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Over the past 12 months...

33b. How often did you eat **corn** (fresh, canned, or frozen) **DURING THE REST OF THE YEAR**?

☐ NEVER

☐ 1–6 times per year ☐ 2 times per week
☐ 7–11 times per year ☐ 3–4 times per week
☐ 1 time per month ☐ 5–6 times per week
☐ 2–3 times per month ☐ 1 time per day
☐ 1 time per week ☐ 2 or more times per day

33c. Each time you ate **corn**, how much did you usually eat?

☐ Less than 1 ear or less than ½ cup
☐ 1 ear or ½ to 1 cup
☐ More than 1 ear or more than 1 cup

34. Over the past 12 months, how often did you eat **broccoli** (fresh or frozen)?

☐ NEVER (GO TO QUESTION 35)

☐ 1–6 times per year ☐ 2 times per week
☐ 7–11 times per year ☐ 3–4 times per week
☐ 1 time per month ☐ 5–6 times per week
☐ 2–3 times per month ☐ 1 time per day
☐ 1 time per week ☐ 2 or more times per day

34a. Each time you ate **broccoli**, how much did you usually eat?

☐ Less than ¼ cup
☐ ¼ to 1 cup
☐ More than 1 cup

35. How often did you eat **cauliflower** or **Brussels sprouts** (fresh or frozen)?

☐ NEVER (GO TO QUESTION 36)

☐ 1–6 times per year ☐ 2 times per week
☐ 7–11 times per year ☐ 3–4 times per week
☐ 1 time per month ☐ 5–6 times per week
☐ 2–3 times per month ☐ 1 time per day
☐ 1 time per week ☐ 2 or more times per day

35a. Each time you ate **cauliflower** or **Brussels sprouts**, how much did you usually eat?

☐ Less than ¼ cup
☐ ¼ to ½ cup
☐ More than ½ cup

Question 36 appears in the next column

36. How often did you eat mixed **vegetables**?

☐ NEVER (GO TO QUESTION 37)

☐ 1–6 times per year ☐ 2 times per week
☐ 7–11 times per year ☐ 3–4 times per week
☐ 1 time per month ☐ 5–6 times per week
☐ 2–3 times per month ☐ 1 time per day
☐ 1 time per week ☐ 2 or more times per day

36a. Each time you ate **mixed vegetables**, how much did you usually eat?

☐ Less than ½ cup
☐ ½ to 1 cup
☐ More than 1 cup

37. How often did you eat **onions**?

☐ NEVER (GO TO QUESTION 38)

☐ 1–6 times per year ☐ 2 times per week
☐ 7–11 times per year ☐ 3–4 times per week
☐ 1 time per month ☐ 5–6 times per week
☐ 2–3 times per month ☐ 1 time per day
☐ 1 time per week ☐ 2 or more times per day

37a. Each time you ate **onions**, how much did you usually eat?

☐ Less than 1 slice or less than 1 tablespoon
☐ 1 slice or 1 to 4 tablespoons
☐ More than 1 slice or more than 4 tablespoons

38. Now think about all the **cooked vegetables** you ate in the past 12 months and how they were prepared. How often were your vegetables **COOKED WITH** some sort of **fat**, including oil spray? *(Please do not include potatoes.)*

☐ NEVER (GO TO QUESTION 39)

☐ 1–6 times per year ☐ 2 times per week
☐ 7–11 times per year ☐ 3–4 times per week
☐ 1 time per month ☐ 5–6 times per week
☐ 2–3 times per month ☐ 1 time per day
☐ 1 time per week ☐ 2 or more times per day

Question 39 appears on the next page

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Over the past 12 months…

38a. Which fats were usually added to your vegetables **DURING COOKING**? *(Please do not include potatoes. **Mark all that apply.**)*

- ☐ Margarine (including low-fat)
- ☐ Butter (including low-fat)
- ☐ Lard, fatback, or bacon fat
- ☐ Olive oil
- ☐ Corn oil
- ☐ Canola or rapeseed oil
- ☐ Oil spray, such as Pam or others
- ☐ Other kinds of oils
- ☐ None of the above

39. Now, thinking again about all the **cooked vegetables** you ate in the past 12 months, how often was some sort of fat, sauce, or dressing added **AFTER COOKING OR AT THE TABLE**? *(Please do not include potatoes.)*

☐ NEVER (GO TO QUESTION 40)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1–2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 times per day
- ☐ 3 or more times per day

39a. Which fats, sauces, or dressings were usually added **AFTER COOKING OR AT THE TABLE**? *(Please do not include potatoes. **Mark all that apply.**)*

- ☐ Margarine (including low-fat)
- ☐ Butter (including low-fat)
- ☐ Lard, fatback, or bacon fat
- ☐ Salad dressing
- ☐ Cheese sauce
- ☐ White sauce
- ☐ Other

39b. If margarine, butter, lard, fatback, or bacon fat was added to your cooked vegetables **AFTER COOKING OR AT THE TABLE**, how much did you usually add?

- ☐ Did not usually add these
- ☐ Less than 1 teaspoon
- ☐ 1 to 3 teaspoons
- ☐ More than 3 teaspoons

39c. If salad dressing, cheese sauce, or white sauce was added to your cooked vegetables **AFTER COOKING OR AT THE TABLE**, how much did you usually add?

- ☐ Did not usually add these
- ☐ Less than 1 tablespoon
- ☐ 1 to 3 tablespoons
- ☐ More than 3 tablespoons

Question 40 appears in the next column

40. Over the past 12 months, how often did you eat **sweet peppers** (green, red, or yellow)?

☐ NEVER (GO TO QUESTION 41)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

40a. Each time you ate **sweet peppers**, how much did you usually eat?

- ☐ Less than ⅛ pepper
- ☐ ⅛ to ¼ pepper
- ☐ More than ¼ pepper

41. Over the past 12 months, did you eat **fresh tomatoes** (including those in salads)?

☐ NO (GO TO QUESTION 42)

☐ YES

41a. How often did you eat **fresh tomatoes** (including those in salads) **WHEN IN SEASON**?

☐ NEVER

- ☐ 1–6 times per season
- ☐ 7–11 times per season
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

41b. How often did you eat **fresh tomatoes** (including those in salads) **DURING THE REST OF THE YEAR**?

☐ NEVER

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

41c. Each time you ate **fresh tomatoes**, how much did you usually eat?

- ☐ Less than ¼ tomato
- ☐ ¼ to ½ tomato
- ☐ More than ½ tomato

Question 42 appears on the next page

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Over the past 12 months…

42. How often did you eat **lettuce salads** (with or without other vegetables)?

☐ NEVER (GO TO QUESTION 43)

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times per day

42a. Each time you ate **lettuce salads**, how much did you usually eat?

☐ Less than ¼ cup
☐ ¼ to 1¼ cups
☐ More than 1¼ cups

43. How often did you eat **salad dressing** (including low-fat) on salads?

☐ NEVER (GO TO QUESTION 44)

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times per day

43a. Each time you ate **salad dressing** on salads, how much did you usually eat?

☐ Less than 2 tablespoons
☐ 2 to 4 tablespoons
☐ More than 4 tablespoons

44. How often did you eat **sweet potatoes** or **yams**?

☐ NEVER (GO TO QUESTION 45)

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times per day

44a. Each time you ate **sweet potatoes** or **yams**, how much did you usually eat?

☐ 1 small potato or less than ¼ cup
☐ 1 medium potato or ¼ to ¾ cup
☐ 1 large potato or more than ¾ cup

Question 45 appears in the next column

45. How often did you eat **French fries, home fries, hash browned potatoes,** or **tater tots**?

☐ NEVER (GO TO QUESTION 46)

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times per day

45a. Each time you ate **French fries**, **home fries**, **hash browned potatoes**, or **tater tots** how much did you usually eat?

☐ Less than 10 fries or less than ½ cup
☐ 10 to 25 fries or ½ to 1 cup
☐ More than 25 fries or more than 1 cup

46. How often did you eat **potato salad**?

☐ NEVER (GO TO QUESTION 47)

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times per day

46a. Each time you ate **potato salad**, how much did you usually eat?

☐ Less than ½ cup
☐ ½ to 1 cup
☐ More than 1 cup

47. How often did you eat **baked, boiled,** or **mashed potatoes**?

☐ NEVER (GO TO QUESTION 48)

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times per day

47a. Each time you ate **baked, boiled,** or **mashed potatoes**, how much did you usually eat?

☐ 1 small potato or less than ½ cup
☐ 1 medium potato or ½ to 1 cup
☐ 1 large potato or more than 1 cup

Question 48 appears on the next page

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Over the past 12 months...

47b. How often was **sour cream** (including low-fat) added to your potatoes, **EITHER IN COOKING OR AT THE TABLE**?

☐ Almost never or never (GO TO QUESTION 47d)
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

47c. Each time **sour cream** was added to your potatoes, how much was usually added?

☐ Less than 1 tablespoon
☐ 1 to 3 tablespoons
☐ More than 3 tablespoons

47d. How often was **margarine** (including low-fat) added to your potatoes, **EITHER IN COOKING OR AT THE TABLE**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

47e. How often was **butter** (including low-fat) added to your potatoes, **EITHER IN COOKING OR AT THE TABLE**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

47f. Each time **margarine** or **butter** was added to your potatoes, how much was usually added?

☐ Never added
☐ Less than 1 teaspoon
☐ 1 to 3 teaspoons
☐ More than 3 teaspoons

47g. How often was **cheese** or **cheese sauce** added to your potatoes, **EITHER IN COOKING OR AT THE TABLE**?

☐ Almost never or never (GO TO QUESTION 48)
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

Question 48 appears in the next column

47h. Each time **cheese** or **cheese sauce** was added to your potatoes, how much was usually added?

☐ Less than 1 tablespoon
☐ 1 to 3 tablespoons
☐ More than 3 tablespoons

48. How often did you eat **salsa**?

☐ NEVER (GO TO QUESTION 49)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

48a. Each time you ate **salsa**, how much did you usually eat?

☐ Less than 1 tablespoon
☐ 1 to 5 tablespoons
☐ More than 5 tablespoons

49. How often did you eat **catsup**?

☐ NEVER (GO TO QUESTION 50)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

49a. Each time you ate **catsup**, how much did you usually eat?

☐ Less than 1 teaspoon
☐ 1 to 6 teaspoons
☐ More than 6 teaspoons

50. How often did you eat **stuffing, dressing,** or **dumplings**?

☐ NEVER (GO TO QUESTION 51)

☐ 1–6 times per year
☐ 7–11 times per year
☐ 1 time per month
☐ 2–3 times per month
☐ 1 time per week
☐ 2 times per week
☐ 3–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2 or more times per day

50a. Each time you ate **stuffing, dressing,** or **dumplings**, how much did you usually eat?

☐ Less than ½ cup
☐ ½ to 1 cup
☐ More than 1 cup

Question 51 appears on the next page

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Over the past 12 months...

51. How often did you eat **chili**?

☐ NEVER (GO TO QUESTION 52)

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times per day

51a. Each time you ate **chili**, how much did you usually eat?

☐ Less than ½ cup
☐ ½ to 1¾ cups
☐ More than 1¾ cups

52. How often did you eat **Mexican foods** (such as tacos, tostados, burritos, tamales, fajitas, enchiladas, quesadillas, and chimichangas)?

☐ NEVER (GO TO QUESTION 53)

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times per day

52a. Each time you ate **Mexican foods**, how much did you usually eat?

☐ Less than 1 taco, burrito, etc.
☐ 1 to 2 tacos, burritos, etc.
☐ More than 2 tacos, burritos, etc.

53. How often did you eat **cooked dried beans** (such as baked beans, pintos, kidney, blackeyed peas, lima, lentils, soybeans, or refried beans)? *(Please don't include bean soups or chili.)*

☐ NEVER (GO TO QUESTION 54)

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times per day

53a. Each time you ate **beans**, how much did you usually eat?

☐ Less than ½ cup
☐ ½ to 1 cup
☐ More than 1 cup

Question 54 appears in the next column

53b. How often were the beans you ate **refried beans, beans prepared with any type of fat**, or **with meat added**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

54. How often did you eat **other kinds of vegetables**?

☐ NEVER (GO TO QUESTION 55)

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times per day

54a. Each time you ate **other kinds of vegetables**, how much did you usually eat?

☐ Less than ¼ cup
☐ ¼ to ½ cup
☐ More than ½ cup

55. How often did you eat **rice** or **other cooked grains** (such as bulgur, cracked wheat, or millet)?

☐ NEVER (GO TO QUESTION 56)

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times per day

55a. Each time you ate **rice** or **other cooked grains**, how much did you usually eat?

☐ Less than ½ cup
☐ ½ to 1½ cups
☐ More than 1½ cups

55b. How often was **butter, margarine**, or **oil** added to your rice **IN COOKING OR AT THE TABLE**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

Question 56 appears on the next page

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Over the past 12 months...

56. How often did you eat **pancakes, waffles,** or **French toast**?

☐ NEVER (GO TO QUESTION 57)

☐ 1–6 times per year ☐ 2 times per week
☐ 7–11 times per year ☐ 3–4 times per week
☐ 1 time per month ☐ 5–6 times per week
☐ 2–3 times per month ☐ 1 time per day
☐ 1 time per week ☐ 2 or more times per day

56a. Each time you ate **pancakes, waffles,** or **French toast**, how much did you usually eat?

☐ Less than 1 medium piece
☐ 1 to 3 medium pieces
☐ More than 3 medium pieces

56b. How often was **margarine** (including low-fat) added to your pancakes, waffles, or French toast **AFTER COOKING OR AT THE TABLE**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

56c. How often was **butter** (including low-fat) added to your pancakes, waffles, or French toast **AFTER COOKING OR AT THE TABLE**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

56d. Each time **margarine** or **butter** was added to your pancakes, waffles, or French toast, how much was usually added?

☐ Never added
☐ Less than 1 teaspoon
☐ 1 to 3 teaspoons
☐ More than 3 teaspoons

56e. How often was **syrup** added to your pancakes, waffles, or French toast?

☐ Almost never or never (GO TO QUESTION 57)
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

Question 57 appears in the next column

56f. Each time **syrup** was added to your pancakes, waffles, or French toast, how much was usually added?

☐ Less than 1 tablespoon
☐ 1 to 4 tablespoons
☐ More than 4 tablespoons

57. How often did you eat **lasagna, stuffed shells, stuffed manicotti, ravioli,** or **tortellini**? *(Please do not include spaghetti or other pasta.)*

☐ NEVER (GO TO QUESTION 58)

☐ 1–6 times per year ☐ 2 times per week
☐ 7–11 times per year ☐ 3–4 times per week
☐ 1 time per month ☐ 5–6 times per week
☐ 2–3 times per month ☐ 1 time per day
☐ 1 time per week ☐ 2 or more times per day

57a. Each time you ate **lasagna, stuffed shells, stuffed manicotti, ravioli,** or **tortellini**, how much did you usually eat?

☐ Less than 1 cup
☐ 1 to 2 cups
☐ More than 2 cups

58. How often did you eat **macaroni and cheese**?

☐ NEVER (GO TO QUESTION 59)

☐ 1–6 times per year ☐ 2 times per week
☐ 7–11 times per year ☐ 3–4 times per week
☐ 1 time per month ☐ 5–6 times per week
☐ 2–3 times per month ☐ 1 time per day
☐ 1 time per week ☐ 2 or more times per day

58a. Each time you ate **macaroni and cheese**, how much did you usually eat?

☐ Less than 1 cup
☐ 1 to 1½ cups
☐ More than 1½ cups

59. How often did you eat **pasta salad** or **macaroni salad**?

☐ NEVER (GO TO QUESTION 60)

☐ 1–6 times per year ☐ 2 times per week
☐ 7–11 times per year ☐ 3–4 times per week
☐ 1 time per month ☐ 5–6 times per week
☐ 2–3 times per month ☐ 1 time per day
☐ 1 time per week ☐ 2 or more times per day

Question 60 appears on the next page

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Over the past 12 months…

59a. Each time you ate **pasta salad** or **macaroni salad**, how much did you usually eat?

☐ Less than ½ cup
☐ ½ to 1 cup
☐ More than 1 cup

60. Other than the pastas listed in Questions 57, 58, and 59, how often did you eat **pasta, spaghetti**, or **other noodles**?

☐ NEVER (GO TO QUESTION 61)

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times per day

60a. Each time you ate **pasta, spaghetti, or other noodles**, how much did you usually eat?

☐ Less than 1 cup
☐ 1 to 3 cups
☐ More than 3 cups

60b. How often did you eat your pasta, spaghetti, or other noodles with **tomato sauce** or **spaghetti sauce made WITH meat**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

60c. How often did you eat your pasta, spaghetti, or other noodles with **tomato sauce** or **spaghetti sauce made WITHOUT meat**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

60d. How often did you eat your pasta, spaghetti, or other noodles with **margarine, butter, oil,** or **cream sauce**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

Question 61 appears in the next column

61. How often did you eat **bagels** or **English muffins**?

☐ NEVER (GO TO INTRODUCTION TO QUESTION 62)

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times per day

61a. Each time you ate **bagels** or **English muffins**, how many did you usually eat?

☐ Less than 1 bagel or English muffin
☐ 1 bagel or English muffin
☐ More than 1 bagel or English muffin

61b. How often was **margarine** (including low-fat) added to your bagels or English muffins?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

61c. How often was **butter** (including low-fat) added to your bagels or English muffins?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

61d. Each time **margarine** or **butter** was added to your bagels or English muffins, how much was usually added?

☐ Never added
☐ Less than 1 teaspoon
☐ 1 to 2 teaspoons
☐ More than 2 teaspoons

61e. How often was **cream cheese** (including low-fat) spread on your bagels or English muffins?

☐ Almost never or never (GO TO INTRODUCTION TO QUESTION 62)
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

Introduction to Question 62 appears on the next page

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Over the past 12 months...

61f. Each time **cream cheese** was added to your bagels or English muffins, how much was usually added?

☐ Less than 1 tablespoon
☐ 1 to 2 tablespoons
☐ More than 2 tablespoons

The next questions ask about your intake of breads other than bagels or English muffins. First, we will ask about bread you ate as part of sandwiches only. Then we will ask about all other bread you ate.

62. How often did you eat **breads** or **rolls AS PART OF SANDWICHES** (including burger and hot dog rolls)?

☐ NEVER (GO TO QUESTION 63)

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times per day

62a. Each time you ate **breads** or **rolls AS PART OF SANDWICHES**, how many did you usually eat?

☐ 1 slice or ½ roll
☐ 2 slices or 1 roll
☐ More than 2 slices or more than 1 roll

62b. How often were the breads or rolls that you used for your sandwiches **white bread** (including burger and hot dog rolls)?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

62c. How often was **mayonnaise** or **mayonnaise-type dressing** (including low-fat) added to your sandwich bread or rolls?

☐ Almost never or never (GO TO QUESTION 62e)
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

Question 62e appears in the next column
Question 63 appears in the next column

62d. Each time **mayonnaise** or **mayonnaise-type dressing** was added to your sandwich breads or rolls, how much was usually added?

☐ Less than 1 teaspoon
☐ 1 to 3 teaspoons
☐ More than 3 teaspoons

62e. How often was **margarine** (including low-fat) added to your sandwich bread or rolls?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

62f. How often was **butter** (including low-fat) added to your sandwich bread or rolls?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

62g. Each time **margarine** or **butter** was added to your sandwich breads or rolls, how much was usually added?

☐ Never added
☐ Less than 1 teaspoon
☐ 1 to 2 teaspoons
☐ More than 2 teaspoons

63. How often did you eat **breads** or **dinner rolls, NOT AS PART OF SANDWICHES**?

☐ NEVER (GO TO QUESTION 64)

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times per day

63a. Each time you ate **breads** or **dinner rolls, NOT AS PART OF SANDWICHES**, how much did you usually eat?

☐ 1 slice or 1 dinner roll
☐ 2 slices or 2 dinner rolls
☐ More than 2 slices or 2 dinner rolls

Question 64 appears on the next page

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Over the past 12 months…

63b. How often were the breads or rolls you ate **white bread**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

63c. How often was **margarine** (including low-fat) added to your breads or rolls?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

63d. How often was **butter** (including low-fat) added to your breads or rolls?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

63e. Each time **margarine** or **butter** was added to your breads or rolls, how much was usually added?

☐ Never added
☐ Less than 1 teaspoon
☐ 1 to 2 teaspoons
☐ More than 2 teaspoons

63f. How often was **cream cheese** (including low-fat) added to your breads or rolls?

☐ Almost never or never (GO TO QUESTION 64)
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

63g. Each time **cream cheese** was added to your breads or rolls, how much was usually added?

☐ Less than 1 tablespoon
☐ 1 to 2 tablespoons
☐ More than 2 tablespoons
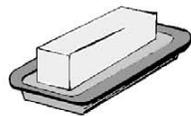
Question 64 appears in the next column

64. How often did you eat **jam, jelly,** or **honey** on bagels, muffins, bread, rolls, or crackers?

☐ NEVER (GO TO QUESTION 65)

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times per day

64a. Each time you ate **jam, jelly,** or **honey,** how much did you usually eat?

☐ Less than 1 teaspoon
☐ 1 to 3 teaspoons
☐ More than 3 teaspoons

65. How often did you eat **peanut butter** or **other nut butter**?

☐ NEVER (GO TO QUESTION 66)

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times per day

65a. Each time you ate **peanut butter** or **other nut butter**, how much did you usually eat?

☐ Less than 1 tablespoon
☐ 1 to 2 tablespoons
☐ More than 2 tablespoons

66. How often did you eat **roast beef** or **steak IN SANDWICHES**?

☐ NEVER (GO TO QUESTION 67)

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times per day

66a. Each time you ate **roast beef** or **steak IN SANDWICHES**, how much did you usually eat?

☐ Less than 1 slice or less than 2 ounces
☐ 1 to 2 slices or 2 to 4 ounces
☐ More than 2 slices or more than 4 ounces

Question 67 appears on the next page

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Over the <u>past 12 months</u>...

67. How often did you eat **turkey** or **chicken COLD CUTS** (such as loaf, luncheon meat, turkey ham, turkey salami, or turkey pastrami)? (*We will ask about other turkey or chicken later.*)

☐ NEVER (GO TO QUESTION 68)

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times per day

67a. Each time you ate **turkey or chicken COLD CUTS**, how much did you usually eat?

☐ Less than 1 slice
☐ 1 to 3 slices
☐ More than 3 slices

68. How often did you eat **luncheon** or **deli-style ham**? (*We will ask about other ham later.*)

☐ NEVER (GO TO QUESTION 69)

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times per day

68a. Each time you ate **luncheon** or **deli-style ham**, how much did you usually eat?

☐ Less than 1 slice
☐ 1 to 3 slices
☐ More than 3 slices

68b. How often was the luncheon or deli-style ham you ate **light**, **low-fat**, or **fat-free**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

69. How often did you eat **other cold cuts** or **luncheon meats** (such as bologna, salami, corned beef, pastrami, or others, including low-fat)? (*Please do not include ham, turkey, or chicken cold cuts.*)

☐ NEVER (GO TO QUESTION 70)

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times per day

69a. Each time you ate **other cold cuts** or **luncheon meats**, how much did you usually eat?

☐ Less than 1 slice
☐ 1 to 3 slices
☐ More than 3 slices

69b. How often were the other cold cuts or luncheon meats you ate **light, low-fat, or fat-free cold cuts** or **luncheon meats**? (*Please do not include ham, turkey, or chicken cold cuts.*)

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

70. How often did you eat **canned tuna** (including in salads, sandwiches, or casseroles)?

☐ NEVER (GO TO QUESTION 71)

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times per day

70a. Each time you ate **canned tuna**, how much did you usually eat?

☐ Less than ¼ cup or less than 2 ounces
☐ ¼ to ½ cup or 2 to 3 ounces
☐ More than ½ cup or more than 3 ounces

70b. How often was the canned tuna you ate **water-packed tuna**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

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Over the past 12 months…

70c. How often was the canned tuna you ate **prepared with mayonnaise** or **other dressing** (including low-fat)?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

71. How often did you eat **GROUND chicken** or **turkey**? *(We will ask about other chicken and turkey later.)*

☐ NEVER (GO TO QUESTION 72)

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times per day

71a. Each time you ate **GROUND chicken** or **turkey**, how much did you usually eat?

☐ Less than 2 ounces or less than ½ cup
☐ 2 to 4 ounces or ½ to 1 cup
☐ More than 4 ounces or more than 1 cup

72. How often did you eat **beef hamburgers** or **cheeseburgers**?

☐ NEVER (GO TO QUESTION 73)

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times per day

72a. Each time you ate **beef hamburgers** or **cheeseburgers**, how much did you usually eat?

☐ Less than 1 patty or less than 2 ounces
☐ 1 patty or 2 to 4 ounces
☐ More than 1 patty or more than 4 ounces

72b. How often were the beef hamburgers or cheeseburgers you ate made with **lean ground beef**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

Question 73 appears in the next column

73. How often did you eat **ground beef in mixtures** (such as meatballs, casseroles, chili, or meatloaf)?

☐ NEVER (GO TO QUESTION 74)

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times per day

73a. Each time you ate **ground beef in mixtures**, how much did you usually eat?

☐ Less than 3 ounces or less than ½ cup
☐ 3 to 8 ounces or ½ to 1 cup
☐ More than 8 ounces or more than 1 cup

74. How often did you eat **hot dogs** or **frankfurters**? *(Please do not include sausages or vegetarian hot dogs.)*

☐ NEVER (GO TO QUESTION 75)

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times per day

74a. Each time you ate **hot dogs** or **frankfurters**, how many did you usually eat?

☐ Less than 1 hot dog
☐ 1 to 2 hot dogs
☐ More than 2 hot dogs

74b. How often were the hot dogs or frankfurters you ate **light** or **low-fat hot dogs**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

Question 75 appears on the next page

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Over the past 12 months…

75. How often did you eat beef mixtures such as **beef stew, beef pot pie, beef and noodles,** or **beef and vegetables**?

    ☐ NEVER (GO TO QUESTION 76)

    ☐ 1–6 times per year   ☐ 2 times per week
    ☐ 7–11 times per year ☐ 3–4 times per week
    ☐ 1 time per month   ☐ 5–6 times per week
    ☐ 2–3 times per month ☐ 1 time per day
    ☐ 1 time per week   ☐ 2 or more times per day

75a. Each time you ate **beef stew, beef pot pie, beef and noodles,** or **beef and vegetables,** how much did you usually eat?

    ☐ Less than 1 cup
    ☐ 1 to 2 cups
    ☐ More than 2 cups

76. How often did you eat **roast beef** or **pot roast**? *(Please do not include roast beef or pot roast in sandwiches.)*

    ☐ NEVER (GO TO QUESTION 77)

    ☐ 1–6 times per year   ☐ 2 times per week
    ☐ 7–11 times per year ☐ 3–4 times per week
    ☐ 1 time per month   ☐ 5–6 times per week
    ☐ 2–3 times per month ☐ 1 time per day
    ☐ 1 time per week   ☐ 2 or more times per day

76a. Each time you ate **roast beef** or **pot roast** (including in mixtures), how much did you usually eat?

    ☐ Less than 2 ounces
    ☐ 2 to 5 ounces
    ☐ More than 5 ounces

77. How often did you eat **steak** (beef)? *(Do not include steak in sandwiches)*

    ☐ NEVER (GO TO QUESTION 78)

    ☐ 1–6 times per year   ☐ 2 times per week
    ☐ 7–11 times per year ☐ 3–4 times per week
    ☐ 1 time per month   ☐ 5–6 times per week
    ☐ 2–3 times per month ☐ 1 time per day
    ☐ 1 time per week   ☐ 2 or more times per day

77a. Each time you ate **steak** (beef), how much did you usually eat?

    ☐ Less than 3 ounces
    ☐ 3 to 7 ounces
    ☐ More than 7 ounces

Question 78 appears in the next column

77b. How often was the steak you ate **lean steak**?

    ☐ Almost never or never
    ☐ About ¼ of the time
    ☐ About ½ of the time
    ☐ About ¾ of the time
    ☐ Almost always or always

78. How often did you eat **pork** or **beef spareribs**?

    ☐ NEVER (GO TO QUESTION 79)

    ☐ 1–6 times per year   ☐ 2 times per week
    ☐ 7–11 times per year ☐ 3–4 times per week
    ☐ 1 time per month   ☐ 5–6 times per week
    ☐ 2–3 times per month ☐ 1 time per day
    ☐ 1 time per week   ☐ 2 or more times per day

78a. Each time you ate **pork** or **beef spareribs,** how much did you usually eat?

    ☐ Less than 4 ribs
    ☐ 4 to 12 ribs
    ☐ More than 12 ribs

79. How often did you eat **roast turkey, turkey cutlets,** or **turkey nuggets** (including in sandwiches)?

    ☐ NEVER (GO TO QUESTION 80)

    ☐ 1–6 times per year   ☐ 2 times per week
    ☐ 7–11 times per year ☐ 3–4 times per week
    ☐ 1 time per month   ☐ 5–6 times per week
    ☐ 2–3 times per month ☐ 1 time per day
    ☐ 1 time per week   ☐ 2 or more times per day

79a. Each time you ate **roast turkey, turkey cutlets,** or **turkey nuggets,** how much did you usually eat? *(Please note: 4 to 8 turkey nuggets = 3 ounces.)*

    ☐ Less than 2 ounces
    ☐ 2 to 4 ounces
    ☐ More than 4 ounces

80. How often did you eat **chicken** as part of **salads, sandwiches, casseroles, stews,** or **other mixtures**?

    ☐ NEVER (GO TO QUESTION 81)

    ☐ 1–6 times per year   ☐ 2 times per week
    ☐ 7–11 times per year ☐ 3–4 times per week
    ☐ 1 time per month   ☐ 5–6 times per week
    ☐ 2–3 times per month ☐ 1 time per day
    ☐ 1 time per week   ☐ 2 or more times per day

Question 81 appears on the next page

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Over the past 12 months...

80a. Each time you ate **chicken** as part of **salads, sandwiches, casseroles, stews,** or **other mixtures,** how much did you usually eat?

☐ Less than ½ cup
☐ ½ to 1½ cups
☐ More than 1½ cups

81. How often did you eat **baked, broiled, roasted, stewed,** or **fried chicken** (including nuggets)? *(Please do not include chicken in mixtures.)*

☐ NEVER (GO TO QUESTION 82)

☐ 1–6 times per year ☐ 2 times per week
☐ 7–11 times per year ☐ 3–4 times per week
☐ 1 time per month ☐ 5–6 times per week
☐ 2–3 times per month ☐ 1 time per day
☐ 1 time per week ☐ 2 or more times per day

81a. Each time you ate **baked, broiled, roasted, stewed,** or **fried chicken** (including nuggets), how much did you usually eat?

☐ Less than 2 drumsticks or wings, less than 1 breast or thigh, or less than 4 nuggets
☐ 2 drumsticks or wings, 1 breast or thigh, or 4 to 8 nuggets
☐ More than 2 drumsticks or wings, more than 1 breast or thigh, or more than 8 nuggets

81b. How often was the chicken you ate **fried chicken** (including deep fried) or **chicken nuggets**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

81c. How often was the chicken you ate **WHITE meat**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

81d. How often did you eat chicken **WITH skin**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

Question 82 appears in the next column

82. How often did you eat **baked ham** or **ham steak**?

☐ NEVER (GO TO QUESTION 83)

☐ 1–6 times per year ☐ 2 times per week
☐ 7–11 times per year ☐ 3–4 times per week
☐ 1 time per month ☐ 5–6 times per week
☐ 2–3 times per month ☐ 1 time per day
☐ 1 time per week ☐ 2 or more times per day

82a. Each time you ate **baked ham** or **ham steak**, how much did you usually eat?

☐ Less than 1 ounce
☐ 1 to 3 ounces
☐ More than 3 ounces

83. How often did you eat **pork** (including chops, roasts, and in mixed dishes)? *(Please do not include ham, ham steak, or sausage.)*

☐ NEVER (GO TO QUESTION 84)

☐ 1–6 times per year ☐ 2 times per week
☐ 7–11 times per year ☐ 3–4 times per week
☐ 1 time per month ☐ 5–6 times per week
☐ 2–3 times per month ☐ 1 time per day
☐ 1 time per week ☐ 2 or more times per day

83a. Each time you ate **pork**, how much did you usually eat?

☐ Less than 2 ounces or less than 1 chop
☐ 2 to 5 ounces or 1 chop
☐ More than 5 ounces or more than 1 chop

84. How often did you eat **gravy** on meat, chicken, potatoes, rice, etc.?

☐ NEVER (GO TO QUESTION 85)

☐ 1–6 times per year ☐ 2 times per week
☐ 7–11 times per year ☐ 3–4 times per week
☐ 1 time per month ☐ 5–6 times per week
☐ 2–3 times per month ☐ 1 time per day
☐ 1 time per week ☐ 2 or more times per day

84a. Each time you ate **gravy** on meat, chicken, potatoes, rice, etc., how much did you usually eat?

☐ Less than ⅛ cup
☐ ⅛ to ½ cup
☐ More than ½ cup

Question 85 appears on the next page

Over the past 12 months…

85. How often did you eat **liver** (all kinds) or **liverwurst**?

☐ NEVER (GO TO QUESTION 86)

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times per day

85a. Each time you ate **liver** or **liverwurst**, how much did you usually eat?

☐ Less than 1 ounce
☐ 1 to 4 ounces
☐ More than 4 ounces

86. How often did you eat **bacon** (including low-fat)?

☐ NEVER (GO TO QUESTION 87)

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times per day

86a. Each time you ate **bacon**, how much did you usually eat?

☐ Fewer than 2 slices
☐ 2 to 3 slices
☐ More than 3 slices

86b. How often was the bacon you ate **light, low-fat**, or **lean bacon**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

87. How often did you eat **sausage** (including low-fat)?

☐ NEVER (GO TO QUESTION 88)

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times per day

Question 88 appears in the next column

87a. Each time you ate **sausage**, how much did you usually eat?

☐ Less than 1 patty or 2 links
☐ 1 to 3 patties or 2 to 5 links
☐ More than 3 patties or 5 links

87b. How often was the sausage you ate **light, low-fat**, or **lean sausage**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

88. How often did you eat **fish sticks** or **fried fish** (including fried seafood or shellfish)?

☐ NEVER (GO TO QUESTION 89)

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times per day

88a. Each time you ate **fish sticks** or **fried fish**, how much did you usually eat?

☐ Less than 2 ounces or less than 1 fillet
☐ 2 to 7 ounces or 1 fillet
☐ More than 7 ounces or more than 1 fillet

89. How often did you eat **fish** or **seafood that was NOT FRIED** (including shellfish)?

☐ NEVER (GO TO INTRODUCTION TO QUESTION 90)

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times per day

89a. Each time you ate eat **fish** or **seafood that was NOT FRIED**, how much did you usually eat?

☐ Less than 2 ounces or less than 1 fillet
☐ 2 to 5 ounces or 1 fillet
☐ More than 5 ounces or more than 1 fillet

Introduction to Question 90 appears on the next page

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Over the past 12 months…

Now think about all the meat, poultry, and fish you ate in the past 12 months and how they were prepared.

90. How often was **oil, butter, margarine, or other fat** used to **FRY, SAUTE, BASTE, OR MARINATE** any meat, poultry, or fish you ate? *(Please do not include deep frying.)*

    ☐ NEVER (GO TO QUESTION 91)

    ☐ 1–6 times per year ☐ 2 times per week
    ☐ 7–11 times per year ☐ 3–4 times per week
    ☐ 1 time per month ☐ 5–6 times per week
    ☐ 2–3 times per month ☐ 1 time per day
    ☐ 1 time per week ☐ 2 or more times per day

90a. Which of the following **fats** were regularly used to prepare your meat, poultry, or fish? ***(Mark all that apply.)***

    ☐ Margarine (including low-fat) ☐ Corn oil
    ☐ Canola or rapeseed oil
    ☐ Butter (including low-fat) ☐ Oil spray, such as Pam or others
    ☐ Lard, fatback, or bacon fat ☐ Other kinds of oils
    ☐ None of the above
    ☐ Olive oil

91. How often did you eat **tofu, soy burgers, or soy meat-substitutes**?

    ☐ NEVER (GO TO QUESTION 92)

    ☐ 1–6 times per year ☐ 2 times per week
    ☐ 7–11 times per year ☐ 3–4 times per week
    ☐ 1 time per month ☐ 5–6 times per week
    ☐ 2–3 times per month ☐ 1 time per day
    ☐ 1 time per week ☐ 2 or more times per day

91a. Each time you ate **tofu, soy burgers, or soy meat-substitutes**, how much did you usually eat?

    ☐ Less than ¼ cup or less than 2 ounces
    ☐ ¼ to ½ cup or 2 to 4 ounces
    ☐ More than ½ cup or more than 4 ounces

92. Over the past 12 months, did you eat **soups**?

    ☐ NO (GO TO QUESTION 93)
    ☐ YES

92a. How often did you eat **soup DURING THE WINTER**?

    ☐ NEVER

    ☐ 1–6 times per winter ☐ 2 times per week
    ☐ 7–11 times per winter ☐ 3–4 times per week
    ☐ 1 time per month ☐ 5–6 times per week
    ☐ 2–3 times per month ☐ 1 time per day
    ☐ 1 time per week ☐ 2 or more times per day

92b. How often did you eat **soup DURING THE REST OF THE YEAR**?

    ☐ NEVER

    ☐ 1–6 times per year ☐ 2 times per week
    ☐ 7–11 times per year ☐ 3–4 times per week
    ☐ 1 time per month ☐ 5–6 times per week
    ☐ 2–3 times per month ☐ 1 time per day
    ☐ 1 time per week ☐ 2 or more times per day

92c. Each time you ate **soup**, how much did you usually eat?

    ☐ Less than 1 cup
    ☐ 1 to 2 cups
    ☐ More than 2 cups

92d. How often were the soups you ate **bean soups**?

    ☐ Almost never or never
    ☐ About ¼ of the time
    ☐ About ½ of the time
    ☐ About ¾ of the time
    ☐ Almost always or always

92e. How often were the soups you ate **cream soups** (including chowders)?

    ☐ Almost never or never
    ☐ About ¼ of the time
    ☐ About ½ of the time
    ☐ About ¾ of the time
    ☐ Almost always or always

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Over the past 12 months...

92f. How often were the soups you ate **tomato** or **vegetable soups**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

92g. How often were the soups you ate **broth soups** (including chicken) **with** or **without noodles** or **rice**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

93. How often did you eat **pizza**?

☐ NEVER (GO TO QUESTION 94)

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times per day

93a. Each time you ate **pizza**, how much did you usually eat?

☐ Less than 1 slice or less than 1 mini pizza
☐ 1 to 3 slices or 1 mini pizza
☐ More than 3 slices or more than 1 mini pizza

93b. How often did you eat pizza with **pepperoni, sausage,** or **other meat**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

94. How often did you eat **crackers**?

☐ NEVER (GO TO QUESTION 95)

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times per day

Question 95 appears in the next column

94a. Each time you ate **crackers**, how many did you usually eat?

☐ Fewer than 4 crackers
☐ 4 to 10 crackers
☐ More than 10 crackers

95. How often did you eat **corn bread** or **corn muffins**?

☐ NEVER (GO TO QUESTION 96)

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times per day

95a. Each time you ate **corn bread** or **corn muffins**, how much did you usually eat?

☐ Less than 1 piece or muffin
☐ 1 to 2 pieces or muffins
☐ More than 2 pieces or muffins

96. How often did you eat **biscuits**?

☐ NEVER (GO TO QUESTION 97)

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times per day

96a. Each time you ate **biscuits**, how many did you usually eat?

☐ Fewer than 1 biscuit
☐ 1 to 2 biscuits
☐ More than 2 biscuits

97. How often did you eat **potato chips, tortilla chips,** or **corn chips** (including low-fat, fat-free, or low-salt)?

☐ NEVER (GO TO QUESTION 98)

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times per day

Question 98 appears on the next page

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Over the past 12 months…

97a. Each time you ate **potato chips, tortilla chips,** or **corn chips**, how much did you usually eat?

☐ Fewer than 10 chips or less than 1 cup
☐ 10 to 25 chips or 1 to 2 cups
☐ More than 25 chips or more than 2 cups

97b. How often were the chips you ate **Wow chips** or other **chips made with fat substitute** (Olean or Olestra)?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

97c. How often were the chips you ate other **low-fat** or **fat-free chips**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

98. How often did you eat **popcorn** (including low-fat)?

☐ NEVER (GO TO QUESTION 99)

☐ 1–6 times per year ☐ 2 times per week
☐ 7–11 times per year ☐ 3–4 times per week
☐ 1 time per month ☐ 5–6 times per week
☐ 2–3 times per month ☐ 1 time per day
☐ 1 time per week ☐ 2 or more times per day

98a. Each time you ate **popcorn**, how much did you usually eat?

☐ Less than 2 cups, popped
☐ 2 to 5 cups, popped
☐ More than 5 cups, popped

99. How often did you eat **pretzels**?

☐ NEVER (GO TO QUESTION 100)

☐ 1–6 times per year ☐ 2 times per week
☐ 7–11 times per year ☐ 3–4 times per week
☐ 1 time per month ☐ 5–6 times per week
☐ 2–3 times per month ☐ 1 time per day
☐ 1 time per week ☐ 2 or more times per day

Question 100 appears in the next column

99a. Each time you ate **pretzels**, how many did you usually eat?

☐ Fewer than 5 average twists
☐ 5 to 20 average twists
☐ More than 20 average twists

100. How often did you eat **peanuts, walnuts, seeds,** or **other nuts**?

☐ NEVER (GO TO QUESTION 101)

☐ 1–6 times per year ☐ 2 times per week
☐ 7–11 times per year ☐ 3–4 times per week
☐ 1 time per month ☐ 5–6 times per week
☐ 2–3 times per month ☐ 1 time per day
☐ 1 time per week ☐ 2 or more times per day

100a. Each time you ate **peanuts, walnuts, seeds,** or **other nuts**, how much did you usually eat?

☐ Less than ¼ cup
☐ ¼ to ½ cup
☐ More than ½ cup

101. How often did you eat **energy, high-protein,** or **breakfast bars** such as **Power Bars, Balance, Clif,** or **others**?

☐ NEVER (GO TO QUESTION 102)

☐ 1–6 times per year ☐ 2 times per week
☐ 7–11 times per year ☐ 3–4 times per week
☐ 1 time per month ☐ 5–6 times per week
☐ 2–3 times per month ☐ 1 time per day
☐ 1 time per week ☐ 2 or more times per day

101a. Each time you ate **energy, high-protein,** or **breakfast bars**, how much did you usually eat?

☐ Less than 1 bar
☐ 1 bar
☐ More than 1 bar

102. How often did you eat **yogurt** (NOT including frozen yogurt)?

☐ NEVER (GO TO QUESTION 103)

☐ 1–6 times per year ☐ 2 times per week
☐ 7–11 times per year ☐ 3–4 times per week
☐ 1 time per month ☐ 5–6 times per week
☐ 2–3 times per month ☐ 1 time per day
☐ 1 time per week ☐ 2 or more times per day

Question 103 appears on the next page

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Over the past 12 months…

102a. Each time you ate **yogurt**, how much did you usually eat?

☐ Less than ½ cup or less than 1 container
☐ ½ to 1 cup or 1 container
☐ More than 1 cup or more than 1 container

103. How often did you eat **cottage cheese** (including low-fat)?

☐ NEVER (GO TO QUESTION 104)

☐ 1–6 times per year ☐ 2 times per week
☐ 7–11 times per year ☐ 3–4 times per week
☐ 1 time per month ☐ 5–6 times per week
☐ 2–3 times per month ☐ 1 time per day
☐ 1 time per week ☐ 2 or more times per day

103a. Each time you ate **cottage cheese**, how much did you usually eat?

☐ Less than ¼ cup
☐ ¼ to 1 cup
☐ More than 1 cup

104. How often did you eat **cheese** (including low-fat; including on cheeseburgers or in sandwiches or subs)?

☐ NEVER (GO TO QUESTION 105)

☐ 1–6 times per year ☐ 2 times per week
☐ 7–11 times per year ☐ 3–4 times per week
☐ 1 time per month ☐ 5–6 times per week
☐ 2–3 times per month ☐ 1 time per day
☐ 1 time per week ☐ 2 or more times per day

104a. Each time you ate **cheese**, how much did you usually eat?

☐ Less than ½ ounce or less than 1 slice
☐ ½ to 1½ ounces or 1 slice
☐ More than 1½ ounces or more than 1 slice

104b. How often was the cheese you ate **light** or **low-fat cheese**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

104c. How often was the cheese you ate **fat-free cheese**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

105. How often did you eat **frozen yogurt, sorbet,** or **ices** (including low-fat or fat-free)?

☐ NEVER (GO TO QUESTION 106)

☐ 1–6 times per year ☐ 2 times per week
☐ 7–11 times per year ☐ 3–4 times per week
☐ 1 time per month ☐ 5–6 times per week
☐ 2–3 times per month ☐ 1 time per day
☐ 1 time per week ☐ 2 or more times per day

105a. Each time you ate **frozen yogurt, sorbet,** or **ices**, how much did you usually eat?

☐ Less than ½ cup or less than 1 scoop
☐ ½ to 1 cup or 1 to 2 scoops
☐ More than 1 cup or more than 2 scoops

106. How often did you eat **ice cream, ice cream bars,** or **sherbet** (including low-fat or fat-free)?

☐ NEVER (GO TO QUESTION 107)

☐ 1–6 times per year ☐ 2 times per week
☐ 7–11 times per year ☐ 3–4 times per week
☐ 1 time per month ☐ 5–6 times per week
☐ 2–3 times per month ☐ 1 time per day
☐ 1 time per week ☐ 2 or more times per day

106a. Each time you ate **ice cream, ice cream bars,** or **sherbet**, how much did you usually eat?

☐ Less than ½ cup or less than 1 scoop
☐ ½ to 1½ cups or 1 to 2 scoops
☐ More than 1½ cups or more than 2 scoops

106b. How often was the ice cream you ate **light, low-fat,** or **fat-free ice cream** or **sherbet**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

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Over the past 12 months…

107. How often did you eat **cake** (including low-fat or fat-free)?

☐ NEVER (GO TO QUESTION 108)

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times per day

107a. Each time you ate **cake**, how much did you usually eat?

☐ Less than 1 medium piece
☐ 1 medium piece
☐ More than 1 medium piece

107b. How often was the cake you ate **light, low-fat,** or **fat-free cake**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

108. How often did you eat **cookies** or **brownies** (including low-fat or fat-free)?

☐ NEVER (GO TO QUESTION 109)

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times per day

108a. Each time you ate **cookies** or **brownies**, how much did you usually eat?

☐ Less than 2 cookies or 1 small brownie
☐ 2 to 4 cookies or 1 medium brownie
☐ More than 4 cookies or 1 large brownie

108b. How often were the cookies or brownies you ate **light, low-fat,** or **fat-free cookies** or **brownies**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

109. How often did you eat **doughnuts, sweet rolls, Danish,** or **pop-tarts**?

☐ NEVER (GO TO QUESTION 110)

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times per day

109a. Each time you ate **doughnuts, sweet rolls, Danish,** or **pop-tarts**, how much did you usually eat?

☐ Less than 1 piece
☐ 1 to 2 pieces
☐ More than 2 pieces

110. How often did you eat **sweet muffins** or **dessert breads** (including low-fat or fat-free)?

☐ NEVER (GO TO QUESTION 111)

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times per day

110a. Each time you ate **sweet muffins or dessert breads**, how much did you usually eat?

☐ Less than 1 medium piece
☐ 1 medium piece
☐ More than 1 medium piece

110b. How often were the sweet muffins or dessert breads you ate **light, low-fat,** or **fat-free sweet muffins** or **dessert breads**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

111. How often did you eat **fruit crisp, cobbler,** or **strudel**?

☐ NEVER (GO TO QUESTION 112)

☐ 1–6 times per year   ☐ 2 times per week
☐ 7–11 times per year   ☐ 3–4 times per week
☐ 1 time per month   ☐ 5–6 times per week
☐ 2–3 times per month   ☐ 1 time per day
☐ 1 time per week   ☐ 2 or more times per day

Over the past 12 months…

111a. Each time you ate **fruit crisp, cobbler,** or **strudel,** how much did you usually eat?

- ☐ Less than ½ cup
- ☐ ½ to 1 cup
- ☐ More than 1 cup

112. How often did you eat **pie**?

- ☐ NEVER (GO TO QUESTION 113)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

112a. Each time you ate **pie**, how much did you usually eat?

- ☐ Less than ⅛ of a pie
- ☐ About ⅛ of a pie
- ☐ More than ⅛ of a pie

The next four questions ask about the kinds of pie you ate. Please read all four questions before answering.

112b. How often were the pies you ate **fruit pie** (such as apple, blueberry, others)?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

112c. How often were the pies you ate **cream, pudding, custard,** or **meringue pie**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

112d. How often were the pies you ate **pumpkin** or **sweet potato pie**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

112e. How often were the pies you ate **pecan pie**?

- ☐ Almost never or never
- ☐ About ¼ of the time
- ☐ About ½ of the time
- ☐ About ¾ of the time
- ☐ Almost always or always

113. How often did you eat **chocolate candy**?

- ☐ NEVER (GO TO QUESTION 114)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

113a. Each time you ate **chocolate candy**, how much did you usually eat?

- ☐ Less than 1 average bar or less than 1 ounce
- ☐ 1 average bar or 1 to 2 ounces
- ☐ More than 1 average bar or more than 2 ounces

114. How often did you eat **other candy**?

- ☐ NEVER (GO TO QUESTION 115)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

114a. Each time you ate **other candy**, how much did you usually eat?

- ☐ Fewer than 2 pieces
- ☐ 2 to 9 pieces
- ☐ More than 9 pieces

115. How often did you eat **eggs, egg whites,** or **egg substitutes** (NOT counting eggs in baked goods and desserts)? *(Please include eggs in salads, quiche, and soufflés.)*

- ☐ NEVER (GO TO QUESTION 116)

- ☐ 1–6 times per year
- ☐ 7–11 times per year
- ☐ 1 time per month
- ☐ 2–3 times per month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3–4 times per week
- ☐ 5–6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

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Over the past 12 months…

115a. Each time you ate **eggs**, how many did you usually eat?

☐ 1 egg
☐ 2 eggs
☐ 3 or more eggs

115b. How often were the eggs you ate **egg substitutes**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

115c. How often were the eggs you ate **egg whites only**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

115d. How often were the eggs you ate **regular whole eggs**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

115e. How often were the eggs you ate **cooked in oil, butter, or margarine**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

115f. How often were the eggs you ate part of **egg salad**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always
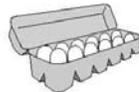
Question 116 appears in the next column

116. How many cups of **coffee**, caffeinated or decaffeinated, did you drink?

☐ NEVER (GO TO QUESTION 117)

☐ Less than 1 cup per month
☐ 1–3 cups per month
☐ 1 cup per week
☐ 2–4 cups per week

☐ 5–6 cups per week
☐ 1 cup per day
☐ 2–3 cups per day
☐ 4–5 cups per day
☐ 6 or more cups per day

116a. How often was the coffee you drank **decaffeinated**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

117. How many glasses of **ICED tea**, caffeinated or decaffeinated, did you drink?

☐ NEVER (GO TO QUESTION 118)

☐ Less than 1 cup per month
☐ 1–3 cups per month
☐ 1 cup per week
☐ 2–4 cups per week

☐ 5–6 cups per week
☐ 1 cup per day
☐ 2–3 cups per day
☐ 4–5 cups per day
☐ 6 or more cups per day

117a. How often was the iced tea you drank **decaffeinated** or **herbal tea**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

118. How many cups of **HOT tea**, caffeinated or decaffeinated, did you drink?

☐ NEVER (GO TO QUESTION 119)

☐ Less than 1 cup per month
☐ 1–3 cups per month
☐ 1 cup per week
☐ 2–4 cups per week

☐ 5–6 cups per week
☐ 1 cup per day
☐ 2–3 cups per day
☐ 4–5 cups per day
☐ 6 or more cups per day

118a. How often was the hot tea you drank **decaffeinated** or **herbal tea**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

Question 119 appears on the next page

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Over the past 12 months…

119. How often did you add **sugar** or **honey** to your coffee or tea?

☐ NEVER (GO TO QUESTION 120)

☐ Less than 1 time per month
☐ 1–3 times per month
☐ 1 time per week
☐ 2–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2–3 times per day
☐ 4–5 times per day
☐ 6 or more times per day

119a. Each time **sugar** or **honey** was added to your coffee or tea, how much was usually added?

☐ Less than 1 teaspoon
☐ 1 to 3 teaspoons
☐ More than 3 teaspoons

120. How often did you add **artificial sweetener** to your coffee or tea?

☐ NEVER (GO TO QUESTION 121)

☐ Less than 1 time per month
☐ 1–3 times per month
☐ 1 time per week
☐ 2–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2–3 times per day
☐ 4–5 times per day
☐ 6 or more times per day

120a. What kind of **artificial sweetener** did you usually use?

☐ Equal or aspartame
☐ Sweet N Low or saccharin

121. How often was **non-dairy creamer** added to your coffee or tea?

☐ NEVER (GO TO QUESTION 122)

☐ Less than 1 time per month
☐ 1–3 times per month
☐ 1 time per week
☐ 2–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2–3 times per day
☐ 4–5 times per day
☐ 6 or more times per day

121a. Each time **non-dairy creamer** was added to your coffee or tea, how much was usually used?

☐ Less than 1 teaspoon
☐ 1 to 3 teaspoons
☐ More than 3 teaspoons

121b. What kind of **non-dairy creamer** did you usually use?

☐ Regular powdered
☐ Low-fat or fat-free powdered
☐ Regular liquid
☐ Low-fat or fat-free liquid

122. How often was **cream** or **half and half** added to your coffee or tea?

☐ NEVER (GO TO QUESTION 123)

☐ Less than 1 time per month
☐ 1–3 times per month
☐ 1 time per week
☐ 2–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2–3 times per day
☐ 4–5 times per day
☐ 6 or more times per day

122a. Each time **cream** or **half and half** was added to your coffee or tea, how much was usually added?

☐ Less than 1 tablespoon
☐ 1 to 2 tablespoons
☐ More than 2 tablespoons

123. How often was **milk** added to your coffee or tea?

☐ NEVER (GO TO QUESTION 124)

☐ Less than 1 time per month
☐ 1–3 times per month
☐ 1 time per week
☐ 2–4 times per week
☐ 5–6 times per week
☐ 1 time per day
☐ 2–3 times per day
☐ 4–5 times per day
☐ 6 or more times per day

123a. Each time **milk** was added to your coffee or tea, how much was usually added?

☐ Less than 1 tablespoon
☐ 1 to 3 tablespoons
☐ More than 3 tablespoons

123b. What kind of **milk** was usually added to your coffee or tea?

☐ Whole milk
☐ 2% milk
☐ 1% milk
☐ Skim, nonfat, or ½% milk
☐ Evaporated or condensed (canned) milk
☐ Soy milk
☐ Rice milk
☐ Other

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Over the past 12 months...

124. How often was **sugar** or **honey** added to foods you ate? (Please do not include sugar in coffee, tea, other beverages, or baked goods.)

☐ NEVER (GO TO INTRODUCTION TO QUESTION 125)

☐ 1–6 times per year ☐ 2 times per week
☐ 7–11 times per year ☐ 3–4 times per week
☐ 1 time per month ☐ 5–6 times per week
☐ 2–3 times per month ☐ 1 time per day
☐ 1 time per week ☐ 2 or more times per day

124a. Each time **sugar** or **honey** was added to foods you ate, how much was usually added?

☐ Less than 1 teaspoon
☐ 1 to 3 teaspoons
☐ More than 3 teaspoons

The following questions are about the kinds of margarine, mayonnaise, sour cream, cream cheese, and salad dressing that you eat. If possible, please check the labels of these foods to help you answer.

125. Over the past 12 months, did you eat **margarine**?

☐ NO (GO TO QUESTION 126)

☐ YES

125a. How often was the margarine you ate **regular-fat margarine** (stick or tub)?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

125b. How often was the margarine you ate **light** or **low-fat margarine** (stick or tub)?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

Question 126 appears in the next column

125c. How often was the margarine you ate **fat-free margarine**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

126. Over the past 12 months, did you eat **butter**?

☐ NO (GO TO QUESTION 127)

☐ YES

126a. How often was the butter you ate **light** or **low-fat butter**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

127. Over the past 12 months, did you eat **mayonnaise** or **mayonnaise-type dressing**?

☐ NO (GO TO QUESTION 128)

☐ YES

127a. How often was the mayonnaise you ate **regular-fat mayonnaise**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

127b. How often was the mayonnaise you ate **light** or **low-fat mayonnaise**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

Question 128 appears on the next page

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Over the past 12 months…

127c. How often was the mayonnaise you ate **fat-free mayonnaise**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

128. Over the past 12 months, did you eat **sour cream**?

☐ NO (GO TO QUESTION 129)

☐ YES

128a. How often was the sour cream you ate **regular-fat sour cream**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

128b. How often was the sour cream you ate **light, low-fat, or fat-free sour cream**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

129. Over the past 12 months, did you eat **cream cheese**?

☐ NO (GO TO QUESTION 130)

☐ YES

129a. How often was the cream cheese you ate **regular-fat cream cheese**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

Question 130 appears in the next column

129b. How often was the cream cheese you ate **light, low-fat, or fat-free cream cheese**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

130. Over the past 12 months, did you eat **salad dressing**?

☐ NO (GO TO INTRODUCTION TO QUESTION 131)

☐ YES

130a. How often was the salad dressing you ate **regular-fat salad dressing** (including oil and vinegar dressing)?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

130b. How often was the salad dressing you ate **light or low-fat salad dressing**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

130c. How often was the salad dressing you ate **fat-free salad dressing**?

☐ Almost never or never
☐ About ¼ of the time
☐ About ½ of the time
☐ About ¾ of the time
☐ Almost always or always

The following two questions ask you to summarize your usual intake of vegetables and fruits. Please do not include salads, potatoes, or juices.

131. Over the past 12 months, how many servings of **vegetables** (not including salad or potatoes) did you eat per week or per day?

☐ Less than 1 per week
☐ 1–2 per week
☐ 3–4 per week
☐ 5–6 per week
☐ 1 per day
☐ 2 per day
☐ 3 per day
☐ 4 per day
☐ 5 or more per day

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Over the <u>past 12 months</u>…

132. Over the <u>past 12 months</u>, how many servings of **fruit** (not including juices) did you eat per week or per day?

☐ Less than 1 per week   ☐ 2 per day
☐ 1–2 per week   ☐ 3 per day
☐ 3–4 per week   ☐ 4 per day
☐ 5–6 per week   ☐ 5 or more per day
☐ 1 per day

133. Over the <u>past month</u>, which of the following foods did you eat **AT LEAST THREE TIMES?** *(Mark all that apply.)*

☐ Avocado, guacamole   ☐ Olives
☐ Cheesecake   ☐ Oysters
☐ Chocolate, fudge, or   ☐ Pickles or pickled
  butterscotch toppings   vegetables or fruit
  or syrups   ☐ Plantains
☐ Chow mein noodles   ☐ Pork neckbones, hock,
☐ Croissants   head, feet
☐ Dried apricots   ☐ Pudding or custard
☐ Egg rolls   ☐ Veal, venison, lamb
☐ Granola bars   ☐ Whipped cream, regular
☐ Hot peppers   ☐ Whipped cream,
☐ Jello, gelatin   substitute
☐ Milkshakes or
  ice-cream sodas   ☐ NONE

134. For **ALL** of the <u>past 12 months</u>, have you followed any type of **vegetarian diet**?

☐ NO (GO TO INTRODUCTION TO QUESTION 135)

☐ YES

134a. Which of the following foods did you **TOTALLY EXCLUDE** from your diet? *(Mark all that apply.)*

☐ Meat (beef, pork, lamb, etc.)
☐ Poultry (chicken, turkey, duck)
☐ Fish and seafood
☐ Eggs
☐ Dairy products (milk, cheese, etc.)
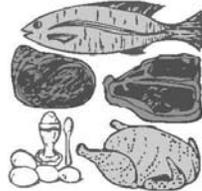
Introduction to Question 135 appears in the next column

The next questions are about your use of fiber supplements or vitamin pills.

135. Over the <u>past 12 months</u>, did you take any of the following types of **fiber or fiber supplements** on a regular basis (more than once per week for at least 6 of the last 12 months)? *(Mark all that apply.)*

☐ NO, didn't take any fiber supplements on a regular basis (GO TO QUESTION 136)
☐ YES, psyllium products (such as Metamucil, Fiberall, Serutan, Perdiem, Correctol)
☐ YES, methylcellulose/cellulose products (such as Citrucel, Unifiber)
☐ YES, Fibercon
☐ YES, Bran (such as wheat bran, oat bran, or bran wafers)

136. Over the <u>past 12 months</u>, did you take any **multivitamins**, such as One-a-Day-, Theragran-, or Centrum-type multivitamins (as pills, liquids, or packets)?

☐ NO (GO TO INTRODUCTION TO QUESTION 138)

☐ YES

137. How often did you take **One-a-day-, Theragran-, or Centrum-type** multivitamins?

☐ Less than 1 day per month
☐ 1–3 days per month
☐ 1–3 days per week
☐ 4–6 days per week
☐ Every day

137a. Does your **multivitamin** usually contain **minerals** (such as iron, zinc, etc.)?

☐ NO
☐ YES
☐ Don't know

137b. For how many years have you taken **multivitamins**?

☐ Less than 1 year
☐ 1–4 years
☐ 5–9 years
☐ 10 or more years

Introduction to Question 138 appears on the next page

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Over the past 12 months...

137c. Over the past 12 months, did you take any vitamins, minerals, or other herbal supplements other than your multivitamin?

☐ NO

> Thank you *very much* for completing this questionnaire! Because we want to be able to use all the information you have provided, we would greatly appreciate it if you would please take a moment to review each page making sure that you:
>
> - **Did not skip any pages and**
> - **Crossed out the incorrect answer and circled the correct answer if you made any changes.**

☐ YES (GO TO INTRODUCTION TO QUESTION 138)

These last questions are about the vitamins, minerals, or herbal supplements you took that are **NOT** part of a One-a-day-, Theragran-, or Centrum-type of multivitamin.

Please include vitamins taken as part of an antioxidant supplement.

138. How often did you take **Beta-carotene** (**NOT** as part of a multivitamin in Question 137)?

☐ NEVER (GO TO QUESTION 139)

☐ Less than 1 day per month
☐ 1–3 days per month
☐ 1–3 days per week
☐ 4–6 days per week
☐ Every day

138a. When you took **Beta-carotene**, about how much did you take in one day?

☐ Less than 10,000 IU
☐ 10,000–14,999 IU
☐ 15,000–19,999 IU
☐ 20,000–24,999 IU
☐ 25,000 IU or more
☐ Don't know

138b. For how many years have you taken **Beta-carotene**?

☐ Less than 1 year
☐ 1–4 years
☐ 5–9 years
☐ 10 or more years

Question 139 appears in the next column

139. How often did you take **Vitamin A** (**NOT** as part of a multivitamin in Question 137)?

☐ NEVER (GO TO QUESTION 140)

☐ Less than 1 day per month
☐ 1–3 days per month
☐ 1–3 days per week
☐ 4–6 days per week
☐ Every day

139a. When you took **Vitamin A**, about how much did you take in one day?

☐ Less than 8,000 IU
☐ 8,000–9,999 IU
☐ 10,000–14,999 IU
☐ 15,000–24,999 IU
☐ 25,000 IU or more
☐ Don't know

139b. For how many years have you taken **Vitamin A**?

☐ Less than 1 year
☐ 1–4 years
☐ 5–9 years
☐ 10 or more years

140. How often did you take **Vitamin C** (**NOT** as part of a multivitamin in Question 137)?

☐ NEVER (GO TO QUESTION 141)

☐ Less than 1 day per month
☐ 1–3 days per month
☐ 1–3 days per week
☐ 4–6 days per week
☐ Every day

140a. When you took **Vitamin C**, about how much did you take in one day?

☐ Less than 500 mg
☐ 500–999 mg
☐ 1,000–1,499 mg
☐ 1,500–1,999 mg
☐ 2,000 mg or more
☐ Don't know

140b. For how many years have you taken **Vitamin C**?

☐ Less than 1 year
☐ 1–4 years
☐ 5–9 years
☐ 10 or more years

Question 141 appears on the next page

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Over the past 12 months…

141. How often did you take **Vitamin E** (**NOT** as part of a multivitamin in Question 137)?

☐ NEVER (GO TO QUESTION 142)

☐ Less than 1 day per month
☐ 1–3 days per month
☐ 1–3 days per week
☐ 4–6 days per week
☐ Every day

141a. When you took **Vitamin E**, about how much did you take in one day?

☐ Less than 400 IU
☐ 400–799 IU
☐ 800–999 IU
☐ 1,000 IU or more
☐ Don't know

141b. For how many years have you taken **Vitamin E**?

☐ Less than 1 year
☐ 1–4 years
☐ 5–9 years
☐ 10 or more years

142. How often did you take **Calcium** or **Calcium-containing antacids** (**NOT** as part of a multivitamin in Question 137)?

☐ NEVER (GO TO QUESTION 143)

☐ Less than 1 day per month
☐ 1–3 days per month
☐ 1–3 days per week
☐ 4–6 days per week
☐ Every day

142a. When you took **Calcium** or **Calcium-containing antacids**, about how much elemental calcium did you take in one day? *(If possible, please check the label for elemental calcium.)*

☐ Less than 500 mg
☐ 500–599 mg
☐ 600–999 mg
☐ 1,000 mg or more
☐ Don't know

142b. For how many years have you taken **Calcium** or **Calcium-containing antacids**?

☐ Less than 1 year
☐ 1–4 years
☐ 5–9 years
☐ 10 or more years

The last two questions ask you about other supplements you took more than once per week.

143. Please mark any of the following **single supplements** you took more than once per week (**NOT** as part of a multivitamin in Question 137):

☐ B-6
☐ B-complex
☐ Brewer's yeast
☐ Cod liver oil
☐ Coenzyme Q
☐ Fish oil
   (Omega-3 fatty acids)

☐ Folic acid/folate
☐ Glucosamine
☐ Hydroxytryptophan (HTP)
☐ Iron
☐ Niacin
☐ Selenium
☐ Zinc

144. Please mark any of the following **herbal** or **botanical supplements** you took more than once per week:

☐ Aloe Vera
☐ Astragalus
☐ Bilberry
☐ Cascara sagrada
☐ Cat's claw
☐ Cayenne
☐ Cranberry
☐ Dong Kuai (Tangkwei)
☐ Echinacea
☐ Evening primrose oil
☐ Feverfew
☐ Garlic

☐ Ginger
☐ Ginkgo biloba
☐ Ginseng (American or Asian)
☐ Goldenseal
☐ Grapeseed extract
☐ Kava, kava
☐ Milk thistle
☐ Saw palmetto
☐ Siberian ginseng
☐ St. John's wort
☐ Valerian
☐ Other

Thank you *very much* for completing this questionnaire! Because we want to be able to use all the information you have provided, we would greatly appreciate it if you would please take a moment to review each page making sure that you:

- **Did not skip any pages and**
- **Crossed out the incorrect answer and circled the correct answer if you made any changes.**

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Appendix 7. Menopause-specific quality of life questionnaire

MENQOL questionnaire

For each of the following items, please indicate whether you have experienced the problem in the PAST WEEK.

If your answer to the problem is "NO", please proceed to the next item.

But if your answer is "YES", please rate how bothered you were by the problem. The rating varies from "Not at all bothered" as zero to "Extremely bothered" as six.

ID Number *

Name *

First   Last

Date *

MM   DD   YYYY

Time point *

○ Month 0

◉ Month 6

○ Month 12

Not at all bothered--> 0--1--2--3--4--5--6--> Extremely Bothered *

	No	0	1	2	3	4	5	6
1. Hot flushes or flashes	○	○	○	○	○	○	○	○

Not at all bothered--> 0--1--2--3--4--5--6--> Extremely Bothered *

	No	0	1	2	3	4	5	6
2. Night sweats	○	○	○	○	○	○	○	○

Not at all bothered--> 0--1--2--3--4--5--6--> Extremely Bothered *

	No	0	1	2	3	4	5	6
3. Sweating	○	○	○	○	○	○	○	○

Not at all bothered--> 0--1--2--3--4--5--6--> Extremely Bothered *

	No	0	1	2	3	4	5	6
4. Dissatisfaction with my personal life	○	○	○	○	○	○	○	○

Not at all bothered--> 0--1--2--3--4--5--6--> Extremely Bothered *

	No	0	1	2	3	4	5	6
5. Feeling anxious or nervous	○	○	○	○	○	○	○	○

Not at all bothered--> 0--1--2--3--4--5--6--> Extremely Bothered *

	No	0	1	2	3	4	5	6
6. Poor memory	○	○	○	○	○	○	○	○

Not at all bothered--> 0--1--2--3--4--5--6--> Extremely Bothered *

	No	0	1	2	3	4	5	6
7. Accomplishing less than I used to	○	○	○	○	○	○	○	○

Not at all bothered--> 0--1--2--3--4--5--6--> Extremely Bothered *

	No	0	1	2	3	4	5	6
8. Feeling depressed, down or blue	○	○	○	○	○	○	○	○

Not at all bothered--> 0--1--2--3--4--5--6--> Extremely Bothered *

	No	0	1	2	3	4	5	6
9. Being impatient with other people	○	○	○	○	○	○	○	○

Not at all bothered--> 0--1--2--3--4--5--6--> Extremely Bothered *

	No	0	1	2	3	4	5	6
10. Feelings of wanting to be alone	○	○	○	○	○	○	○	○

Not at all bothered--> 0--1--2--3--4--5--6--> Extremely Bothered *

	No	0	1	2	3	4	5	6
11. Flatulence (wind) or gas pains	○	○	○	○	○	○	○	○

Not at all bothered--> 0--1--2--3--4--5--6--> Extremely Bothered *

	No	0	1	2	3	4	5	6
12. Aching in muscles and joints	○	○	○	○	○	○	○	○

Not at all bothered--> 0--1--2--3--4--5--6--> Extremely Bothered *

	No	0	1	2	3	4	5	6
13. Feeling tired or worn out	○	○	○	○	○	○	○	○

Not at all bothered--> 0--1--2--3--4--5--6--> Extremely Bothered *

	No	0	1	2	3	4	5	6
14. Difficulty sleeping	○	○	○	○	○	○	○	○

Not at all bothered--> 0--1--2--3--4--5--6--> Extremely Bothered *

	No	0	1	2	3	4	5	6
15. Aches in back of neck or head	○	○	○	○	○	○	○	○

Not at all bothered--> 0--1--2--3--4--5--6--> Extremely Bothered *

	No	0	1	2	3	4	5	6
16. Decrease in physical strength	○	○	○	○	○	○	○	○

Not at all bothered--> 0--1--2--3--4--5--6--> Extremely Bothered *

	No	0	1	2	3	4	5	6
17. Decrease in stamina	○	○	○	○	○	○	○	○

Not at all bothered--> 0--1--2--3--4--5--6--> Extremely Bothered *

	No	0	1	2	3	4	5	6
18. Lack of energy	○	○	○	○	○	○	○	○

Not at all bothered--> 0--1--2--3--4--5--6--> Extremely Bothered *

	No	0	1	2	3	4	5	6
19. Dry skin	○	○	○	○	○	○	○	○

Not at all bothered--> 0--1--2--3--4--5--6--> Extremely Bothered *

	No	0	1	2	3	4	5	6
20. Weight gain	○	○	○	○	○	○	○	○

Not at all bothered--> 0--1--2--3--4--5--6--> Extremely Bothered *

	No	0	1	2	3	4	5	6
21. Increased facial hair	○	○	○	○	○	○	○	○

Not at all bothered--> 0--1--2--3--4--5--6--> Extremely Bothered *

	No	0	1	2	3	4	5	6
22. Changes in appearance, texture or tone of my skin	○	○	○	○	○	○	○	○

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Not at all bothered--> 0--1--2--3--4--5--6--> Extremely Bothered *

	No	0	1	2	3	4	5	6
23. Feeling bloated	○	○	○	○	○	○	○	○

Not at all bothered--> 0--1--2--3--4--5--6--> Extremely Bothered *

	No	0	1	2	3	4	5	6
24. Low backache	○	○	○	○	○	○	○	○

Not at all bothered--> 0--1--2--3--4--5--6--> Extremely Bothered *

	No	0	1	2	3	4	5	6
25. Frequent urination	○	○	○	○	○	○	○	○

Not at all bothered--> 0--1--2--3--4--5--6--> Extremely Bothered *

	No	0	1	2	3	4	5	6
26. Involuntary urination when laughing or coughing	○	○	○	○	○	○	○	○

Not at all bothered--> 0--1--2--3--4--5--6--> Extremely Bothered *

	No	0	1	2	3	4	5	6
27. Breast pain or tnederness	○	○	○	○	○	○	○	○

Not at all bothered--> 0--1--2--3--4--5--6--> Extremely Bothered *

	No	0	1	2	3	4	5	6
28. Vaginal bleeding or spotting	○	○	○	○	○	○	○	○

Not at all bothered--> 0--1--2--3--4--5--6--> Extremely Bothered *

	No	0	1	2	3	4	5	6
29. Leg pains or cramps	○	○	○	○	○	○	○	○

Not at all bothered--> 0--1--2--3--4--5--6--> Extremely Bothered *

	No	0	1	2	3	4	5	6
30. Decrease in my sexual desire	○	○	○	○	○	○	○	○

Not at all bothered--> 0--1--2--3--4--5--6--> Extremely Bothered *

	No	0	1	2	3	4	5	6
31. Vaginal dryness	○	○	○	○	○	○	○	○

Not at all bothered--> 0--1--2--3--4--5--6--> Extremely Bothered *

	No	0	1	2	3	4	5	6
32. Avoiding intimacy	○	○	○	○	○	○	○	○

Appendix 8. Participant handbook for the green tea study.

Participant handbook

For The green tea study

PLEASE NOTE:

If at any time you have questions or concerns regarding the study, please contact any of the GREEN TEA STUDY staff immediately.
Contact information is included in this handbook and on the website (www.greenteastudy.umn.edu).
GREEN TEA STUDY staff members are here to assist you throughout the study process. Please do not hesitate to call for any reason (612) 624-3412 ext. 1

Table of Contents

Clinic Visit 1 (screening visit):

- After receiving your consent at the orientation session, your first clinic visit will be scheduled.

- Measurements taken during this visit will be used to make the final judgment of your eligibility.

- This clinic visit must take place at the _Human Nutrition Research Clinic_ (HNRC) located at the University of Minnesota in Saint Paul campus.

What to expect at your Screening Visit:

Our trained Staff will collect several measurements which include:

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

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

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

- 3-liter urine jug
- 1-liter travel jug
- 1-cooler bag
- 2-collection "hats"
- 1-small bag of foil-wrapped vitamin C

- 2-Ice-packs for the 24-hr urine collection.

- 1-Health History Questionnaire

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

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

Clinic Visit 2 (baseline visit):

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

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

What to do to prepare for this visit:

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

Measurements at this clinic visit include:

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

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

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

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

Clinic Visit 3:

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

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

What to do to prepare for this visit:

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

Measurements at this clinic visit include:

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

If you are choosing to use the Fairview Clinics for part of your study visits, please schedule your next month's clinic visit and notify Green Tea Study Staff of this time.
A study staff member will contact you several days prior to your next visit as a reminder.

Clinic Visit 4:

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

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

<u>What to do to prepare for this visit:</u>

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

<u>Measurements at this clinic visit include:</u>

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

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

Clinic Visit 5:

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

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

What to do to prepare for this visit:

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

Measurements at this clinic visit include:

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

Study Supplement and Supplies:

- At this visit you will receive 3 new bottles of our study supplement. Please return any pills that you may still have along with the bottles they came in.

- You will receive 24-hour urinary collection materials for your 8th clinic visit.

  - This includes: one 3-liter urine jug, one small bag of foil-wrapped vitamin C. Of other supplies are needed please let study staff know and they will provide it to you.

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

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

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

Clinic Visit 6:

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

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

<u>What to do to prepare for this visit:</u>

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

<u>Measurements at this clinic visit include:</u>

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

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

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

Clinic Visit 7:

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

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

What to do to prepare for this visit:

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

Measurements at this clinic visit include:

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

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

Clinic Visit 8:

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

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

What to do to prepare for this visit:

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

Measurements at this clinic visit include:

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

Study Supplement and Questionnaires:

- At this visit you will receive 3 new bottles of our study supplement. Please return any pills that you may still have along with the bottles they came in.

- You will need to complete an online Menopause-Specific Quality of Life questionnaire (MENQOL) prior or the day of your clinic visit. This questionnaire may be completed at home or at the HNRC.

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

Clinic Visit 9:

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

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

What to do to prepare for this visit:

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

Measurements at this clinic visit include:

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

Study Supplement and Supplies:

- At this visit you will receive 3 new bottles of our study supplement. Please return any pills that you may still have along with the bottles they came in.

- You will receive 24-hour urinary collection materials for your 8th clinic visit.

  o This includes: one 3-liter urine jug, one small bag of foil-wrapped vitamin C. Of other supplies are needed please let study staff know and they will provide it to you.

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

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

Clinic Visit 10 (Final Visit):

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

WHAT TO DO TO PREPARE FOR THIS VISIT:

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

What to expect at your Final Visit:

Our trained Staff will collect several measurements which include:

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

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

- This appointment should take about 30 minutes.

Summary of Clinical Measurements

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

Green Tea Study Staff

Principle Investigator:

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Study Contact Information

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<u>Telephone:</u> 612-624-3412
<u>Fax:</u>   612-626-3037
<u>E-mail:</u>   greentea@umn.edu

Scheduling Questions and Study Concerns:

PLEASE NOTE: If at any time you have questions or problems arise, please contact any member of the Green Tea Study staff immediately.
The Green Tea Study staff members are here to assist you through the study process; do not hesitate to call for any reason.

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

If leaving a message via telephone or email please provide:

- Your name and

- Contact information

- Brief description about the reason for your contacting the study.

- If you have a preferred time for us to contact you.

If we do not hear from you:

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

HNRC Location, Transportation, and Parking

Human Nutrition Research Clinic (HNRC)
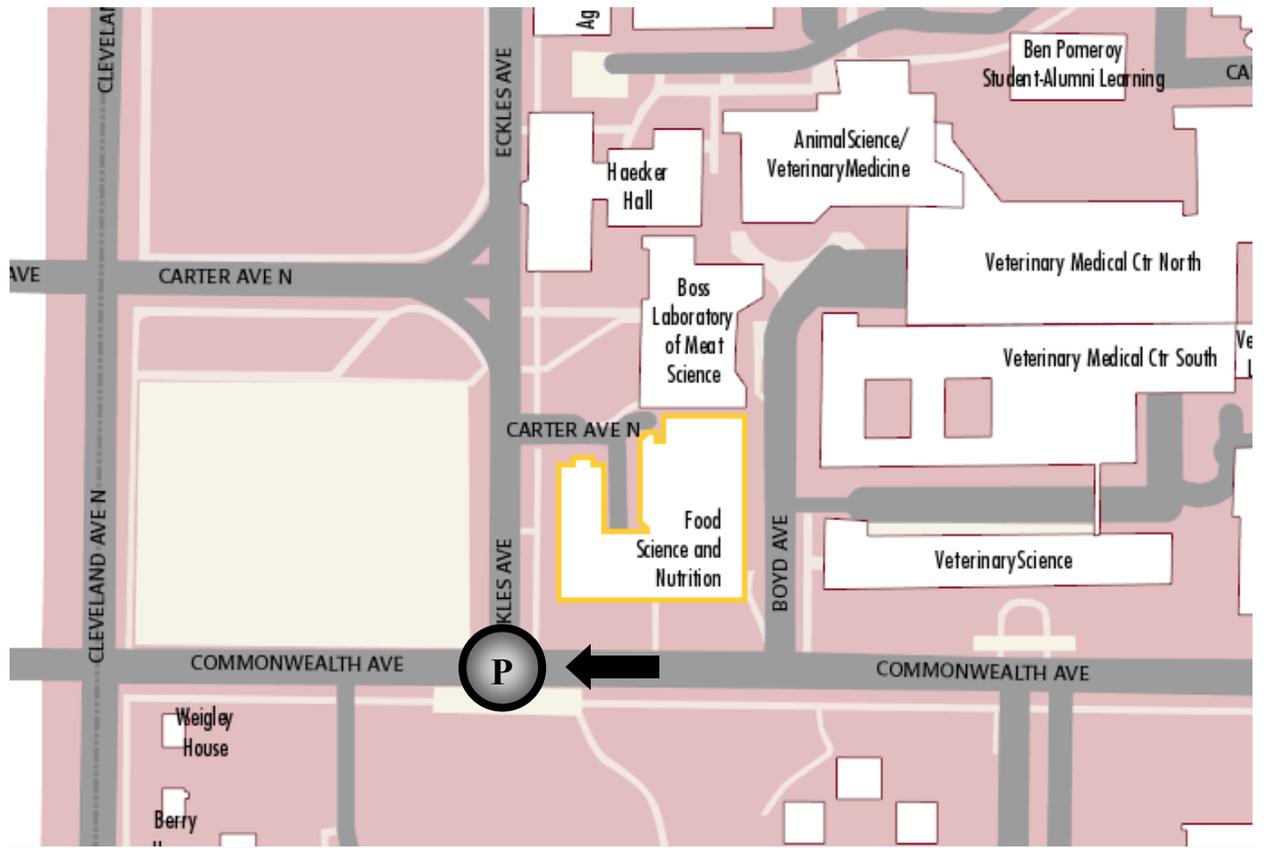
Location

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

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

FREE PARKING AVAILABLE TO STUDY PARTICIPANTS

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

Public Transportation

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

Directions to HNRC from I-35W

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

Directions to HNRC from I-94

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

Parking near HNRC

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

(1) Metered parking is available on Eckles Ave.

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

Fairview Clinics

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

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

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

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

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

Current Participating Fairview Clinics

- ### Crosstown Clinic/ Southdale Medical Center
  6545 France Ave, Edina, MN 55435

  Hours: Monday - Friday: 8 AM - 5 PM

  Appointment Line: (952)- 848 - 5600

- ### Maple Grove Family Medical Center

  14500 99th Ave N, Maple Grove, MN 55369

  Hours: Monday – Friday 7 AM - 5 PM & Saturday: 8 AM - 5 PM

  Appointment Line: (763) – 898-1000

- ## Jonathan Clinic

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

Current Participating Fairview Clinics - Continued

- ## Oxboro Clinic

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

- ## Farmington Clinic

  19685 Pilot Knob Road, Farmington, MN 55024

  Hours: Monday, Wednesday & Thursday: 8 AM – 4:30 PM
         Tuesday: 8 AM – 6:30 PM
         Friday: 8 AM – 4 PM
  Appointment Line: (651) – 463-5100

Blood Draws

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

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

<u>What are we drawing your blood for? :</u>
Blood is drawn to measure the following biomarkers during the aforementioned clinic visits: sex hormones (estrogen, estradiol, androstendione hormones), oxidative stress biomarkers, green tea catechins, and enzymes related to liver function.

<u>Are there any risks? :</u>
There is a small risk of infection when blood is taken, but the risk is minimal as all

needles and equipment are sterilized and the procedures are performed by trained clinical

staff at the human Nutrition Research Center (HNRC) or a partnering Fairview Clinic.

You may experience some mild to moderate pain lasting a few seconds upon insertion of

the needle used to draw the blood. You may also get a bruise from the blood draw.

Please inform the clinical staff if you feel faint or nauseous.

24-Hour Urine Collection

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

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

<u>Collection supplies:</u>

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

- 1-3 Liter plastic jug

- 1 Liter brown jug

- 1 Baggie containing 3 grams powdered ascorbic acid (Vitamin C)

- 1 thermal/lunch bag

- Ice packs

- Urine collection hats

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

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

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

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

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

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

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

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

4. When you arrive home, immediately transfer the urine to the large 3-liter container labeled for that day. Label your 3-liter urine container with the start and end times as

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well as the appropriate dates. You will be provided with labels to attach to all your containers. Keep all containers filled with urine in a refrigerator or cooler with ice. DO NOT STORE OUTSIDE DURING WINTER MONTHS.

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

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

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

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

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

What if…

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

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

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

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

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

Example of: CORRECT URINE LABEL

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

Completing Questionnaires

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

Health History Questionnaire (HHQ)

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

Dietary History Questionnaire (DHQ)

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

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

Menopause-Specific Quality Of Life (MENQOL) Questionnaire

The Menopause-Specific Quality of Life questionnaire assesses the effects of menopausal symptoms on health-related quality of life. The collected information from this questionnaire will help us to study the possible effects of the supplement on the quality of your life. This is a self-administered questionnaire including 32 questions, which ask about your experience of certain physical, psychosocial, and sexual symptoms over the previous week. If you have experienced any of the questioned items, answer "Yes" and then rate the experience from zero (not at all bothered) to six (extremely bothered),

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otherwise check the "No" box and proceed to the following item. This questionnaire will be online at the beginning, month 6, and the end of study, and can be accomplished in less than 15 minutes.

Taking the Study Supplement

Instructions for Taking the Green Tea Supplement

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

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

What if:

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

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

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

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

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

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

Example of Study Log:

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

If leaving a message, give:

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

THANK YOU FOR YOUR
PARTICIPATION.