Chronic and Acute Effects of Green Tea Extract and Catechol-*O*-methyltransferase Genotype on Body Composition and Obesity-Associated Hormones in Overweight and Obese Postmenopausal Women

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Allison M. Dostal

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Mindy S. Kurzer

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Dedication

"i would not trade a single highway or city or moment or person i met for anything. i have loved it all."

- St. Vincent

To my parents, for their enduring patience and confidence that I would be done with school…someday. Thank you for never losing faith and always giving me strength.

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Abstract

This dissertation details the chronic and acute effects of green tea extract (GTE) supplementation (1315 mg green tea catechins/day, 843 mg as (-)-epigallocatechin-3-gallate, [EGCG]) on body composition, obesity-associated hormones, glucose homeostasis, and satiety in overweight and obese postmenopausal women at increased risk for breast cancer due to high mammographic density. Participants in the forthcoming studies were a subset of participants drawn from the Minnesota Green Tea Trial (MGTT), which was a randomized, placebo-controlled, double-blind, phase II clinical trial designed to determine the effects of supplementing GTE for one year on breast cancer risk factors including mammographic density, reproductive hormones, insulin-like growth factor (IGF) axis proteins, and F2-isoprostanes, a recognized biomarker of oxidative stress. Effect modification by catechol-O-methyltransferase (COMT), an enzyme involved in the metabolism of green tea catechins, estrogens, and norepinephrine, was also analyzed for all endpoints, due to its potential role in modulating the impact of GTE on breast cancer risk factors.

Chapter 1 provides a brief introduction to the MGTT and the forthcoming chapters. Chapter 2 presents a review of the literature, providing context for the MGTT and ancillary studies. Chapter 3 describes the effect of GTE on anthropometric variables, obesity-associated hormones (leptin, ghrelin, adiponectin, and insulin) and markers of glucose homeostasis (blood glucose concentrations and the homeostasis measure of insulin resistance [HOMA-IR]) in 237 participants. In this study, no changes in energy intake or anthropometric measurements were observed in women taking GTE or placebo. Similarly, no changes were seen in circulating leptin, ghrelin, adiponectin, or glucose concentrations. However, among participants with baseline insulin ≥10 μIU/mL, there was a reduction in insulin concentration in the GTE group over 12 months compared to the placebo group and participants with baseline insulin < 10 μIU/mL in either group ($P < 0.01$). Participants with the homozygous high-activity (G/G) form of COMT showed significantly lower adiponectin and higher insulin concentrations at month 12 as compared to those with the low-activity (A/A) genotype, regardless of treatment group.

Chapter 4 describes the more specific effects of GTE on body composition as measured by dual-energy x-ray absorptiometry (DXA), including total body fat, % body fat, region-specific adiposity, and bone mineral density (BMD) in 121 participants. These results were correlated with measures of leptin, adiponectin, and insulin. No changes in BMI, total fat mass, % body fat, or BMD were observed in women taking GTE compared to placebo; however, a reduction in visceral adipose tissue mass in GTE participants as compared to the placebo group nearly reached significance. Interactions were observed between treatment, time, and baseline BMI for gynoid % fat and tissue % fat, with more favorable results seen in the GTE group. No changes were seen in circulating leptin, adiponectin, or insulin concentrations. COMT genotype did not modify the effect of GTE on any variable.

Chapter 5 details the acute postprandial effects of GTE administration in 60 participants who were administered a high-carbohydrate breakfast meal in the final months of their participation in the MGTT. Leptin, ghrelin, and adiponectin were not different between GTE and placebo at any time point and COMT genotype did not modify these results. Participants randomized to GTE with the high-activity form of the COMT enzyme (GTE-high COMT) had higher insulin concentrations immediately after the test meal (time 0) and at 0.5 and 1.0 hours post-meal compared to all COMT groups randomized to placebo. The GTE-high COMT group had higher insulin concentrations at times 0, 0.5, 1.0, 1.5, and 2.0 h compared to the GTE-low COMT group. Nine markers of satiety and appetite, as measured through comparison of mean area under the curve (cm/hr), were not different between GTE and placebo.

The results of these three studies demonstrate that daily supplementation of 1315 mg GTE, independent of caffeine, does not influence long-term energy intake, satiety, body weight, or obesity-associated hormones, though it may be beneficial for individuals with a higher degree of visceral adiposity and with increased circulating insulin concentrations. This suggests benefit for those at risk for metabolic syndrome or type 2 diabetes. Given the association of these conditions with breast cancer risk, GTE may be a beneficial dietary supplement for overweight and obese postmenopausal women.

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List of Abbreviations

AE: adverse event

AgRP: agouti-related protein

AICR: American Institute for Cancer Research

ALT: alanine aminotransferase

BAP: bone-specific alkaline phosphatase

BMD: bone mineral density

BMI: body mass index

cAMP: cyclic adenosine monophosphate

CART: cocaine-amphetamine-regulated transcript

CI: confidence interval

CNS: central nervous system

COMT: catechol-O-methyltranferase

DXA: dual-energy x-ray absorptiometry

EC: epicatechin

ECG: epicatechin gallate

EE: energy expenditure

EGC: epigallocatechin

EGCG: (-)-epigallocatechin-3-gallate

FA: fatty acid

FFQ: food frequency questionnaire

GTC: green tea catechins

GTE: green tea extract

HbA1c: glycated hemoglobin; used to identify plasma glucose concentrations over time

HDL-C: high-density lipoprotein cholesterol

HOMA-IR: homeostasis model of assessment for insulin resistance

IGF: insulin-like growth factor

IGT: impaired glucose tolerance

IR: insulin resistance

IU: international units; used to describe blood insulin concentrations

MGTT: Minnesota Green Tea Trial

MET-hr: metabolic equivalent hours; defined as the amount of oxygen consumed while sitting at rest, equal to 3.5 mL O_2 per kg body weight x min.

MHT: menopausal hormone therapy

MUFA: monounsaturated fatty acid

NHS: Nurses' Health Study

NIH: National Institutes of Health

NPY: neuropeptide-Y

PDE: phosphodiesterase

POMC: pro-opiomelanocortin

PUFA: polyunsaturated fatty acid

RQ: respiratory quotient

SEM: standard error of the mean

SNS: sympathetic nervous system

T2D: type 2 diabetes

VAS: visual analog scale, used to assess satiety

VAT: visceral adipose tissue

VEGF: vascular endothelial growth factor

WC: waist circumference

WCRF: World Cancer Research Fund

WHR: waist-to-hip ratio

Chapter 1 - Introduction

Breast cancer is the most common type of non-skin cancer worldwide and is a significant cause of cancer-related mortality in women. Results of many epidemiologic studies have determined that obesity is a strong risk factor for postmenopausal breast cancer, which is concerning given the increasingly widespread epidemic of this metabolic condition. While risk factors for breast cancer are both genetic and lifestyle-related, recent research has focused on those determinants that may be modifiable, such as body weight, adiposity, and dietary intake of certain foods and beverages.

Green tea catechin consumption has been suggested as a strategy for weight loss and weight maintenance in overweight and obese women. While some positive results of green tea on body weight and adiposity have been reported, other studies have demonstrated null results, making the current data inconclusive. Additionally, it is undetermined if any beneficial effects of green tea on body weight are due to its catechin content, caffeine (which is naturally present in tea), or a synergistic relationship between the two compounds. To date, the number of long-term randomized trials (>12 weeks) examining these relationships is few, and clinical relevance of any observed weight loss is uncertain. Rate of metabolism of green tea catechins may also have an impact on the influence of green tea on these variables. *O*-methylation, catalyzed by catechol-*O*-methyltransferase (COMT) is one pathway of catechin metabolism, and polymorphisms of this enzyme may influence the duration of exposure to green tea catechins.

This dissertation addresses four general questions related to obesity and decaffeinated green tea extract (GTE) supplementation. First, does GTE produce favorable changes in body weight and body composition in overweight and obese postmenopausal women? Second, are circulating concentrations of obesity-associated hormones and markers of glucose homeostasis altered with chronic ingestion of GTE? Third, does GTE supplementation impact postprandial satiety or concentrations of obesity-associated hormones and glucose homeostasis markers? Lastly, are these effects modified by COMT genotype? Answers to these four questions will provide a comprehensive analysis of the role of long-term green tea catechin consumption and COMT genotype on obesity, adiposity, and obesity-associated hormone concentrations in

postmenopausal women, which may lead to targeted strategies for prevention of obesity-related health conditions, including postmenopausal breast cancer.

Research reported in this dissertation is the result of three ancillary studies that were conducted in a subset of overweight and obese participants enrolled in the Minnesota Green Tea Trial (MGTT). The MGTT was a randomized, double-blind, placebo-controlled, phase II clinical trial investigating the effect of 12 months of daily decaffeinated GTE supplementation on breast cancer risk factors in postmenopausal women. Participants in the MGTT were healthy women between the ages of 50-70 years who were at high risk of breast cancer due to dense breast tissue. The intervention was a green tea extract supplement containing a daily dosage of 1315 mg green tea catechins (843 mg as (-)-epigallocatechin-3-gallate) or placebo, taken in capsule form for one year. The primary outcomes included change from baseline in percent mammographic density, circulating endogenous sex hormones, and insulin-like growth factor axis proteins; the secondary endpoints were urinary estrogens and metabolites and plasma F2-isoprostanes (a recognized biomarker of oxidative stress). The MGTT screened more than 100,000 mammograms and randomized 1075 participants into 6 groups based on treatment (GTE vs. placebo) and COMT genotype (high-, intermediate-, or low-activity). Among enrolled participants, 937 women successfully completed the study and 139 withdrew from the study (attrition rate: 12.9%).

Chapter 2 presents a review of the literature on green tea and its associations with obesity and breast cancer, which will assemble the framework upon which the rationale for the forthcoming chapters will be constructed. Chapter 3 describes in detail a study designed to examine the impact of GTE on anthropometric variables, obesity-associated hormone concentrations, and glucose homeostasis markers over the 12-month intervention period. The second study, comprising Chapter 4, analyzed the effect of GTE on specific measures of body composition, adiposity, and skeletal health and correlated these results with circulating concentrations of obesity-associated hormones. Chapter 5 details research that aimed to determine the effect of GTE consumption on acute postprandial concentrations of obesity-associated hormones, markers of glucose homeostasis, and satiety after a high-carbohydrate breakfast meal. Lastly, Chapter 6

summarizes the knowledge gained from these studies and assesses this research in the context of the current literature on the relationship between green tea, obesity, and postmenopausal breast cancer.

Chapter 2 - Literature Review

I. Breast Cancer

Breast cancer is the most common type of non-skin cancer in women worldwide and is the second leading cause of cancer-related deaths amid women in Western countries. In 2015, an estimated 231,840 new cases of breast cancer will be diagnosed in the United States and it is projected that 40,290 deaths will occur as a result of the disease. Approximately 1 in 8 women in the U.S. will develop breast cancer during their lifetime (1).

A. Risk Factors

Numerous risk factors for breast cancer have been identified and can be classified into modifiable or non-modifiable categories. Non-modifiable risk factors, such as increasing age or female gender, cannot be changed. Risk factors that are linked to environmental causes or personal health behaviors are modifiable and may change over time. Therefore, a woman's risk of developing breast cancer continues to evolve through the life span.

1. Non-modifiable Risk Factors

a) Age

Breast cancer risk increases with increasing age. According to the American Cancer Society, approximately 1 in 8 invasive breast cancers are diagnosed in women younger than 45 years, while 2 of 3 cases of breast cancer are found in women 55 years or older (2).

b) Gender

Breast cancer occurs nearly 100 times more often in women than in men, likely due to increased exposure to estrogen and progesterone, which are known to promote breast carcinogenesis (3).

c) Race and Ethnicity

Breast cancer incidence varies by race and ethnicity. In 2011, the most recent year for which numbers have been reported for U.S. populations, white women had the highest breast cancer incidence rates, followed by black, Hispanic, Asian/Pacific Islander, and American Indian/Alaska Native women (4).

d) Genetic Factors

It is estimated that 5 to 10% of all breast cancers can be directly attributed to inherited gene mutations, most often to mutations in the *BRCA1* or *BRCA2* genes. Mutations in other genes, including *ATM, TP53, CHEK2, PTEN, CDH1*, *PALB2*, and *STK11* can also increase breast cancer risk, though these mutations are much more rare and do not increase risk to the same extent as BRCA genes (2).

e) Family History

A woman's risk for breast cancer doubles if she has one first-degree relative with the disease. If a woman has 2 first-degree relatives with breast cancer, her risk is three times higher. However, the number of women diagnosed with breast cancer who have a family member with the disease (15%) is relatively small compared to those diagnosed without a family history (85%) (3).

f) Breast Density

Women classified as having dense breasts on mammogram, indicating an increased amount of fibrous or glandular breast tissue as compared to fatty tissue, have a 1.2- to 2-fold increased risk of breast cancer as compared to women with average breast density. In addition, dense breast tissue can make detecting tumors via mammogram more difficult (5).

2. Lifestyle-related Risk Factors

a) Reproductive History and Estrogen Exposure

Lifetime exposure to estrogens is an established risk factor for breast cancer. Having several pregnancies, becoming pregnant at a young age, and breastfeeding are known to reduce risk, attributed to exposure to lower levels of endogenous estrogens during pregnancy and lactation (6). Nulliparous women or those who had their first child after the age of 30 have a modestly increased risk of breast cancer. Additional risk factors include early age of menarche (before age 12) and entering menopause after age 55, both of which are associated with longer duration of estrogen exposure (3).

Hormone therapy after menopause, referred to as menopausal hormone therapy (MHT) in this manuscript, is used to relieve adverse symptoms after menopause and prevent loss of bone mineral density (BMD). Research has shown that using combined MHT (both estrogen and progesterone) increases breast cancer incidence and mortality, especially if used for longer than 2 to 3 years. Use of estrogen alone has not been associated with these effects (7). Use of oral contraceptives may also increase risk, though this association diminishes if a woman has discontinued use for over 10 years (8).

b) Alcohol and Tobacco Use

Compared with non-drinkers, women who consume 1 alcoholic drink per day have an increased risk of breast cancer, and this risk increases with the amount of alcohol consumed (9). Both mainstream and secondhand smoke contain chemicals that have been shown to cause breast cancer in rodents, and chemicals present in tobacco smoke have been found in breast milk (10). Yet, the association between tobacco use and breast cancer remains inconclusive, as the 2014 U.S. Surgeon General's report concluded that there is "suggestive but not sufficient" evidence that smoking increases breast cancer risk (11).

c) Physical Activity

Regular physical activity has been shown to have a protective effect against postmenopausal breast cancer: risk reduction for physically active individuals compared with those who are least active may be as much as 25% (12).

d) Dietary Intake

The influence of diet on postmenopausal breast cancer risk remains inconclusive. While some studies have found that a diet rich in fruits, vegetables, poultry, fish, and low-fat dairy is associated with reduced breast cancer risk, others have found null effects (9). Intake of foods high in dietary flavonoids, such as soy and green tea, may also have preventive effects against breast cancer occurrence (9). Epidemiologic studies have mainly determined that breast cancer is less common in countries where the typical diet is low in total fat,

polyunsaturated fat, and saturated fat (13). Yet, these results have not been seen in studies conducted in the United States (14). Reasons for this discrepancy may involve variations in typical dietary intake, physical activity, and genetic factors when comparing women in the U.S. versus those in other countries. Another important consideration is the strong association between dietary and total energy intake with body weight and obesity, which itself is an independent risk factor for postmenopausal breast cancer.

e) Obesity

A vast body of knowledge has been amassed regarding the epidemiologic associations of body weight, body fatness (as measured by body mass index [BMI]), and postmenopausal breast cancer risk. It is widely known that obesity increases risk for postmenopausal breast cancer (15-23) and is associated with poorer prognosis (24). A pooled analysis of seven prospective cohort studies comprised of 337,819 women and 4,385 incident breast cancer cases indicated that the relative risk of breast cancer was 1.26 (95% CI: 1.09-1.46) when a BMI > 28 kg/m^2 was used as the highest category in the analyses of postmenopausal women (21). Interestingly, overweight and obesity have been found to be moderately protective in premenopausal women (21). This reversal of effect may be explained by differing methods of estrogen production in pre- versus postmenopausal women. Estrogen production in premenopausal women mainly occurs in the ovaries; in contrast, estrogen is produced primarily by adipose tissue after menopause (25). Since epidemiologic studies have suggested that serum estrogen concentration is a risk factor for breast cancer in postmenopausal women (26) increased body fat, leading to a higher serum estrogen concentration, may be an independent risk factor for breast carcinogenesis. Additionally, central adiposity increases risk of postmenopausal breast cancer independent of body weight (15,20,27). Conversely, maintenance of body weight throughout the adult years and weight loss after menopause are associated with decreased risk of postmenopausal breast cancer (18,28).

In 2007, the World Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR) issued the Second Expert Report "Food, Nutrition, Physical Activity, and the Prevention of Cancer: a Global Perspective"(29), which concluded that adult weight gain, body fatness, and abdominal body fatness are causally linked to breast cancer incidence in postmenopausal women. These conclusions continue to be supported by the AICR's Continuous Update Project, whose last publication in 2010 classified the evidence of body fatness as a risk factor for postmenopausal breast cancer as "convincing" and abdominal fatness and adult weight gain as "probable" (30).

B. Section Summary

There are a number of modifiable and non-modifiable risk factors for breast cancer. While characteristics such as age, gender, race and ethnicity, genetics, and family history are unable to be impacted by prevention strategies, several lifestyle modifications can be made to reduce postmenopausal breast cancer risk, including decreasing body weight and adiposity.

II. Mechanisms of the Relationship between Obesity, Body Composition and Breast Cancer Risk

A. Introduction and Definitions

Over the past several decades, obesity has become a significant public health concern due to its association with many chronic diseases and cancers, including postmenopausal breast cancer. It is estimated that more than one-third (34.9% or 78.6 million) of U.S. adults are obese, with the highest rates in middle-age adults (40-59 years old, 39.5% obesity rate) as compared to younger adults (20-39 years old, 30.3%) and those aged 60 or above (35.4%) (31). As of 2008, the annual burden of obesity on the U.S.'s health care system was estimated to be $147 billion; annual medical costs for people who are obese are approximately $1,429 higher than those of normal weight (32).

The World Health Organization defines obesity in terms of the body mass index (BMI), measured by weight in kilograms (kg) divided by height in meters squared (m^2). An adult with a BMI between 25.0 and 29.9 kg/m^2 is considered overweight, and a BMI

>30.0 kg/m^2 is considered obese. While BMI does not directly measure adiposity, its use is widespread due to its simple calculation and high correlation with body fatness. Most epidemiologic studies use simple measurements such as BMI, waist circumference (WC), and waist-to-hip ratio (WHR) as proxies for obesity due to the inherent difficulty and expense in gathering more detailed and specific data from large study populations.

Fat is principally deposited in two compartments; subcutaneously and viscerally. Visceral adipose tissue (VAT), which is comprised of intra-abdominal fat, is known to be more metabolically active than subcutaneous fat and has multiple endocrine, metabolic, and immunological functions. It has also been shown to be central to the pathogenesis of the metabolic syndrome, a constellation of disorders diagnosed by possessing 3 of the 5 following characteristics: WC >88 cm (women); triglycerides ≥ 150 mg/dL; high-density lipoprotein (HDL) cholesterol < 50 mg/dL (women); blood pressure ≥ 130/85 mmHg; and fasting glucose ≥ 110 mg/dL (33).

It is well established that obesity is associated with cancer development at numerous sites, including the esophagus, pancreas, colorectum, endometrium, kidney, and breast (in postmenopausal women) (29). The AICR has estimated that 38% of all breast cancer cases in the U.S. could be prevented with lifestyle modifications including maintaining a healthy weight – this amounts to approximately 89,300 women being spared of breast cancer every year (30). The mechanisms by which obesity increases breast cancer incidence and mortality, and the role of visceral adipose tissue in disease pathogenesis, have become the focus of considerable research.

B. Visceral Adipose Tissue and Breast Cancer Pathogenesis

The importance of adipose tissue location is evident, as an increased ratio of VAT area to subcutaneous fat area has been shown to be strongly related to glucose and lipid metabolism disorders in obese subjects (34). VAT has also been identified as an independent risk factor for breast cancer, largely attributed to the state of chronic systemic low-grade inflammation brought about by excess visceral obesity. As VAT expands, it becomes infiltrated with macrophages and T-cells. These immune cells and the adipocytes produce adipokines including leptin, interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α). In addition, increasing visceral adiposity leads to

hyperinsulinemia and increased concentrations of free insulin-like growth factor-1 (IGF-1) (35). Consequently, there is a pro-tumorigenic state of inflammation, angiogenesis and insulin resistance, a condition that will be discussed in the forthcoming section.

C. Obesity- and Appetite-associated Hormones

Several hormones associated with obesity and markers of glucose homeostasis also play a critical role in communicating nutritional status to the central nervous system (CNS), such as the hypothalamus and the brainstem. In addition to their involvement in obesity, IR, and breast cancer, hormonal regulation of appetite by these peptide hormones may play a significant role in appetite control and energy intake. Several hormones have demonstrated these dual effects, including leptin, adiponectin, ghrelin, and insulin.

1. Leptin

Adipose tissue is a dynamic endocrine organ that releases several adipokines, including leptin, which was first identified by Zhang et al. (36) in 1994. Leptin is a 166-amino acid protein with well-characterized effects on regulation of energy balance. Leptin concentrations increase after a meal, resulting in reduced food intake (37) and increased energy expenditure (38) to maintain the body's energy stores. This is largely due to increased expression of anorexigenic peptides such as pro-opiomelanocortin (POMC) and cocaine-amphetamine-regulated transcript (CART) modulated by leptin (39). Circulating concentrations of the hormone are proportional to fat mass (40); however, obese individuals have demonstrated a resistance to the protein's effects (41). Therefore, its appetite-reductive and satiation effects are reduced in individuals with a higher degree of adiposity.

Apart from its effects on appetite and metabolic rate, leptin has recently been associated with the pro-inflammatory properties of obesity and insulin resistance, which have been correlated with breast cancer risk (42). Higher blood leptin concentrations have been observed in breast cancer patients as compared to controls and Garafalo et al. (43) found overexpression of leptin and its receptors in primary and metastatic breast cancer as compared to non-cancer tissues. Potential mechanisms for carcinogenic effects of leptin include increasing cancer cell

migration and invasion (44), increasing angiogenesis via vascular endothelial growth factor (VEGF)(45), and induction of pro-inflammatory cytokines and macrophage stimulation(46). Therefore, interventions that reduce circulating leptin concentrations may have a risk-reductive effect for breast cancer.

2. Adiponectin

Adiponectin is the most abundant adipokine and is secreted mainly from adipocytes in visceral fat. In contrast to leptin, circulating concentrations are inversely correlated with various measures of obesity, including BMI and abdominal adiposity (47). Adiponectin modulates several metabolic processes, including glucose regulation (48) and fatty acid metabolism (49). Adiponectin has known insulin-sensitizing effects, and circulating concentrations of this hormone have been found to predict the development of type 2 diabetes (50). Circulating adiponectin and adiponectin gene expression in adipose tissue are reduced in patients with type 2 diabetes and obese populations (51-53). This hormone may also have centrally mediated effects on food intake and energy expenditure, though animal studies examining this relationship have shown conflicting results (54,55).

Adiponectin has been shown to be both anti-angiogenic and anti-inflammatory, and can inhibit tumor growth in animal models (56). Adiponectin concentrations have been shown to be an independent risk factor for breast cancer (57). In a prospective case-control study nested within the Nurses' Health Study (NHS) and NHS II cohorts, prediagnostic adiponectin levels were inversely associated with the risk of postmenopausal breast cancer (58).

3. Ghrelin

Ghrelin is a 28-amino acid peptide produced mainly in the stomach and is a potent stimulator of food intake (59). Ghrelin secretion is primarily mediated by an individual's nutritional state – concentrations increase in the fasted state and decrease after feeding. After its release into the bloodstream, ghrelin crosses the blood brain barrier and exerts its orexigenic effects on the hypothalamus. This stimulates the activity of neurons expressing neuropeptide-Y (NPY), agouti-related

protein (AgRP), and orexin, leading to increased appetite (60,61). At the same time, ghrelin reduces the activity of neurons expressing POMC and CART, which normally act to decrease food intake and body weight (62). Increased preprandial ghrelin concentrations have been correlated with hunger scores in humans (59), and intravenous infusion of ghrelin can induce hunger and food intake in both normal-weight and obese adults (63,64). Circulating ghrelin concentrations are negatively correlated with BMI and typically increase with weight loss (65).

Several studies have correlated ghrelin levels to breast cancer risk – ghrelin treatment has been shown to increase proliferation of breast cancer cell lines *in vitro* (66). Conflicting evidence exists for the association of polymorphisms in the gene coding for ghrelin (*GHRL*) or the ghrelin receptor (*GHSR*) with breast cancer risk (67-69).

4. Insulin

Insulin is a 51-amino acid peptide hormone produced in the beta cells of the pancreas. Its primary role is maintaining glucose homeostasis mainly in the postprandial period, though it is also an important anorexigenic hormone involved in food intake. Animal studies have shown that central administration of insulin decreases food intake in primates (70) and rodents (71). Concentrations of insulin vary with adiposity (72), and this hormone is known to have growth-stimulatory effects (73).

a) Methods of Assessment of Insulin Sensitivity

Though no reference range is established for optimal fasting insulin concentrations, objective assessment of insulin sensitivity is important for diagnosis of health conditions such as IR and diabetes. Direct measures of insulin sensitivity include the euglycemic glucose clamp, which is considered to be the gold standard of measurement but is also time-consuming, labor intensive, expensive and impractical for large clinical investigations and epidemiologic studies (74). Therefore, indirect measurements are typically used to assess insulin sensitivity. The homeostasis model assessment of insulin resistance (HOMA-IR), which is equivalent to ([fasting insulin

(μIU/mL)] × [fasting glucose (mg/dL)]) / 405, is commonly used in clinical trials. The denominator of 405 is the product of a standard fasting plasma insulin (5 μIU/mL) and fasting plasma glucose (81 mg/dL), typical of a "normal" healthy individual (74). Therefore, for an individual with "normal" insulin sensitivity, HOMA-IR = 1.

b) Insulin, IR, and Breast Cancer Risk

Higher concentrations of fasting insulin have been associated with a 2- to 3-fold increased risk of breast cancer mortality in several studies (75-78), including a Women's Health Initiative random sample of female participants with a baseline measurement for glucose and insulin, in which the risk ratio for the highest tertile of insulin was 2.22 (95% CI: 1.39-3.53) (79). Yet, associations between fasting insulin concentrations and breast cancer risk are not entirely consistent: one meta-analysis (80) provided data from five studies, in which two (81,82) found a direct relationship between increased fasting insulin and breast cancer risk and three studies (83-85) found no association. A meta-analysis specific to the association between metabolic syndrome and postmenopausal breast cancer was published in 2013. The authors determined that among 9 articles comprised of 6,417 cancer cases, metabolic syndrome was associated with a 52% increased risk for breast cancer, which was mainly attributed to the results of case-control studies rather than prospective cohort studies (86). Importantly, no single component explained the risk conveyed by the full syndrome, indicating that there is a complex, synergistic negative effect of the health conditions contributing to the metabolic syndrome.

D. Obesity and Skeletal Health

Osteoporosis is a skeletal disorder characterized by low bone mass and bone tissue deterioration that results in reduced bone strength and increased fracture risk. Several epidemiologic studies have highlighted the correlation between obesity and osteoporosis (87-91), with many indicating a significant positive relationship between body weight and bone mineral density (BMD). This correlation is largely attributed to both the increased mechanical load of excess weight and hormonal changes associated

with obesity, including increased estrogen and leptin production by adipose tissue, which have been shown to suppress bone resorption and stimulate osteoblastogenesis (92-94). However, recent evidence has emerged demonstrating that increased body weight may actually be negatively correlated with BMD after correction for the mechanical loading effect of excess body weight (95), and that obese individuals with high BMD are still at increased risk for fractures (88). Research on the association between obesity and metabolic bone diseases such as osteoporosis, a major cause of morbidity and mortality in postmenopausal women, remains inconclusive.

1. Dual-energy X-ray Absorptiometry for Measurement of Bone Mineral Density and Body Composition

Although there are several methods for studying human body composition, dual-energy X-ray absorptiometry (DXA) has emerged as one of the most commonly used clinical standards (96). Using this technique, an energy source produces photons at two different energy levels, 40 and 70 keV, which pass through tissues and diminish at rates related to its elemental composition. Bone is rich in calcium and phosphorous, which are highly attenuating minerals, and therefore it is easily distinguished from soft tissues. The elemental profiles of bone, fat, and non-bone lean tissue are different from each other, which allows for visualization and separate analysis of each tissue type (97). Most currently marketed DXA instruments use standardized calibrations, are able to scan quickly (within 5 to 20 min), and use standard software for analyses (97). The ability to study body composition in the whole body and individual body segments is useful in determining not only BMD, but also body fat distribution. The DXA is considered to be one of the gold standards of body composition assessment (98).

E. Section Summary

Obesity is a significant public health concern and is associated with insulin resistance, the metabolic syndrome, and increased risk for postmenopausal breast cancer. Intra-abdominal fat, known as VAT, has been identified as an independent risk factor for breast cancer, largely attributed to the state of chronic systemic low-grade inflammation

brought about by excess abdominal obesity. Several hormones associated with obesity and breast cancer also communicate nutritional status to areas of the central nervous system, including leptin, adiponectin, ghrelin, and insulin. Osteoporosis and obesity are typically negatively correlated, though research has indicated that obese individuals with high BMD are still at increased risk for fractures. DXA has emerged as one of the most commonly used methods of body composition assessment in randomized trials. Taken together, interventions that reduce body weight and adiposity may reduce risk for metabolic diseases, osteoporosis, and postmenopausal breast cancer via several mechanisms, including alterations in obesity-associated hormone concentrations and reductions in VAT.

III. Green Tea Catechins

A. Description and Chemical Composition

Tea is one of the most widely consumed beverages in the world, second only to water (99). There are over 300 types of tea, each derived from the leaves of the *Camellia sinensis* plant, which are commonly divided into three types: green tea (non-fermented), oolong tea (semi-fermented), and black tea (fermented). Green tea accounts for approximately 20% of the world's tea production, with black tea comprising 78% and oolong teas accounting for the remaining 2%. Green tea is the main tea consumed in Japan and China but has been relatively uncommon in western countries, where black tea is more frequently consumed (99). Green tea is manufactured by steaming and heating fresh tea leaves immediately after harvesting; this process avoids oxidation of the naturally occurring polyphenols in the plant, the most abundant of which are known as catechins. In contrast, the processing of black tea involves oxidization of dried and crushed tea leaves by polyphenol oxidase and peroxidase, which convert catechins to other compounds (mainly theaflavins and thearubigens) (99,100).

Green tea has been associated with myriad health benefits which have been attributed mainly to its catechin content. Catechins belong to a specific class of flavonoid, the flavan-3-ols, and are characterized by a di- or tri-hydroxyl group substitution of the B ring and the meta-5,7-dihydroxy substitution of the A ring (101,102). These compounds

account for 30-42% of the dry weight of the solids in brewed green tea. Though there are many different forms of catechins, the four predominantly found in green tea are (-)-epicatechin (EC), (-)-epicatechin gallate (ECG), (-)-epigallocatechin (EGC), and (-)-epigallocatechin-3-gallate (EGCG). Of these, EGCG accounts for 50-80% of the total catechins in green tea, making it the most abundant polyphenol (103). The structures of these four major catechins are shown in **Figure 2.2** (104). Green tea also contains caffeine as 2-5% of the water-extractable solids. The amount of caffeine in a tea beverage is determined by leaf size, brewing time, and temperature of the steeping liquid and is relatively consistent between types of tea, ranging from 14 to 61 mg per 6-8 ounce serving (105). Flavonols and flavones make up a small percentage of the polyphenolic content (106); other compounds include gallic acid, theanine, theogallin, theobromine, theophyilline, nitrogenous compounds, and several minerals (potassium, calcium, magnesium, aluminum, and fluoride) (106).

B. Green Tea Catechin Biotransformation and Metabolism

Animal studies have determined that green tea catechins (GTC) and their downstream metabolites are widely distributed in a broad range of tissues after oral administration (107,108). Catechin metabolites reach peak plasma nanomolar concentrations 1.6-2.3 hours after intake, which then rapidly decline - only trace amounts remain 8 hours after consumption (109). GTC go through extensive biotransformation following ingestion, which is primarily accomplished through methylation, sulfation, glucuronidation, and ring-fission metabolism by colonic bacteria (110). EC and ECG metabolites are primarily excreted in the urine. In contrast, EGCG appears unmetabolized in low concentrations in plasma and its metabolites are typically undetectable in urine (111). It is largely believed that enterohepatic recirculation of EGCG occurs and that it is primarily excreted in the bile (112). Approximately 69% of GTC and their metabolites reach the large intestine, where they are converted into phenylvalerolactones and phenylvaleric acids, which then leave the body through urinary excretion (109). Clifford et al. have noted that based on 24-hour urinary excretion, gut microflora-associated metabolites account for substantially more of the recovered flavan-3-ol dose than from strictly sulfated, glucuronidated, or methylated forms of GTC (109).

1. Catechol-*O*-methyltranferase (COMT)

Catechol-*O*-methyltransferase (COMT), which catalyzes *O*-methylation reactions, is of considerable research interest as it is known to have widespread influence on several physiological processes and disease conditions. COMT is ubiquitously present in human tissues, with its highest activity in the liver, kidney, and gastrointestinal tract (113) and the enzyme is also known to metabolize catecholamines (dopamine, norepinephrine, and epinephrine, which are significantly involved in sympathetic nervous system stimulation), catecholestrogens, neurotransmitters, and other dietary flavonoids (114). COMT is present in both soluble and membrane-bound forms, with the soluble form predominating in most tissues (113). While *O*-methylation of GTC occurs very rapidly, inhibition of the COMT enzyme by EGCG and its metabolites has also been demonstrated (113), indicating that (1) ingestion of GTC may decrease the degradation rate of catecholamines and other substrates of COMT; and (2) alternate pathways of catechin metabolism play a significant role in the biotransformation of green tea constituents. Indeed, Zhu and colleagues found that ECG and EGCG were methylated at much lower rates as compared to EC and ECG (115), while glucuronidation and sulfation may play a larger role in EGCG metabolism (116).

Genetic variations in COMT enzyme activity have been widely noted. One common polymorphism, a guanine (G) to adenine (A) substitution, at codon 108 (rs4680) of the soluble form of the enzyme (codon 158 of the membrane-bound form) has been identified that produces an amino acid change from valine to methionine, which has been shown to reduce the thermostability of the enzyme and is associated with a 3- to 4-fold decrease in enzymatic activity (117,118). Given these effects, COMT genotype may influence the rate of catechin metabolism, stimulation of the sympathetic nervous system, and the functional response to dietary catechins.

Since the health effects of green tea are largely attributed to catechins, factors that modulate the bioavailability of these compounds or their downstream metabolites could have a significant impact on the health properties associated with tea consumption. Polymorphisms in COMT have also been studied in direct relation to breast cancer risk, though conflicting hypotheses exist for this association. Wu et al. have described an

interaction between tea intake, COMT genotype, and breast cancer risk, in which the protective effect of tea consumption on breast cancer risk was mainly limited to women carrying at least one copy of the low-activity (A) allele (119). These results suggest that individuals with the low-activity COMT genotype metabolize tea catechins at a slower rate than those with the high-activity (G) allele, which increases the *in vivo* exposure of these flavonoids within the body. Therefore, a stronger beneficial effect of catechins may be observed. This hypothesis has proven to be inconsistent, as some studies have found no evidence that COMT genotype is independently associated with breast cancer risk among Caucasian and Asian women (120,121). It has also been suggested that individuals who inherit the homozygous low-activity genotype may be at increased risk for breast cancer due to an increased accumulation of the catechol estrogen intermediates, methoxyestradiols and methoxyestrones (122-124). A meta-analysis completed in 2012 encompassing 51 case-control studies, with a total of 34,358 cases and 45,429 controls, concluded that the COMT Val158Met polymorphism was not associated with breast cancer risk in women either overall or among subgroups of ethnicity, menopausal status, or sources of the control population (125). Yet, dietary interactions between COMT genotype and breast cancer risk remain understudied.

a) Insulin and COMT

Immunohistochemical staining has indicated that the COMT enzyme is present in the beta cells of the pancreas (126), therefore demonstrating significant potential for cross-talk between its enzymatic activity and glucose homeostasis. In an animal model of diabetes, COMT activity was markedly decreased in the liver, and correction of hyperglycemia restored activity of the enzyme (127). Hyperglycemia is associated with beta cell destruction; therefore, high blood glucose or an insulin deficit may account for the abnormality in COMT activity. The neurotransmitters norepinephrine and dopamine are important regulators of many physiological processes - including glucose homeostasis (128) - and are metabolized by COMT, so polymorphisms in COMT and potential interactions with dietary intake may impact the insulin response.

Translation of this body of research to humans has yielded evidence of an increased risk of impaired glucose tolerance (IGT) and type 2 diabetes (T2D) in

individuals with the homozygous high-activity (G/G) genotype. In a cohort study of Danish Caucasian men, an 11.6% increased frequency of the G/G genotype was noted in those with IGT and T2D as compared to the homozygous low-activity (A/A) genotype (129). Similarly, Miller et al. showed that individuals with the G/G genotype (n = 10) had a greater increase from baseline in postprandial insulin concentrations 2 and 3 hours after a high-carbohydrate breakfast meal as compared to those with the A/A genotype (n = 10), despite similar glucose concentrations at these time points (130). However, an association between COMT genotype and circulating insulin concentrations was not seen in a crossover study of 64 overweight and obese males who consumed 800 mg decaffeinated GTC daily for 6 weeks (131). Notably, there is a lack of research – in both epidemiologic and randomized controlled trials - on the role of COMT genotype in insulin sensitivity in female subjects, indicating the need for additional research in this area.

C. Section Summary

Green tea is a popular beverage and is believed to have health benefits, which have been attributed its high content of polyphenolic catechins. Catechins are metabolized through methylation, sulfation, glucuronidation, and ring-fission metabolism, and the COMT enzyme is known to be involved in degradation of catechins as well as catecholamines, including norepinephrine. This duality of effect is believed to be vital in green tea's effects on body weight and adiposity. Polymorphisms in COMT are thought to play a role in the functional response to dietary catechins, and may also impact green tea's effect on breast cancer and obesity.

IV. Effect of Green Tea on Obesity and Body Composition

Given their modifiable nature, obesity and body composition may be influenced by dietary intake of foods and beverages, such as green tea. The following sections will detail the biological mechanisms behind green tea's effects on body weight and body composition, results of randomized trials assessing body weight and body composition as clinical endpoints, and previous research on the impact of GTC on obesity-associated hormone concentrations and insulin sensitivity.

A. Biological Mechanisms of Green Tea Catechins on Changes in Body Composition

Specific mechanisms by which GTC may influence body weight and adiposity continue to be actively researched. Research in humans has primarily focused on four main areas: increases in energy expenditure (EE) with GTC administration; increased fat oxidation; decreased nutrient absorption, and modifications to satiety and appetite control.

1. Increased Energy Expenditure

The sympathetic nervous system (SNS) is highly involved in regulation of EE and lipolysis. Norepinephrine, a catecholamine produced in the adrenal glands, is a key mediator of SNS activity and is known to increase EE and promote fat oxidation (132). As stated previously, degradation of norepinephrine is mediated primarily by COMT, an enzyme whose activity may be inhibited by GTC. Therefore, GTC, through inhibition of COMT activity, may prolong norephinephrine activity and lead to increases in EE and fat oxidation. In addition to catechins, green tea also naturally contains caffeine, which is independently known to increase EE. Caffeine inhibits phosphodiesterase (PDE), an enzyme that rapidly degrades cyclic adenosine monophosphate (cAMP), which is released in response to norepinephrine (133) . Therefore, through the dual actions of GTC and caffeine, green tea consumption may increase an individual's metabolic rate, leading to weight loss and reductions in adiposity.

Many randomized trials examining the acute effects of green tea on EE have been conducted, with most using interventions including a combination of GTC + caffeine. Most of these studies have demonstrated modest increases (3-4%) in 24-hour EE relative to caffeine-free control (134-139). A widely cited paper by Dulloo et al. (138) demonstrated a 4% increase in 24-hour EE following treatment with 270 mg GTC + 150 mg caffeine, while there was no change in the group that received caffeine without GTC. This finding suggested that GTC had an independent effect on EE. Yet, these results have proven difficult to replicate, as additional studies comparing EGCG + caffeine to caffeine only and placebo are notably lacking. Further, randomized trials that include comparisons

of placebo, caffeine alone, caffeine + EGCG, and EGCG alone (i.e. a 2 x 2 factorial design) have not been conducted.

There is a scarcity of research of the effects of GTC on EE independent of caffeine. Only a few studies in total have examined caffeine-free GTC compared to a caffeine-free control (140,141) and they have not included EE assessment. While this is certainly an important area of active study, measurement of EE was beyond the scope of the current project and will not be addressed in the forthcoming research chapters.

2. Increased Fat Oxidation

Green tea may also influence body weight and adiposity through changes in fatty acid oxidation and metabolism. Norepinephrine stimulates lipolysis in peripheral tissues (including adipose, the liver, and skeletal muscle), which releases fatty acids into the circulation and up-regulates hepatic lipid metabolism (142). Inhibition of COMT (by GTC) and PDE (by caffeine) is theorized to increase fat oxidation. Animal studies support this hypothesis (143,144); yet, human studies have shown mixed results.

The respiratory quotient (RQ) is an indicator of the ratio of fat to carbohydrate oxidation, in which a lower RQ (close to 0.7 to 0.8) indicates greater fatty acid oxidation as compared to a higher RQ of 1.0, indicating pure carbohydrate oxidation. In humans, this is measured through indirect calorimetry (145) . Dulloo et al. (138) first demonstrated that 375 mg GTC + 150 mg caffeine decreased RQ by 3.4% as compared to a caffeine-free placebo in a small sample of young male subjects (n=10), which was suggestive of increased fat oxidation. Importantly, they did not see an effect of caffeine alone, which suggested that a combination of GTC and caffeine was necessary to elicit increased FA oxidation. As highlighted in the section above, the findings of this study have been widely cited in the literature in support of synergism between catechins and caffeine; yet, despite many attempts to replicate these findings, it is the only acute feeding study to show preferential effects of GTC in addition to caffeine on RQ and fat oxidation.

It has also been suggested that the effects of GTC on fat oxidation may be cumulative over time – one animal study showed that EGCG upregulated gene expression of acyl-CoA oxidase and medium chain acyl-CoA dehydrogenase and downregulated

expression of hepatic fatty acid synthase following one month of feeding. This resulted in a 285% increase in beta-oxidation as compared to control mice on a low-fat diet. These results were not noted 24 hours after feeding (144).

Human studies of long-term (>4 weeks) effects of GTC on fatty acid oxidation are inconclusive, and research using female participants is very limited. Mielgo-Ayuso studied the effect of 300 mg/day EGCG on body weight and adiposity in a randomized trial of 83 obese pre-menopausal Caucasian women over 12 weeks. They did not find any significant difference in change in fat mass or fat metabolism between the EGCG and placebo groups at the end of the intervention (146). This agrees with previous work, which showed that RQ was not different at baseline or between groups after GTC ingestion (141 mg GTC + 87 mg caffeine/day) for 4 weeks versus a caffeine-free control, declined at week 8, and returned to baseline levels by week 12 (134).

Human intervention trials have indirectly demonstrated that GTC may promote lipolysis from specific fat depots. Abdominal fat is known to be more lipolytically active than lower body adipose; therefore, abdominal adipose tissue could be selectively reduced more than peripheral fat stores (142). It is possible that GTC, by enhancing sympathetic effects, may have differential influences on lipid storage in various fat depots. Several randomized trials have demonstrated reductions in measures of central adiposity independent of weight loss in subjects receiving GTC interventions. In a sample of Japanese type 2 diabetics (men and women), Nagao et al. noted that while body weight did not differ between treatment groups after 12 weeks, WC was significantly reduced (-3.3 cm) in those consuming GTC (583 mg GTC + 75 mg caffeine/day) as compared to the control (96 mg GTC + 75 mg caffeine/day). Similar results were shown in Caucasian men and women following a 12-week study with in subjects receiving 624 mg GTC + 39 mg caffeine along with an exercise program: Maki et al. (147) determined that total abdominal fat area (measured by computed tomography) was significantly reduced (−7.7 cm^2) versus the control group, despite there being no significant difference between groups in body weight loss (−2.2 vs. −1.0 kg). More recently, another 12-week study with 60 healthy Caucasian men and women found no difference between groups receiving either placebo or GTE (560 mg EGCG + 280-450 mg caffeine/day) for body weight, fat

mass (assessed by BodPod), WC, or RQ (148). While increased fat oxidation and mobilization of VAT seems to be a promising mechanism of GTC's effects on body composition, several studies have based their conclusions of a beneficial effect on crude measurements such as WC and WHR, while others have used a variety of technology-based techniques, making aggregation of this data difficult. Research using precise measurements of body composition are needed to draw definitive conclusions of the effect of GTC on changes in adiposity.

3. Decreased Nutrient Absorption

Green tea catechins may inhibit gastrointestinal enzymes involved in nutrient absorption. Chan et al. demonstrated that hamsters given GTC produced feces with increased concentrations of total fatty acids and sterols compared with a control group (149). These results were recently confirmed in another study (150). With regard to human studies, Zhong, et al. reported that meals containing 100 mg ECG and 300 mg EGCG, black tea, and mulberry tea resulted in malabsorption of carbohydrates of approximately 60 calories compared to placebo, as assessed by hydrogen breath test (151). However, this same study failed to see any difference in fat malabsorption. Another study determined that a single dose of GTE taken with a test meal decreased lipid digestion and absorption in 32 healthy subjects (152). Though beyond the scope of the forthcoming research chapters in this dissertation, alteration of nutrient absorption by GTC remains a topic of current research, and its clinical relevance is in need of further study.

4. Appetite Inhibition

Green tea consumption may impact body weight through inhibition of appetite, though this remains a somewhat understudied area of research. Mechanisms for this effect include green tea's ability to increase hepatic fatty acid oxidation, which has been shown to decrease voluntary food intake (153). Early animal studies suggested that administration of GTC decreased food intake as compared to controls (154). However, the intervention was given intraperitoneally, rendering these results untranslatable to humans due to the administration of a catechin dose higher than physiologically

achievable through oral intake. When GTC were given orally, there was no difference in food intake between treatment and control groups (143). .

Randomized trials of human subjects have yielded mixed results, dependent on the variation in methods of assessing satiety and prospective food intake as well as the experimental conditions. Most studies have implemented the 100 mm visual analog scale (VAS) to measure satiety parameters, with the test anchored by 0 ("not at all hungry") and 100 ("extremely hungry"), as described by Flint (155). Using this method, Auvichayapat et al. measured appetite in subjects taking GTC or placebo for 12 weeks – appetite scores did not differ from baseline or between treatment groups over time (134). A lack of difference in satiety scores between green tea catechin interventions and placebo has been noted in several other randomized trials (139,156). Somewhat unexpectedly, Diepvens et al. showed that in a study of 46 overweight women randomized to receive 1125 mg catechins + 225 mg caffeine daily for 3 months or placebo, those randomized to receive catechins became more hungry over time and showed increased prospective food consumption compared with the placebo group (157). Other studies have used 3-day diet records to analyze food intake over time (147,158). While these studies have failed to show any change in food intake when comparing green tea interventions to the control group, self-reported diet recalls may not be sensitive enough to detect small changes that may impact long-term body weight and fat mass. Further, studies assessing the long-term (>12 weeks) impact of GTC consumption on appetite and food intake have not been conducted.

To date, only three studies have examined the acute postprandial effects of green tea catechins on satiety. One of these (159) demonstrated increased satiety after consuming a beverage containing 10 g soluble fiber, 167 mg catechins and 100 mg caffeine as compared to catechin-free beverage (10 g fiber + 46 mg caffeine), water, or no beverage control. These results are similar to those of Josic (160), who found increased satiety in a crossover study of 14 healthy volunteers who consumed 300 mL green tea beverage (approximately 148 mg catechins) with a breakfast meal as compared to water. Lastly, Belza et al. (136), also using a crossover study design, compared satiety and next-meal energy intake in 12 healthy men who randomly consumed 400 mg GTE,

400 mg tyrosine, 50 mg caffeine, or placebo on 4 separate occasions. They found that *ad libitum* energy intake 4 hours after administration of the intervention was reduced by 96 calories in the GTE group as compared to the placebo, despite no difference in VAS ratings of satiety and prospective food intake.

B. Epidemiologic Studies of Green Tea Consumption and Body Composition

A few epidemiologic studies have examined the impact of tea on body weight and other obesity-related end points. A 2003 cross-sectional epidemiologic study of 1103 Taiwanese adults found that habitual tea drinkers (defined as tea consumption at least once per week for 6 months) who consumed tea for >10 years had 19.6% lower body fat percentage and 2.1% lower WHR compared with non-habitual consumers (161). While this study did not separate consumers of different tea types, they did note that green tea and oolong tea were consumed more frequently than black tea. A longitudinal analysis within The Netherlands Cohort study of 4280 adults aged 55-69 years found an inverse relationship between catechin consumption and BMI increase over the 14-year study period in women only (162). Women with the highest intake of total flavonols/flavones and total catechins experienced a significantly lower increase in BMI of 0.40 and 0.31 kg/m^2, respectively (162). These population-based studies indicate that regular consumption of green tea may have beneficial effects on adiposity and body weight.

C. Randomized Trials and Meta-analyses of Green Tea Interventions and Body Weight-Related Endpoints

Over the last decade, many human intervention studies have assessed the effects of green tea catechins on body weight and related outcomes. Four major meta-analyses (156,163-165) have summarized these results, with the most recent being published in 2012. Since then, several more articles have been published on the subject of green tea and body composition. The following section highlights the results of the previous meta-analyses and describes in more detail the studies that have been published within the last 3 years.

1. Randomized Trials and Meta-analyses of Green Tea Interventions and Body Weight-Related Endpoints

a) Hursel et al., 2009

Hursel et al. (156) published the first meta-analysis of green tea and body weight in 2009. Their study aim was to determine the effects of EGCG on weight loss and weight maintenance and included possible modifiers, including regular caffeine intake and ethnicity. Trials were included if they had a randomized, double-blinded design, compared effects of GTC on weight loss or weight maintenance to different catechin doses or no catechins, and were at least 12 weeks in duration. Eleven articles fulfilled all criteria, 9 of which were weight loss studies (134,157,166-171) and 2 (139,172) were weight maintenance. Their results showed that GTC significantly decreased body weight and significantly maintained body weight after weight loss (-1.31 kg; 95% CI: -2.05, -0.57). While not reaching statistical significance, their results also demonstrated that high habitual caffeine intake may inhibit these effects on body weight, and that GTC had a larger effect on subjects of Asian ethnicity as compared to Caucasian subjects. Authors speculated that this could be due to different distributions of COMT genotype in these two populations, with Asian populations known to have a higher proportion of individuals with the G/G genotype as compared to Caucasians, who exhibit a higher frequency of the low-activity COMT enzyme allele (A/A or A/G) (173). A potential explanation for why studies examining the effects of GTC on body weight and adiposity -related variables in Asian populations have shown more favorable results may be that individuals of Asian descent are more sensitive to the effects of GTC on EE and other SNS-mediated outcomes. This outcome was also noted in the meta-analysis by Jurgens et al., below.

b) Phung et al., 2010

The results of the meta-analysis by Phung et al. were published in 2010 (163). Their objective was to characterize the relationship between GTC with and without caffeine and changes in anthropometric variables, including BMI, body weight, WC, and WHR. To account for possible confounding with caffeine in the results, they

conducted 3 separate analyses: (1) trials with GTC + caffeine compared to a caffeine-matched control; (2) GTC + caffeine compared with a caffeine-free control; and (3) caffeine-free GTC compared with a caffeine-free control. A total of 15 trials (n = 1243) met their inclusion criteria, 7 of which evaluated GTC + caffeine compared with caffeine-matched control (147,167,174-178), 6 that evaluated GTC + caffeine compared with caffeine-free control (134,157,170,179-181), and 2 (140,141) with caffeine-free GTC compared with a caffeine-free control. The authors determined that administration of catechins with caffeine significantly decreased body weight (-1.38 kg, 95% CI: -1.70, -1.06), BMI (-0.55; 95% CI: -0.65, -0.40), and WC (-1.93 cm; 95% CI: -2.82, -1.04) when compared with caffeine alone, suggesting an independent beneficial effect of catechins on body weight and central and total adiposity. This contrasts with the literature highlighted above on the effect of GTC on EE and fat oxidation, as most studies of specific mechanisms have failed to show positive results of GTC administration apart from the effects of caffeine. A significant reduction in body weight was observed in studies that compared GTC + caffeine to a caffeine-free control (-0.44 kg; 95% CI: -0.72, -0.15), but had no effect on BMI, WC, or WHR. The two studies that compared caffeine-free catechins with placebo did not find a significant effect of green tea alone on body weight or related outcomes. This lack of effect may be due to the small number of studies, small sample sizes (n=38 and n=81), short duration of supplementation (12 weeks), and moderate catechin dose provided (280 mg EGCG/day, equal to 2-3 four-ounce cups of green tea per day). Aside from the study by Matsuyama (176), which was conducted in children, the longest study duration was 12 weeks, so any effect of GTC extending past this point was unable to be determined.

c) Jurgens et al., 2012

A comprehensive review of green tea's effects on weight loss and weight maintenance specific to overweight or obese adults was conducted in 2012 (164). This meta-analysis used Cochrane methodology and looked at 15 weight loss studies (134,140,141,147,157,158,166-168,170,182-186) and 3 weight maintenance studies (139,172,187) (total n = 1945). Each of the included randomized trials was at least

12 weeks in duration and study size ranged from 19 to 270 participants. Nine of the 18 took place in Japan (140,166-168,182-186), which were separated from the remaining 9 studies due to significant heterogeneity between Japanese and non-Japanese studies. Daily dose of GTC ranged from 141 to 1207 mg, with 16 of the 18 doses ranging from 270 to 646 mg. Overall, more favorable changes in body weight, BMI, WC, and WHR were seen in Japanese populations as compared to those from other countries. In studies conducted outside of Japan, the mean difference in weight loss when comparing the green tea intervention to control was -0.04 kg (95% CI: -0.5, 0.4; $P = 0.88$), whereas mean difference in weight loss in Japanese populations ranged from -0.2 kg to -3.5 kg in favor of green tea preparations (results could not be pooled for Japanese studies, so a mean difference and 95% CI could not be calculated). When assessing BMI, mean difference in studies conducted outside of Japan was -0.2 kg/m^2 (95% CI: -0.5, 0.1, $P = 0.21$), while the range for Japanese studies was from no effect to -1.3 kg/m^2 in favor of green tea over control. WC showed similar findings, in that a small but insignificant reduction was seen in groups both inside (range from -3.3 cm to +1 cm) and outside of Japan (mean difference = -0.2 cm, 95% CI: -1.5, 0.9; $P = 0.70$; 404 participants). In the three studies that measured WHR, no significant change was seen (mean difference = 0; 95% CI: -0.02, 0.01). Authors concluded that green tea preparations appeared to induce a small, though statistically non-significant weight loss in overweight and obese adults. They further stated that because the amount of weight loss was small, it was unlikely to be clinically significant. While this meta-analysis included a large number of subjects and addressed a number of body weight-related variables, they did not separate out studies that included caffeine in the intervention from those that did not, therefore rendering the results incomparable to those of Phung et al. Additionally, they used crude measurements of abdominal adiposity (WC and WHR) that are prone to measurement error, so precision in these measurement is uncertain. Lastly, only 5 (141,157,170,184,185) of the 18 studies included only women, while 10 included both males and females, making it impossible to separate any gender-specific differences on weight and adiposity variables.

d) Baladia et al., 2014

Baladia et al. conducted a meta-analysis (165) whose aim was to evaluate the magnitude of the effect of green tea or its extracts on body weight and body composition in overweight or obese individuals. In the 5 studies that were included in their analysis (134,158,170,172,187), authors concluded that there was not a statistically significant mean difference in weight loss, BMI, WC, or hip circumference in the overall sample or in Caucasian or Asian subgroups when comparing green tea interventions to control. Despite a lack of statistically significant change in body fat percentage in either Asian or Caucasian subgroups, a small, statistically significant decrease was seen in those randomized to green tea intervention as compared to control when the subgroups were pooled (mean difference = -0.76% [95% CI: -1.44% to -0.09%; $P = 0.03$]; n = 260). Though this analysis is limited by the small number of studies included, it is the only review to date that has included measurement of percent body fat, which contributes added precision to adiposity measurement as compared to WC or WHR. In support of this, assessment of WC and hip circumference did not yield significant mean differences between treatment and control groups despite a significant reduction in % fat mass.

2. Studies Conducted since 2013

Since the last meta-analysis was conducted, two studies (146,148) examining the effect of GTC on body weight and adiposity have been conducted. One of these measured changes in body composition in an energy-restricted diet intervention in 83 obese pre-menopausal women (146). Subjects consumed 300 mg/d of EGCG or placebo for 12 weeks. Body weight and adiposity were assessed through DXA. Authors did not find any significant difference in the changes in body weight (-0.3 kg, 95% CI -5.0, 4.3) between the EGCG and control groups. Janssens et al. (148) measured body composition in 60 healthy subjects (50 female, 10 male) who received 560 g EGCG + 280-450 g caffeine/day for 12 weeks. No significant differences between groups and no changes over time (baseline vs. week 12) were observed for body weight, body fat percentage, and WHR. There were no significant differences in the response to EGCG between subjects with a BMI of 18–25 kg/m^2 and subjects

with a BMI >25 kg/m^2, and no differences were seen when comparing men and women.

D. Green Tea and Adiposity Measurements

Several randomized trials have examined the impact of GTC on measures of adiposity. Overall, most studies have observed a small effect of GTC interventions on total body and abdominal fat reduction, which supports the hypothesis that GTC may selectively reduce VAT through SNS-mediated increases in fat oxidation. However, comparisons between studies have been difficult to draw, due to the wide variety in measurement techniques. Westerterp-Platenga, using the deuterium (^2H_2O) dilution technique, determined that subjects randomized to the GTC intervention lost significantly more body fat after an energy-restricted diet as compared to the control group (139). Similar results were obtained by another study using the same technique (157). Nagao et al. (177) used computed tomography to measure total, abdominal, visceral, and subcutaneous fat area in a 12-week study including 240 obese participants. Decreases in body weight, BMI, body fat mass, visceral fat area, and subcutaneous fat area were found to be greater in the catechin group than in the control group. Another 12-week study (175) used bioimpedance techniques and determined that a significant time effect was detected for percent body fat: in the control group, percent body fat had significantly increased at week 12 compared with weeks 0, 4, and 8; no significant within-group changes in percent body fat were detected in the catechin group. No significant differences between the two groups were noted. More recently, DXA technology has been used in randomized trials to measure changes in adiposity. Maki et al. (147) found that while percent changes in fat mass did not differ between the catechin (625 mg catechins + 39 mg caffeine) and control groups after 12 weeks, percent change in total abdominal fat area were greater in the catechin group (-7.7 [-11.7, -3.8] vs. -0.3 [-4.4, 3.9]; $P = 0.013$). In contrast, another more recent study found that consumption of 300 mg EGCG/day for 12 weeks did not lead to significant loss of fat mass as compared to the control in a sample of obese premenopausal women (-0.7 kg, 95% CI -3.5, 2.1) (146). However, this dose of catechins is lower than seen in other interventions and may not have been high enough to induce an effect.

E. Effects of Green Tea on Bone Mineral Density and Skeletal Health

1. Pre-Clinical Studies of Green Tea and Bone

Evidence from *in vitro* and animal studies strongly suggests that green tea polyphenols are effective in preventing osteoporosis. This is mainly mitigated through the ability of GTCs to enhance osteoblastogenesis (188) (leading to bone formation) and inhibit osteoclastic activity (189), thereby reducing bone resorption. These *in vitro* effects have been replicated in animal studies using various models for bone loss. Supplementation of 3.4 mg/kg/day EGCG has been shown to reduce the rate of decrease in BMD and improve parameters of bone volume in ovariectomized rats, which serve as an animal model for osteoporosis (190). Song, et al. (191) confirmed these results and further demonstrated that 10 mg/kg/day EGCG had a positive effect on reducing - though not entirely eliminating - bone loss in recently ovariectomized rats as compared to those without EGCG supplementation and rats who had undergone a sham operation.

2. Human Studies of Green Tea Intake and Bone

While evidence from animal studies indicates a positive relationship between tea consumption and skeletal health, results of human studies have been less consistent, yet suggestive of a beneficial effect. A positive association between tea drinking and BMD has been reported among postmenopausal women in epidemiologic studies in several countries, including the United States (192), United Kingdom (193), Australia (194), and Japan (195). Another large case-control study showed that tea drinking was associated with a 30% reduced risk for hip fractures in women >50 years old (196). Yet, no association was seen in a study that examined tea consumption patterns in postmenopausal Turkish women (197). These discrepant results may be due to several challenging features of epidemiologic studies, including variation in study designs, inconsistent dietary intake methods, and incomplete adjustment for confounding lifestyle characteristics, as well as measurement of BMD at different skeletal sites. Further, several of these studies failed to differentiate between types of tea, which makes it impossible to know the specific association of green tea to BMD.

To date, only one randomized trial has examined green tea's effects on bone health. Shen, et al., conducted a study with a 2 x 2 factorial design of green tea supplementation and tai chi exercise in 150 postmenopausal osteopenic women (198) and found that compared to the control group, subjects assigned to receive 500 mg GTC/day for 6 months had significantly increased bone-specific alkaline phosphatase (BAP), a glycoprotein found on the surface of osteoblasts that is indicative of bone biosynthesis. However, this research group did not correlate serum biomarkers of bone metabolism with measurements of BMD, so the clinical relevance of these results is uncertain.

F. Section Summary

Obesity and body composition may be influenced by green tea consumption. Biologic mechanisms of this effect include increases in EE, increased fat oxidation, decreased nutrient absorption, and appetite inhibition. Many randomized trials have noted a small beneficial effect of GTC on body weight and measures of adiposity, while others have reported null results. Four meta-analyses of green tea's effect on body weight and adiposity have been conducted; however, several challenges make aggregation of the results difficult. Most studies have used different inclusion criteria, with many failing to separate the effects of GTC from that of caffeine, which is naturally present in green tea. This makes it difficult to determine whether the responsible agent is the GTC or caffeine - which by itself is known to increase EE – or if a synergistic relationship exists between the two compounds. Many studies have included both men and women of various ages in their participant pool, which limits the generalizability to specific age and gender groups. Lastly, the intervention periods have been mainly 12 weeks or less, so any longer-term effect of GTC on body weight and adiposity remains unclear. The shortcomings of the literature published to date indicate a need for studies that specifically address the impact of GTC – independent of caffeine - on body weight and adiposity, use sophisticated techniques for body composition analysis, include specific population groups (such as postmenopausal women), and are longer in duration.

V. Green Tea's Effects on Obesity-related Peptide Hormone Concentrations

GTC may influence body weight and breast cancer risk through modifying concentrations of obesity- and appetite-associated hormones, including leptin, adiponectin, ghrelin, and insulin.

A. Leptin

Animal studies have shown that green tea catechins can lower leptin concentrations (154,199-201). However, these reductions in leptin were associated with corresponding reductions in body fat. Therefore, green tea has not demonstrated an effect on leptin independent of the hormone's relationship with changes in fat mass. Human studies have demonstrated similar results. Basu et al. (202) randomized 35 obese subjects to receive either green tea beverage (4 cups/day), GTE (2 capsules and 4 cups water/day), or no treatment (4 cups water/day) with similar dosing of EGCG in both green tea treatment groups. Leptin concentrations did not change from baseline to the end of the 8-week intervention. Another study showed that a 4-week weight loss period reduced leptin concentrations, though this reduction did not differ between treatment (270 mg EGCG + 150 mg caffeine/day) and placebo groups, since all groups lost weight (203). These results correlate with Diepvens et al., who observed a reduction in leptin with a corresponding decrease in body fat in 46 overweight female subjects assigned to follow a low-energy diet and either 402 mg GTC + 78 mg caffeine/day or placebo for 12 weeks; no differences were observed between those assigned to GTC and those assigned to placebo (204). The acute postprandial response of leptin concentrations after a meal has not been studied, so it is unclear if leptin secretion and its related appetite-control effects are influenced by GTC.

B. Adiponectin

Since adiponectin has been investigated as a biomarker of breast cancer risk and insulin resistance, an association between green tea intake and adiponectin may have implications for prevention of these conditions. GTC have been shown to up-regulate adiponectin expression in mouse preadipocytes (205), and animal studies have confirmed

that administration of EGCG can increase adiponectin concentrations (206,207). However, results of studies involving human subjects are mixed.

1. Epidemiologic Studies

The relationship between adiponectin levels and green tea intake in humans has been studied in four cross-sectional studies. One study of Japanese males (n = 665) showed that adiponectin levels did not differ by green tea intake after adjusting for age, smoking, BMI, and other covariates (P_{trend} = 0.55) (208). Similarly, green tea consumption was not associated with adiponectin in a multi-ethnic Asian population (n = 4139) (209). Recently published results of a cross-sectional survey of Japanese workers (ages 20-68 years) reported no association of green tea with adiponectin concentrations (210). In contrast, a study conducted in Asian American women (211) (n = 219) demonstrated that regular weekly or daily green tea drinkers had 25% higher adiponectin concentrations than non-green drinkers (P = 0.03). This difference remained after adjustment for age, Asian ethnicity, parity, BMI, and other covariates.

Results from a large nested case-control study within the NHS involving 1477 breast cancer cases and 2196 matched controls reported risk reduction (Odds Ratio = 0.73, 95% CI=0.55–0.98) comparing postmenopausal women in the highest versus lowest quartile of adiponectin levels (58) but this result was not confirmed in two smaller nested case-control studies (212,213).

2. Human Interventions

To date, 6 randomized trials examining the effect of GTC on adiponectin concentrations have been conducted (170,175,202,214-216). Four of these studies (170,202,214,216) found no difference in adiponectin between treatment and control groups at the end of the intervention. In a study of type 2 diabetics not receiving insulin therapy, Nagao et al. found that adiponectin concentrations increased in the group receiving 583 g GTC/day for 12 weeks (n = 23) as compared to the control group (n=20, who received 96.3 mg catechins daily). This increase was correlated with a reduction in WC (175). In contrast, a study conducted in 103 postmenopausal women concluded that adiponectin non-significantly decreased in the GTE groups

(400 mg or 800 mg EGCG) as compared to the placebo ($P = 0.084$) (215), despite no change in weight or anthropometric measurements. These 6 studies ranged from 4 to 16 weeks in duration, administered different amounts of GTC in various preparations, and subjects were of disparate clinical and demographic groups, making it difficult to draw conclusions on any impact that GTC may have on adiponectin. In addition, the postprandial response of adiponectin has not been studied, independent of or together with administration of GTC.

C. Ghrelin

There is a paucity of research on green tea's effect on circulating ghrelin concentrations. One randomized, double blind, placebo-controlled clinical trial in 78 obese women found a significant within-group increase in ghrelin after 12 weeks of daily supplementation with 1200 mg GTE (170). However, increased ghrelin was also observed in the placebo group, which may be related to the modest weight loss demonstrated for both groups. This same effect was seen in another study of 68 overweight subjects with type 2 diabetes (217). No postprandial studies with GTC administration have measured circulating ghrelin concentrations after a meal and its relationship to appetite and hunger.

D. Insulin and the Glycemic Response

Green tea ingestion has demonstrated an association with modulating blood glucose homeostasis, and inclusion of this beverage in the diet may promote reductions in glucose and insulin concentrations among people at risk for or with diagnosed diabetes or insulin resistance. Proposed mechanisms include inhibition of hepatic gluconeogenesis (218), inhibition of glucose uptake in the intestine (219), enhanced insulin sensitivity (220), and protection of pancreatic beta cells by inhibiting nuclear factor-κB (221). Epidemiologic evidence of a beneficial effect was noted by Iso et al., who found a dose-dependent, inverse relationship between green tea intake and diabetes risk in Japanese women (222) . These results were corroborated by a cross-sectional study in Japan that revealed an inverse correlation between daily green tea consumption and fasting glucose concentrations in males, though associations were not seen in mean blood glucose or

glycated hemoglobin (HbA1c), a form of hemoglobin measured primarily to identify average plasma glucose concentrations over time (223).

In vitro and animal studies have shown beneficial effects of green tea consumption on glucose and insulin sensitivity (224,225), but results of human studies are inconsistent and based on short-term intervention studies with small sample sizes. Decreased glucose concentrations have been observed in several studies with green tea catechin interventions (168,202,214,215), while others showed no effect of green tea consumption on blood glucose (170,226). Inconclusive results have also been presented for insulin (160,170,214,226). Wu et al. (215) demonstrated reductions in insulin concentrations ($P = 0.010$) and glucose concentrations ($P = 0.008$) in postmenopausal women given either 400 mg GTE or 800 mg GTE for 8 weeks as compared to placebo, whose insulin concentrations increased over the intervention period. A trial of obese adult males was unable to demonstrate a significant change in oral glucose tolerance test in response to 8 weeks of supplementation with 800 mg EGCG compared to placebo (227), while a trial involving type 2 diabetics showed a significant improvement in insulin secretion and blood glucose concentrations in patients who received green tea (583 mg catechins) versus placebo tea (96 mg catechins) daily for 12 weeks (177). Many studies have not shown a benefit of green tea consumption on insulin sensitivity independent of reductions in body weight or carbohydrate intake (146,180,203,228,229). However, one study found a significant reduction in fasting insulin concentrations and HOMA-IR in subjects randomized to receive 379 mg GTE for 12 weeks, despite non-significant differences in BMI change from baseline as compared to placebo (230).

Research is very limited on the effect of GTC on the acute postprandial insulin response. No significant differences were found in serum insulin concentrations or the AUC for insulin or glucose after administration of a high-carbohydrate meal in 14 healthy volunteers with either 300 mL green tea or water (160).

1. Interaction between GTC, Insulin, and COMT Genotype

COMT genotype has also been suggested as a modifier of insulin sensitivity and the acute postprandial response. Miller et al. (130), whose primary aim was to investigate the impact of green tea and COMT genotype on vascular reactivity,

discovered that participants with the homozygous G/G genotype showed greater postprandial insulin concentrations as compared to those with the homozygous A/A genotype, irrespective of the green tea intervention. Further, Kring et al. (129), found that the frequency of the G/G genotype was 11.6% higher in males with insulin resistance and type 2 diabetes as compared to the G/A and A/A genotypes (n = 451). They suggested that this may be a result of the role of catecholamines in energy and glucose homeostasis. Therefore, it is very plausible that GTC and COMT genotype may both have a clinically meaningful physiologic effect on insulin sensitivity, though additional studies in humans are needed to confirm this hypothesis.

E. Section Summary

Green tea may influence body weight and breast cancer risk through modifying concentrations of leptin, adiponectin, ghrelin, and insulin. Green tea has not demonstrated an effect on leptin independent of the hormone's relationship with changes in fat mass, and a limited body of knowledge has not shown a benefit of GTC on ghrelin concentrations. In contrast, green tea has demonstrated beneficial effects on adiponectin and insulin concentrations in both epidemiologic and human intervention studies. COMT genotype may also modify the insulin response to GTC administration, though this remains inconclusive. With the exception of insulin, the acute postprandial effects of GTC on these hormones has not been studied, indicating the need for further research.

VI. Literature Review Summary and Dissertation Rationale

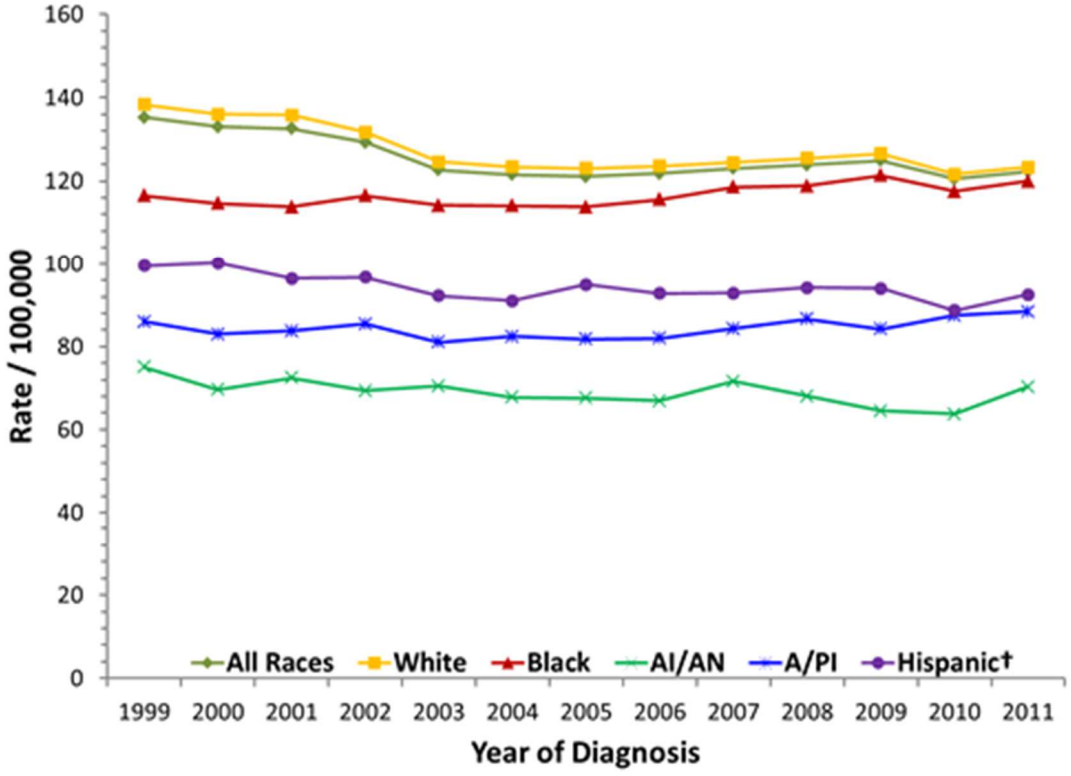
It is well known that postmenopausal breast cancer, obesity, and metabolic diseases are inextricably linked, and that this relationship is exceedingly complex. Green tea consumption may aid in preventing these conditions through a variety of different mechanisms, including reducing body weight and adiposity, altering concentrations of obesity- and appetite-associated hormones, inducing postprandial satiety, and reducing food intake. Further, polymorphisms in COMT are thought to play a role in the functional response to dietary catechins and may also impact green tea's effect on breast cancer and obesity. A vast body of literature detailing the impact of green tea and GTC on body weight and adiposity has been amassed. Yet, research gaps still remain. Most studies conducted to date have not separated catechins from caffeine, so it remains to be seen if

GTC have an independent effect on obesity-related endpoints. Research specific to overweight and obese postmenopausal women is particularly important, given that this population is at increased risk not only for breast cancer, but also for osteoporosis, IR, and other metabolic conditions. Since much of the literature behind green tea's health effects has come from studies less than 12 weeks in duration, it is critical that the long-term implications of GTC consumption be assessed. Lastly, the postprandial response to GTC administration has not been studied in obesity- and appetite-related hormones such as leptin, ghrelin, and adiponectin, and the acute glycemic effects of GTC with a meal remains inconclusive.

The MGTT is the first long-term clinical intervention to evaluate the effects of GTC, independent of caffeine, on parameters related to body composition, glucose homeostasis, and appetite regulation in overweight and obese postmenopausal women. The ultimate goal of this research is to gain a full understanding of the anti-obesity effects of GTC. The findings of the forthcoming chapters in this dissertation will contribute extensively to our understanding of the role of green tea in risk reduction for obesity, metabolic disorders and breast cancer.

VII. Figures

Figure 2.1. Female Breast Cancer Incidence Rates by Race and Ethnicity, U.S., 1999-2011, reprinted from the Centers for Disease Control and Prevention.

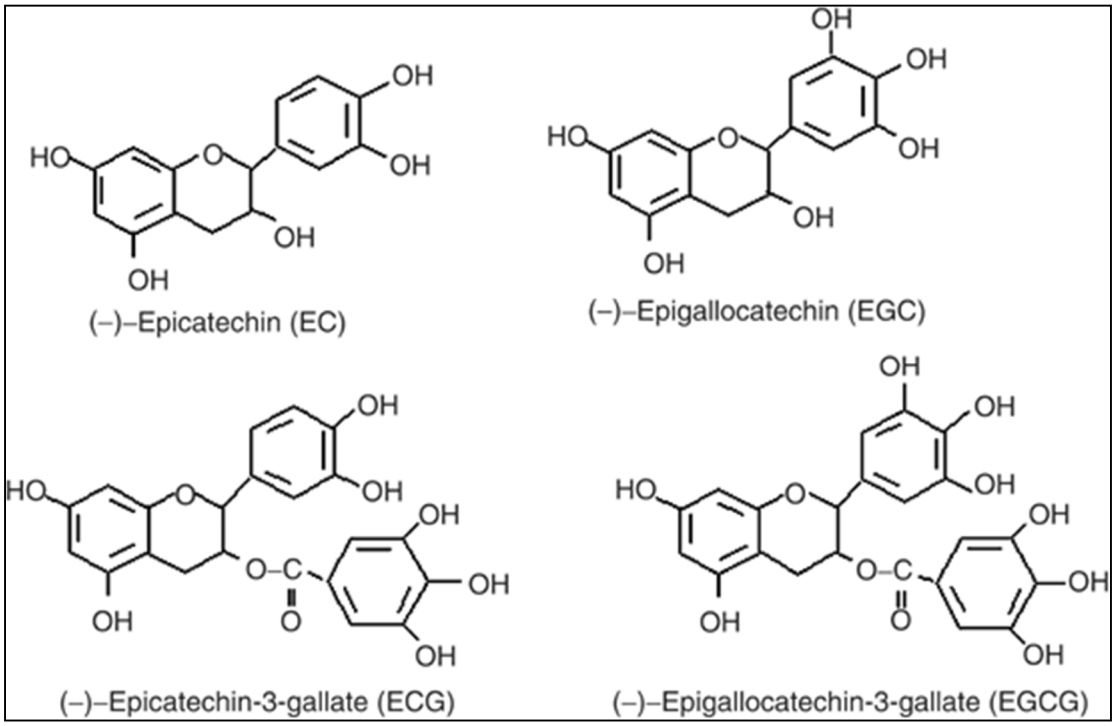


Incidence source: Combined data from the National Program of Cancer Registries as submitted to CDC and from the Surveillance, Epidemiology and End Results program as submitted to the National Cancer Institute in November 2013.

*Rates are per 100,000 and are age-adjusted to the 2000 U.S. standard population (19 age groups – Census P25-1130). Incidence rates are for state registries that meet USCS publication criteria for all years, 1999–2011. Incidence rates cover about 99% of the U.S. population.

†Hispanic origin is not mutually exclusive from race categories (white, black, Asian/Pacific Islander, American Indian/Alaska Native).

Figure 2.2. **Structure of Green Tea Catechins (104)**



Chapter 3 - Effect of Green Tea Extract on Anthropometric Variables, Obesity-Associated Hormones, and Glucose Homeostasis in Overweight and Obese Postmenopausal Women: a Randomized, Controlled Clinical Trial

I. Overview

Background: Green tea consumption has been associated with favorable changes in body weight and obesity-related hormones, though it is unknown if this is due to green tea polyphenols or caffeine. **Objective:** We examined the impact of decaffeinated green tea extract (GTE) containing 843 mg (-)-epigallocatechin-3-gallate (EGCG) on anthropometric variables, obesity-associated hormones, and glucose homeostasis. **Methods:** The Minnesota Green Tea Trial was a 12-month randomized, double-blind, placebo-controlled clinical trial of 937 healthy postmenopausal women assigned to either GTE or placebo based on catechol-*O*-methyltransferase (COMT) genotype. This study was conducted in a subset of 237 overweight and obese participants. **Results:** No changes in energy intake, body weight, BMI, or waist circumference (WC) were observed in women taking GTE (n=117) or placebo (n=120). No changes were seen in circulating leptin, ghrelin, adiponectin, or glucose concentrations. Among participants with baseline insulin ≥10 μIU/mL, there was a reduction in insulin concentrations in the GTE group over 12 months compared to the placebo group and participants with baseline insulin < 10 μIU/mL in either group ($P < 0.01$). Participants with the homozygous high-activity (G/G) form of COMT showed significantly lower adiponectin (5.97 ± 0.50 vs. 7.58 ± 0.53 μg/mL, $P = 0.03$) and increased insulin concentrations (7.63 ± 0.53 vs. 6.18 ± 0.36 μIU/mL, $P = 0.02$) at month 12 as compared to those with the low-activity (A/A) genotype, regardless of treatment group. **Conclusions:** Decaffeinated GTE was not associated with reductions in body weight, BMI, or WC and did not alter energy intake or mean hormone concentrations over 12 months. GTE decreased fasting insulin concentrations in those with elevated baseline fasting concentrations. The high-activity form of the COMT enzyme may be associated with elevations in insulin and reduction in adiponectin concentrations over time. This clinical trial was registered at http://www.clinicaltrials.gov as NCT00917735.

II. Introduction

Excess adiposity, especially visceral adiposity, has been accepted as a risk factor for both postmenopausal breast carcinogenesis and insulin resistance, a condition associated with development of the metabolic syndrome and type 2 diabetes mellitus (231). Numerous studies have indicated that green tea consumption may have beneficial effects on weight loss and weight maintenance as well as breast cancer risk (119,163,232,233). Catechins, a class of flavan-3-ol polyphenols, are major constituents of green tea; these include epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), and (-)-epigallocatechin-3-gallate (EGCG) (154). Among these, EGCG is the most abundant and is thought to be a major contributor to many of the beneficial effects of green tea, including its anti-obesity and anti-diabetic properties (234,235). Green tea also contains caffeine (99), and it remains unclear if green tea's health benefits are due to its catechin content, caffeine content, or synergism between the two compounds.

One of the major metabolic pathways of green tea catechin degradation is *O*-methylation, catalyzed by catechol-*O*-methyltransferase (COMT). At the same time, flavanolic compounds such as catechins reduce the activity of COMT. This enzyme also metabolizes a number of other compounds, including catecholamines. One of the proposed mechanisms by which green tea consumption may influence body weight is through inhibition of COMT and the resulting increased and/or prolonged effects of norepinephrine, including increased energy expenditure and fat oxidation (236). Genetic variability may also have an impact on this relationship, since the COMT enzyme is polymorphic - a single nucleotide polymorphism (SNP) at codon 108/158 (rs4680) results in a guanine (G) to adenine (A) transition which results in a 3- to 4-fold decrease in enzymatic activity (20, 21). Individuals with the low-activity COMT genotype may metabolize tea catechins slower than those with the high-activity genotype, allowing the bioactive components to be retained longer and resulting in greater benefits from green tea intake.

Though there has been significant focus on green tea's regulation of energy expenditure (237) and fat oxidation (132,238), less research has been directed toward its potential effects on glucose homeostasis and adiposity-associated hormones, such as

leptin, ghrelin, and adiponectin, in postmenopausal women. This study aimed to clarify the effects of 12 months of caffeine-free green tea extract (GTE) supplementation on obesity-related hormones, anthropometrics, and glucose homeostasis in postmenopausal women in the absence of other lifestyle–related changes (diet and physical activity). We hypothesized that GTE supplementation would cause reduced body weight, body mass index (BMI), waist circumference (WC), and waist-to-hip ratio (WHR), and parallel changes in obesity-related hormones and glucose homeostasis. We also hypothesized that participants with the low-activity COMT genotype would experience greater effects than those with the high-activity genotype.

III. Methods

A. Study Design

The Minnesota Green Tea Trial (MGTT) is a 12-month randomized, double-blinded, placebo-controlled study designed to examine the effect of high-dose GTE on breast cancer risk factors. Details of the study design, eligibility criteria, randomization, blinding, study conduct, and patient flow through the trial will be published separately (Samavat, et al, *Cancer Causes and Control*). Briefly, healthy postmenopausal women aged 50 to 70 years and classified as having high mammographic density (a breast cancer risk factor) were recruited via mailed letter (example in **Appendix 1**) from 2009 to 2013 after having a routine screening mammogram at one of 8 clinical centers in the Minneapolis-St. Paul metropolitan area. If interested in participation, women contacted the study through telephone or online screening questionnaire.

Exclusionary criteria included: any history of breast cancer, proliferative breast disease, or ovarian cancer; any cancer diagnosis within 5 years; regular consumption of >7 alcoholic drinks per week; regular consumption of green tea (>1 cup per week); BMI < 25.0 or >40 kg/m^2; weight change >4.5 kg during the previous year; current or recent use (within 6 months) of menopausal hormone therapy or chemopreventive agents; current use of methotrexate or Enbrel (etanercept) (anti-inflammatory agents); current smoking; history of breast augmentation; positive serology for Hepatitis B or C antibodies; or alanine aminotransferase higher than 1.5 times the upper limit of normal

(defined as 60 U/L). The blood chemistry variables were determined by a blood sample taken at the screening clinic visit prior to randomization. The telephone/online screening questionnaire used to determine eligibility is included in **Appendix 1**.

Of 1075 randomized women, 538 were assigned to receive four oral GTE capsules containing a total of 1315 mg ± 116 total catechins per day (843 ± 44 mg as EGCG) and 537 were randomized to receive placebo. Nine hundred thirty-seven women (87.2%) completed the study. Of these, 162 overweight (BMI = 25.0-29.9 kg/m^2) and obese (BMI = 30.0-40.0 kg/m^2) women were randomly selected for appetite hormone analysis through a computer-generated random number sequence; an additional 75 overweight and obese participants enrolled in another MGTT ancillary study of body composition variables were included in this analysis. The final sample size was *n* (GTE) = 117 and *n* (placebo) = 120 (total n = 237). Of these, 5 from GTE and 5 from the placebo groups discontinued the intervention at some point during the study period, though they remained in the study in accordance with the intention to treat (ITT) analytic model, wherein all participants originally randomized into the study were included in the analyses, regardless of if they consumed the study supplement for the full 12-month duration.

B. Randomization and Blinding

Participants were randomly assigned to receive oral GTE or placebo for 12 months. Randomization was performed by the Investigational Drug Services (IDS) pharmacy at University of Minnesota Medical Center-Fairview using a computer-generated permuted block randomization scheme with blocks of 8 stratified by COMT genotype activity: low (A/A), intermediate (A/G), or high (G/G). Participants and study staff were blind to treatment allocation throughout the trial.

C. Ethics

Institutional Review Board (IRB) approval was obtained at each clinical center and all participants provided written informed consent. This trial was registered at clinicaltrials.gov as NCT00917735.

D. COMT Genotyping

COMT genotype was determined by the University of Minnesota Genomics Center. DNA was extracted from buffy coat samples by the Qiagen DNAeasy Blood and Tissue Kit method (Qiagen Inc., Gaithersburg, MD, USA). A TaqMan assay was developed for defining the COMT H/L polymorphism using a TaqMan PCR Core Reagent kit (Applied Biosystems, Foster City, CA). Corriell cell lines with known COMT genotype were used as quality controls with each PCR run.

E. Study Supplement

Decaffeinated Green Tea Extract Catechin Complex (GTE) and placebo capsules were supplied by Corban Laboratories (Eniva Nutraceutics, Plymouth, MN) and dispensed by the IDS Pharmacy in 3-month increments. Mean total catechin content of each GTE capsule was 328 ± 30 mg, including 211 ± 11 mg EGCG. The total daily catechin dose was 1315 ± 116 mg, with 843 ± 44 mg as EGCG, which is equivalent to five 8-ounce (240 mL) cups of brewed green tea/day (239) (**Table 3.1**). Placebo capsules were identical in appearance to GTE and contained 816 mg maltodextrin, 808 mg cellulose, and 8 mg magnesium stearate as a flow agent. Each GTE capsule contained less than 4 mg caffeine. GTE ingredients were analyzed by high-performance liquid chromatography (HPLC) (Rutgers University, Piscataway, NJ) to demonstrate comparability with the stated catechin contents of the manufacturer. Participants were instructed to consume two capsules, twice daily with morning and evening meals (4 capsules/day).

F. Anthropometry and Body Composition

Body weight was recorded at screening, baseline, and months 3, 6, 9, and 12 using a stand-on, calibrated digital scale (Scale-Tronix Inc., White Plains, NY). Standing height was assessed by wall-mounted stadiometer (Seca, Hanover, MD) to the nearest 0.1 cm at the screening clinic visit and month 12 following standard protocols. BMI was calculated by dividing weight in kg by height in meters squared (kg/m^2). Waist and hip circumference were recorded to the nearest 0.1 cm in duplicate using a flexible body tape at baseline and month 12. WC was measured at the uppermost lateral border of the iliac

crest at the narrowest point of torso. Hip circumference was measured at the widest part of the buttocks. WHR was calculated by dividing WC by hip circumference.

G. Dietary Assessment

Participants completed a food frequency questionnaire (FFQ) at the beginning and end of the study to capture dietary patterns from the previous 12 months. The Diet History Questionnaire (DHQ) is a FFQ that consists of 124 food items and includes both portion size and dietary supplement questions. The food list and nutrient database used with the DHQ are based on national dietary data (USDA's 1994-96 Continuing Survey of Food Intakes by Individuals, available from the USDA Food Surveys Research Group). FFQ data was analyzed using Diet*Calc software developed at the National Cancer Institute (NCI).

H. Physical Activity

Recreational physical activity was assessed at baseline and month 12 by a health history questionnaire (**Appendix 1**), in which participants were asked about frequency and duration of several types of physical activity. Metabolic equivalent hours (MET-hours), defined as the ratio of work metabolic rate to a standard resting metabolic rate, were computed as the product of average hours per week of each activity multiplied by its MET-hour equivalent. All recorded activities were summed to obtain the total MET-hours of activity performed per week for a given participant.

I. Obesity-associated Hormone and Glucose Homeostasis Marker Analysis

Blood samples for obesity-associated hormone and glucose homeostasis marker assessment were collected at baseline and month 12 by a research nurse or licensed phlebotomist after an overnight fast of >10 hours. Whole blood samples were separated into plasma and serum, aliquoted in 1.5 mL volumes, and stored at -80°C until sample analysis was performed. Plasma leptin, ghrelin, and adiponectin were measured using radioimmunoassay kits manufactured by EMD Millipore (Billerica, MA) [inter-assay % coefficient of variation (% CV): leptin = 7.1%, ghrelin = 7.8%; adiponectin = 8.9%; intra-assay % CV: leptin = 6.6%; ghrelin = 5.5%; adiponectin = 7.4%]. Serum insulin was measured via a simultaneous one-step immunoenzymatic, chemiluminescent assay

(Access Ultrasensitive Insulin assay, Quest Diagnostics, Wood Dale, IL, intra-assay % CV: 3-5.0%; inter-assay % CV = 3.9%). Serum glucose concentrations were measured using a hexokinase enzymatic reference method (Quest Diagnostics, Wood Dale, IL, monthly % CV = 1.4%). Homeostasis model assessment for insulin resistance (HOMA-IR) was used to evaluate insulin resistance: (fasting insulin [μIU/mL] x fasting glucose [mg/dL]) / 405.

J. Adverse Event Reporting

Information on adverse events (AEs) occurring during the study was obtained at clinic visits or by self-report via phone or e-mail. Hepatic function was measured monthly during the first six months of the study and at months 9 and 12 due to GTE's association with hepatotoxicity (240,241). AE information was coded and graded using the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE), version 4.03 (242). The clinical course of each event was tracked in an ongoing AE record in the study clinic and each event was followed until resolution.

K. Statistical Analyses

Differences in baseline demographic and anthropometric characteristics between groups and baseline, month 12 and changes from baseline in dietary intake and anthropometrics were assessed by non-paired Student's *t* tests. Change from baseline was calculated by subtracting the baseline from the month 12 values. A repeated measures linear regression was used to assess within-treatment group and within-COMT genotype differences in anthropometric variables between baseline and month 12. Obesity-associated hormones, glucose, and HOMA-IR were natural log-transformed and a repeated measures 2-way ANOVA was used to compare geometric means between and within groups at baseline and month 12. The pairwise differences between groups were used to compute the *P*-values. Similar analysis was performed to compare geometric means between and within COMT genotype at baseline and month 12.

Mean change in the obesity-associated hormones, glucose, and HOMA-IR at 12 months was analyzed using linear regression and was calculated by subtracting the baseline value from the value at 12 months from the baseline value. The explanatory variables included treatment, baseline value of the variable, change in BMI, and all 2-way

interactions. Main effects were kept in the model. Model reduction was considered using backward elimination for 2-way interactions having *P*-values > 0.05. All-randomized (intention-to-treat) and per-protocol analyses were performed and data are reported for the all-randomized data set unless otherwise noted. To examine the difference in baseline characteristics and AEs by treatment group and AE status, the Chi-square test or Fisher's exact test was used for categorical variables and Student's *t*-test was used for continuous variables. Data are presented as arithmetic or geometric means and standard errors of the mean (SEM) or 95% confidence intervals for continuous variables or as counts and percentages for categorical variables. Sample size calculations were done using power analysis based on previous obesity study reports. The present study had greater than 80% power to detect a 0.5 kg difference in body weight and 0.5 kg/m^2 difference in BMI between treatment groups, and sufficient power to detect an adiponectin increase of 0.62 μg/mL in participants randomized to GTE as compared to placebo. There was also sufficient power to detect an interaction effect between GTE and COMT genotype with >81 participants per group. All analyses were performed using the Mixed procedure of SAS, version 9.3 (SAS, Inc.).

IV. Results

A. Participant Characteristics

Demographic and baseline characteristics were similar for both groups (

Table 3.2). The mean age of the study sample was 60.8 years and most subjects were of non-Hispanic white race/ethnicity (94.0%). Mean BMI did not differ between groups ($P = 0.13$), though the GTE group had a higher proportion of obese participants at baseline as compared to placebo (32.4% vs. 19.2%, $P = 0.02$). Mean total energy intake and intake of caffeine and macro- and micronutrients were similar at baseline.

B. Change in Dietary Intake and Anthropometric Variables

Comparison of dietary intake and anthropometric changes from baseline to month 12 is shown in **Table 3.3**. Total energy intake non-significantly decreased similarly in both groups from baseline to month 12 (-115 ± 505 vs. -108 ± 416 kcal/day in GTE and placebo, respectively). Caffeine intake did not appreciably change from baseline to month 12 in either group. Weight change from baseline to month 12 was not significantly different between GTE and placebo, though slightly greater weight loss was observed in GTE participants (-0.28 ± 2.2 vs. -0.14 ± 3.2 kg, respectively; $P = 0.13$). Similarly, a non-significant reduction in BMI was seen in GTE participants as compared to placebo (-0.10 ± 0.8 vs. -0.05 ± 1.2 kg/m^2, $P = 0.14$). We did not observe any differences in WC or WHR change between GTE and placebo groups. No differences in within-treatment group comparisons of baseline and month 12 values were observed for any anthropometric variable. Results did not differ when analyzed by COMT genotype. These anthropometric outcomes for this subset of participants are consistent with those seen for the full study population (n=937, data not shown).

C. Comparison of Obesity-associated Hormones and Glucose Homeostasis Markers

No baseline or month 12 differences were observed in measurements of leptin, ghrelin, adiponectin, insulin, glucose, or HOMA-IR between treatment groups (**Table 3.4**). There were also no significant within-group differences from baseline at month 12 for any of the hormones or glucose homeostasis variables. Changes in the obesity-associated hormones, glucose, and HOMA-IR after 12 months were not associated with treatment, change in BMI or baseline values. An interaction was observed between treatment group and insulin concentration at baseline, such that baseline insulin was significantly correlated with change in insulin in GTE participants only (Figure 3.1, $P < 0.01$). When analyzed categorically, participants randomized to GTE with baseline

fasting serum insulin ≥10 μIU/mL (n=23) showed a reduction in fasting serum insulin from baseline to month 12 (-1.43 ± 0.59 μIU/mL), while those randomized to placebo with baseline fasting serum insulin ≥10 μIU/mL (n=19) showed an increase in fasting insulin over the same time period (0.55 ± 0.64 μIU/mL) ($P < 0.01$ for comparison of GTE (high insulin) to all other categories). Similarly, participants with baseline fasting insulin < 10 μIU/mL in either study group (GTE: n = 97; placebo: n=101) increased fasting insulin concentrations from baseline to month 12 (0.53 ± 0.30 and 0.24 ± 0.29 μIU/mL in GTE and placebo, respectively). When compared to participants with insulin < 10 μIU/mL, those with baseline insulin concentrations ≥10 μIU/mL had significantly higher body weight, BMI, fasting glucose concentrations, and HOMA-IR at baseline (data not shown).

No treatment by genotype interactions were observed. To examine the effect of COMT genotype independent of GTE supplementation, an exploratory analysis was conducted in which participants were stratified into three groups based on their COMT genotype (high-, intermediate-, or low-activity). When analyzed by COMT genotype irrespective of treatment group, participants with the homozygous high-activity (G/G) form of the enzyme showed lower adiponectin concentrations at month 12 compared to participants with the low (A/A) genotype (5.97 ± 0.50 vs. 7.58 ± 0.53 μg/mL, $P = 0.03$) (**Table 3.5**). Adiponectin concentrations at month 12 also differed between low and intermediate (G/A) participants (7.58 ± 0.53 vs. 6.34 ± 0.37 μg/mL, $P = 0.05$). Mean insulin concentrations were significantly higher in participants with the high-activity COMT genotype compared to those with the low-activity genotype in the all-randomized model (7.63 ± 0.53 vs. 6.18 ± 0.36 μIU/mL; $P = 0.02$); this comparison was only marginally significant in the per-protocol analysis (n = 107, GTE; n=110, placebo) (7.56 ± 0.53 vs. 6.32 ± 0.38 μIU/mL; $P = 0.06$). HOMA-IR was significantly different at month 12 when comparing G/G vs. G/A (1.82 ± 0.14 vs. 1.52 ± 0.08, $P = 0.05$) and G/G vs. A/A (1.82 ± 0.14 vs. 1.44 ± 0.09, P = 0.02) genotypes. Mean baseline glucose concentrations were significantly different between participants with high- and intermediate-activity COMT genotypes (99.75 ± 1.78 vs. 95.11 ± 1.16 mg/dL; $P = 0.03$) at baseline but not at

month 12. No significant differences were observed at month 12 for fasting leptin, ghrelin, or glucose concentrations among COMT genotypes.

D. Tolerability/AEs

Adverse events (AEs) in MGTT participants have been previously reported (Dostal, et al., under review). In this subset of participants, no difference was seen in numbers of participants experiencing any AE (22 vs. 25 in GTE and placebo, respectively; $P = 0.70$) or in numbers of AEs per participant ($P = 0.74$) (data not shown).

V. Discussion

The aim of this study was to evaluate the effects of decaffeinated GTE containing 1315 mg total catechins (843 mg as EGCG) on anthropometric variables, obesity-associated hormones, and glucose homeostasis markers in overweight and obese, free-living, postmenopausal women. Our results indicate that oral decaffeinated GTE supplementation for one year does not alter total energy intake, anthropometric variables, or obesity-associated hormones, though GTE was shown to reduce fasting insulin concentrations in participants with higher insulin at baseline. Additionally, women with the high-activity form of the COMT enzyme showed reductions in fasting plasma adiponectin and increased insulin over 12 months compared to participants with an intermediate- or low-activity form of the enzyme regardless of treatment group.

Green tea and GTEs have recently gained popularity as dietary aids for weight reduction and weight maintenance. Several randomized trials have examined the association between GTE and body weight and results have been largely inconclusive, likely due to differences in study design, short durations of intervention, variable study populations, and differences in green tea preparations and caffeine content. The GTE used in this study contained a high amount of catechins and less than 16 mg of caffeine per day, with the intention of testing the independent effects of green tea catechins. Results indicated that decaffeinated GTE did not significantly reduce energy intake, body weight, WC, or WHR as compared to placebo, although there was a trend toward decreased body weight and BMI. These results are comparable to those of a recent Cochrane review of effects of green tea on weight loss in overweight and obese adults (164), which concluded that green tea was associated with small, non-significant

decreases in body weight. Since weight loss of 5% to 10% of body weight is considered to be beneficial for reducing several disease risk factors associated with overweight and obesity (243), small losses resulting from green tea preparations are not likely to be clinically meaningful.

A significant association between high baseline insulin concentrations and reduction in insulin in GTE participants only was observed, suggesting that GTE is most effective at lowering insulin in those with baseline fasting insulin ≥10 μIU/L. Given that women with baseline insulin >10 μIU/mL tended to have higher fasting glucose concentrations, increased body weight, and increased BMI at baseline as compared to those with baseline insulin < 10 μIU/mL, it could be hypothesized that they may be at risk for developing metabolic syndrome or may already meet criteria for diagnosis. Further studies are needed on this group of participants to combine the present results with lipid profiles (triglycerides and high-density lipoprotein cholesterol, HDL-C) and blood pressure measurements to gain a full picture of the prevalence of metabolic syndrome in this population.

GTE supplementation has been associated with reductions in fasting insulin in several high-quality randomized trials (215,230,244), though this study is the largest, with the longest duration of intervention that has demonstrated an association specifically in overweight or obese postmenopausal women. Lending strength to the observed results, animal studies have suggested that green tea catechins may prevent hyperglycemia by enhancing insulin activity (245,246), and epidemiologic studies have noted lower incidence of type 2 diabetes in individuals with longer duration of green tea consumption and in women consuming > 3 cups of tea/day (222,247). It should be noted that the proportion of participants in the present study with fasting insulin >10 μIU/mL was small (n = 23 [19.7%] and n = 19 [15.8%] in GTE and placebo, respectively) compared to those with concentrations < 10 μIU/mL. Further research in participants with insulin resistance or those with obesity-associated metabolic abnormalities is needed to confirm this effect.

The effect of COMT genotype on hormone concentrations and glucose homeostasis both independently and in interaction with GTE supplementation was also investigated. No treatment by genotype effects were seen. Although there were no

differences in hormones by COMT genotype at baseline, aside from fasting glucose concentrations at baseline between participants with high- and intermediate-activity COMT genotypes, it was noted that fasting insulin increased and fasting plasma adiponectin decreased in women who were homozygous for the high-activity (G) allele of the COMT enzyme, when compared to women with the intermediate- (G/A) and low-activity (A/A) forms of the enzyme, a direction consistent with development of insulin resistance and increased risk for type II diabetes (248,249). These results are consistent with the findings of Kring, et al. (129), who noted that the frequency of the COMT G/G genotype was 11.6% higher in insulin-resistant and type 2 diabetics compared with that of the COMT A/A and G/A genotypes. To our knowledge, this is the first study that has correlated COMT genotype with change in insulin and adiponectin concentrations over time. Since this adipose-derived hormone has notable insulin sensitizing actions (250,251), it is possible that the observed relationship of COMT genotype with insulin sensitivity could be mediated through the enzyme's interaction with adiponectin. However, since this analysis was done irrespective of the intervention, it represents a cross-sectional reporting of differences between COMT genotypes and it is unclear if the changes we observed from baseline to month 12 were already on this trajectory prior to the study, and if they would continue in the same direction after conclusion of the intervention.

The MGTT is the first long-term randomized trial to evaluate the effects of green tea catechins, independent of caffeine, on obesity-associated hormones, glucose homeostasis markers, and anthropometric variables. In addition to having the longest study duration to date (12 months), this intervention provided the highest catechin dose and randomized the largest number of subjects as compared to previous trials. Since study participants were in free-living conditions and were encouraged to maintain typical dietary and physical activity habits, the results observed likely represent translatable effects for a wider population of postmenopausal, Caucasian women. Given that this group is one of the largest users of dietary supplements (252), this research is of particular importance. This study was also powered to detect an effect of COMT polymorphisms on response to GTE supplementation. Genetic variation in response to

dietary intake is increasingly becoming a topic of interest in nutrition research, and this study is one of the first to suggest a relationship between GTE supplementation and possible insulin-reductive effects for those with higher fasting insulin concentrations and of different COMT genotypes. However, since a specific reference range has not been set for this hormone, additional research is needed to confirm these results and determine which individuals might benefit most from GTE supplementation.

Several limitations of this study should be addressed. Aside from being categorized as overweight or obese, participants were generally healthy, Caucasian women free of diagnosed diabetes or other metabolic diseases and average circulating hormones and glucose concentrations were within normal ranges. Therefore, extrapolation of results to diabetic populations or those with metabolic syndrome cannot be confirmed, though several studies in diabetic populations have noted similar results (217,253). Assessment of the variables of interest at time points in addition to baseline and month 12 would lend strength to these results and allow for the determination of the short-term effects of GTE on anthropometrics or peptide hormones. However, the importance of the long-term impact of dietary interventions, in contrast to those of shorter durations, cannot be underscored.

VI. Conclusion

In conclusion, these results show that decaffeinated GTE is not associated with reductions in WC, or WHR and did not alter energy intake or obesity-associated hormone concentrations. GTE may result in small changes in body weight and BMI that are unlikely to be of clinical significance. However, these data suggest that GTE supplementation may decrease fasting insulin concentrations in those with high circulating concentrations and that possessing a high-activity form of the COMT enzyme may result in decreased adiponectin and increased fasting insulin concentrations over time. Thus, GTE supplementation may be particularly beneficial for individuals with elevated insulin concentrations and those possessing the high-activity COMT genotype. Additional research is needed to confirm these results and to identify the feasibility of incorporating GTE supplementation into treatment and prevention strategies for breast cancer and obesity-related metabolic disorders.

VII. Tables and Figures

Table 3.1. Catechin and caffeine content of GTE[a]

Component	Quantity per Capsule[b] (mg)	Dose per Day (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

[a]Values are presented as means ± SD from eight supplement batches. Catechin composition analyses were conducted by Covance Laboratories (Madison, WI) and results were confirmed by the lab of CS Yang lab (Rutgers University, Piscataway, NJ).

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

Abbreviation: GTE, green tea extract.

Table 3.2. Baseline characteristics of study participants.

Characteristic[a]	GTE (n=117)	Placebo (n=120)	P-value
Age at baseline, y	60.9 (0.45)	60.6 (0.47)	0.64
Race/ethnicity, n (%)			0.52
Non-Hispanic White	111 (46.8)	112 (47.2)	
African American	2 (0.8)	5 (2.1)	
Hispanic	1 (0.4)	0 (0)	
Other	3 (1.3)	3 (1.3)	
COMT Genotype, n (%)			0.65
Low (A/A)	38 (16.0)	37 (15.6)	
Intermediate (A/G)	51 (21.6)	59 (24.9)	
High (G/G)	28 (11.8)	24 (10.1)	
Weight, kg	75.6 (0.87)	74.3 (0.86)	0.27
Height, cm	162.9 (0.59)	163 (0.53)	0.94
BMI, kg/m^2	28.5 (0.28)	27.9 (0.25)	0.13
BMI, kg/m^2, n (%)			0.02
25.0-29.9	79 (67.6)	97 (80.8)	
30.0-40.0	38 (32.4)	23 (19.2)	
Waist circumference, cm	91.9 ± 0.8	90.5 ± 0.8	0.23
Waist-hip ratio	0.86 (0.01)	0.86 (0.01)	0.43
Age at menopause, y	47.2 (46.0, 48.4)	48.3 (47.1, 49.6)	0.20
Physical Activity, MET-hr/week	26.1 (21.6, 31.6)	28.3 (23.5, 34.1)	0.55
Alcohol consumption, n (%)			0.79
Yes	96 (40.5)	100 (42.2)	
No	21 (8.9)	20 (8.4)	
Alcohol, drinks/week (drinkers only)	2.0 (1.6, 2.4)	1.8 (1.5, 2.2)	0.53
Energy intake, kcal/day	1386 (1297, 1480)	1397 (1309, 1491)	0.87
Caffeine, mg/day	152 (112, 207)	202 (149, 273)	0.20

[a]Baseline age, weight, height, BMI, waist circumference, waist-hip ratio are presented as arithmetic mean (SEM); Age at menopause, physical activity, alcohol drinks/wk, energy intake, and caffeine intake are presented as geometric mean (95% confidence interval).
Abbreviations: COMT, catechol-*O*-methyltransferase; GTE, green tea extract; MET-hr/week, metabolic equivalent hours per week.

Table 3.3. **Change in energy intake and anthropometric variables over 12 months, by treatment group**.

Variable	GTE (n=117)	Placebo (n=120)	P-value
Total energy intake, kcal/day			
Baseline	1477 (52.3)	1499 (51.7)	0.77
Month 12	1362 (52.3)	1392 (51.9)	0.69
Change from baseline	-115 (505)	-108 (416)	1.0
Body weight, kg			
Baseline	75.6 (0.9)	74.3 (0.9)	0.27
Month 12	75.4 (0.9)	74.3 (0.9)	0.39
Change from baseline	-0.28 (2.2)	-0.14 (3.2)	0.13
BMI, kg/m^2			
Baseline	28.5 (0.3)	27.9 (0.3)	0.13
Month 12	28.5 (0.3)	27.9 (0.3)	0.14
Change from baseline	-0.10 (0.8)	-0.05 (1.2)	0.14
Waist circumference, cm			
Baseline	91.9 (0.8)	90.5 (0.8)	0.24
Month 12	93.2 (0.8)	91.6 (0.8)	0.17
Change from baseline	1.45 (0.5)	1.29 (0.5)	0.82
Waist-to-Hip Ratio			
Baseline	0.86 (0.01)	0.86 (0.01)	0.41
Month 12	0.86 (0.01)	0.86 (0.01)	0.34
Change from baseline	-0.001 (0.005)	-0.001 (0.005)	0.98

Data presented as arithmetic mean (SEM).
Abbreviation: GTE, green tea extract.

Table 3.4. **Unadjusted mean concentrations of obesity-associated hormones, glucose, and HOMA-IR, by treatment group.**

Variable	GTE (n=117)	Placebo (n=120)	P-value[1]
Insulin, µIU/mL			
Baseline	6.66 (6.05, 7.27)	6.23 (5.66, 6.80)	0.31
Month 12	6.82 (6.19, 7.45)	6.45 (5.86, 7.04)	0.44
Glucose, mg/dL			
Baseline	97.17 (94.94, 99.40)	96.44 (94.23, 98.65)	0.65
Month 12	95.06 (92.83, 97.29)	94.52 (92.34, 96.70)	0.74
HOMA-IR			
Baseline	1.60 (1.44, 1.76)	1.48 (1.34, 1.62)	0.30
Month 12	1.60 (1.44, 1.76)	1.51 (1.35, 1.67)	0.43
Adiponectin, µg/mL			
Baseline	6.29 (5.58, 7.00)	6.72 (5.98, 7.46)	0.41
Month 12	6.60 (5.87, 7.33)	6.66 (5.93, 7.39)	0.91
Ghrelin, pg/mL			
Baseline	1131 (1019.65, 1242.35)	1117 (1007.89, 1226.11)	0.87
Month 12	1126 (1015.08, 1236.92)	1161 (1047.14, 1274.86)	0.67
Leptin, ng/mL			
Baseline	32.14 (28.59, 35.69)	29.87 (26.62, 33.12)	0.36
Month 12	30.86 (27.45, 34.27)	30.86 (27.49, 34.23)	0.57

Data presented as geometric mean (95% confidence interval).
[1]*P*-value for the comparison of the means between GTE and placebo.
Abbreviations: GTE, green tea extract; HOMA-IR, homeostatic model assessment for insulin resistance.

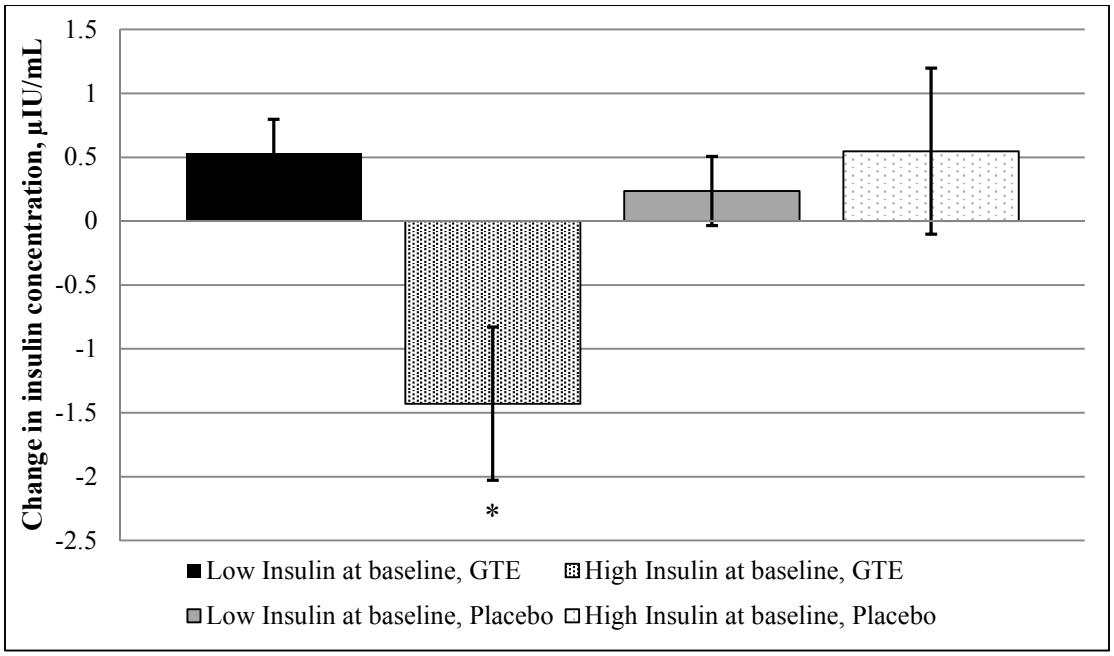
Table 3.5. **Unadjusted geometric means of obesity-associated hormones, insulin, and glucose, by COMT genotype.**

Variable	High (G/G) (n=52)	Intermediate (G/A) (n=110)	Low (A/A) (n=75)
Insulin, µIU/mL			
Baseline	6.65 (5.75, 7.55)	6.36 (5.77, 6.95)	6.41 (5.68, 7.14)
Month 12	7.63[a] (6.59, 8.67)	6.53[ab] (5.92, 7.14)	6.18[b] (5.47, 6.89)
Glucose, mg/dL			
Baseline	99.75[a] (96.26, 103.24)	95.11[b] (92.84, 97.38)	97.32[ab] (94.52, 100.12)
Month 12	96.37 (93.0, 99.74)	94.17 (91.94, 96.40)	94.61 (91.87, 97.35)
HOMA-IR			
Baseline	1.64 (1.40, 1.88)	1.49 (1.33, 1.65)	1.54 (1.34, 1.74)
Month 12	1.82[a] (1.55, 2.09)	1.52[b] (1.36, 1.68)	1.44[b] (1.26, 1.62)
Adiponectin, µg/mL			
Baseline	6.64 (5.54, 7.74)	6.19 (5.48, 6.90)	6.89 (5.95, 7.83)
Month 12	5.97[a] (4.99, 6.95)	6.34[a] (5.61, 7.07)	7.58[b] (6.54, 8.62)
Ghrelin, pg/mL			
Baseline	1062 (906.63, 1217.37)	1101 (989.79, 1212.21)	1205 (1058.31, 1351.69)
Month 12	1092 (932.30, 1251.70)	1093 (982.12, 1203.88)	1261 (1107.41, 1414.59)
Leptin, ng/mL			
Baseline	30.36 (25.36, 35.36)	31.16(27.63, 34.69)	31.12 (26.85, 35.39)
Month 12	30.03 (25.07, 34.99)	30.55 (27.08, 34.02)	29.71 (25.63, 33.79)

Data presented as geometric mean (95% confidence interval). Within a row, values with different superscripts are significantly different ($P < 0.05$).

Abbreviations: COMT, catechol-*O*-methyltransferase; GTE, green tea extract; HOMA-IR, homeostatic model assessment for insulin resistance.

Figure 3.1. Effect of GTE on mean change in fasting serum insulin, by baseline insulin concentration.



High insulin at baseline defined as ≥10.0 µIU/mL; low insulin < 10.0 µIU/mL. Low insulin, GTE: n = 97; low insulin, placebo: n = 101. High insulin, GTE: n = 23; high insulin, placebo: n = 19.

*$P < 0.01$ for comparison between high insulin-GTE group and all other groups. P-value > 0.2 for comparison between all other groups.

Abbreviations: GTE, green tea extract.

Chapter 4 - Effect of Green Tea Extract on Adiposity, Obesity-Associated Hormones, and Bone Mineral Density in Overweight and Obese Postmenopausal Women

I. Overview

Background: Green tea extract (GTE) consumption has been linked to favorable changes in adiposity and bone mineral density (BMD), though it is unknown if these effects are due to green tea catechins or caffeine. Catechol-*O*-methyltransferase (COMT) genotype may also be a modifier of these associations. **Objective:** We examined the impact of decaffeinated GTE containing 843 mg (-)-epigallocatechin-3-gallate (EGCG) on body composition (using dual-energy x-ray absorptiometry [DXA]) and obesity-associated hormones. **Design:** The Minnesota Green Tea Trial was a 12-month randomized, double-blind, placebo-controlled clinical trial of 937 healthy postmenopausal women at elevated risk for breast cancer assigned to either GTE or placebo. This sub-study was conducted in 121 overweight and obese participants. **Results:** No changes in BMI, total fat mass, % body fat, or BMD were observed in women taking GTE (n=61) compared to placebo (n=60). A non-significant reduction in visceral adipose tissue mass was observed in GTE participants as compared to the placebo group (-0.02 ± 0.02 vs. 0.03 ± 0.02 kg; $P = 0.13$). Interactions were observed between treatment and time for gynoid % fat and tissue % fat. Tissue % fat and gynoid % fat increased from baseline to month 12 in the placebo group as baseline BMI increased. Conversely, in the GTE group, tissue % fat and gynoid % fat decreased during the intervention as baseline BMI increased ($P_{interaction}=0.04$ and $P_{interaction}=0.02$, respectively). No changes were seen in circulating leptin, ghrelin, adiponectin, or insulin concentrations. COMT genotype did not modify the effect of GTE on any variable. **Conclusions:** Decaffeinated GTE was not associated with overall reductions in adiposity or improvements in BMD. However, GTE may be more beneficial for body fat reduction in individuals with higher BMI.

II. Introduction

Despite significant research efforts and public health campaigns, overweight and obesity remain prevalent worldwide (254). Excess adiposity is associated with increased risk for serious health conditions such as cardiovascular disease, type 2 diabetes, and cancer. Conversely, loss of excess fat mass and/or maintenance of a healthy body weight are known to reduce risk for these diseases (255,256).

Research on the associations between obesity and metabolic bone diseases such as osteoporosis, a major cause of morbidity and mortality in postmenopausal women, remains inconclusive. Several epidemiologic studies have highlighted the correlation between these conditions (87-91), with many indicating a significant positive relationship between body weight and bone mineral density (BMD). This correlation is largely attributed to both the increased mechanical load of excess weight and hormonal changes associated with obesity, including increased estrogen and leptin production by adipose tissue, which have been shown to suppress bone resorption and stimulate osteoblastogenesis (92-94). However, recent evidence has emerged demonstrating that increased body weight may actually be negatively correlated with BMD after correction for the mechanical loading effect of excess body weight (95), and that obese individuals with high BMD are still at increased risk for fractures (88).

The etiologies of obesity and metabolic bone disorders involve factors that are both modifiable and non-modifiable. Dietary intake of natural bioactive foods and beverages, such as green tea, is an adaptable habit that may play a role in reducing risk of these chronic diseases. Epidemiologic research has suggested that tea intake is associated with both reductions in adiposity and increased BMD (195,238). Green tea's anti-obesity effects are thought to be due to increases in thermogenesis and fat oxidation through inhibition of the catechol-*O*-methyltransferase (COMT) enzyme by GTC, resulting in prolonged sympathetic nervous system (SNS) stimulation (132,257). Proposed mechanisms for improving and/or maintaining bone health include reducing chronic inflammation and oxidative stress, conditions that are also tightly linked with obesity and excess adipose tissue (258).

Genetic variations in COMT enzyme activity have been widely noted. One common polymorphism, a guanine (G) to adenine (A) substitution at position rs4680 of the enzyme has been identified that produces an amino acid change from valine to methionine. The homozygous low-activity (A/A) genotype is associated with a 3- to 4-fold decrease in enzymatic activity when compared to the high-activity (G/G) genotype (117,118). Given these effects, COMT genotype may influence the rate of catechin metabolism and the functional response to dietary catechins, wherein individuals with the low-activity form of the enzyme may have increased thermogenesis, reduced adiposity, and lower body weight due to prolonged SNS effects as compared to individuals with the high-activity genotype. Yet, those with the high-activity form of COMT may benefit more from interventions that inhibit the enzyme, since the higher rate of substrate metabolism would be reduced to a greater degree.

Most of green tea's benefits are attributed to its high concentration of polyphenolic catechins, of which (-)-epigallocatechin-3-gallate (EGCG) is the most abundant and also believed to be the most bioactive (154). On the basis of its potential anti-obesity effects, extracts of green tea catechins (GTC) have been marketed as herbal supplements for the control of body weight. However, its efficacy has not been consistently proven, and the potential beneficial effects of EGCG on obesity, adipose-related hormone concentrations, and skeletal health remain controversial. Additionally, most randomized trials using green tea have included caffeine in the intervention, which is independently known to increase energy expenditure and fat oxidation (259). Therefore, the independent effects of green tea catechins on these end points remain unclear.

The aim of the present study was to determine the effect of caffeine-free green tea extract (GTE) consumption on body composition and BMD in free-living, overweight and obese postmenopausal women, a population at risk for both excess weight gain and rapid bone loss. Secondary aims included measurement of the effects of GTE on obesity-related hormones (insulin, leptin, and adiponectin), correlation of these hormones with body composition parameters, and testing for effect modification by COMT genotype. We hypothesized that GTE supplementation would (1) cause favorable changes in body

composition, (2) enhance or maintain measures of BMD, (3) suppress insulin and leptin concentrations and (4) increase adiponectin concentrations as compared to placebo. We also proposed that GTE supplementation would be particularly beneficial in those with the high-activity form of COMT, due to the inhibitory effects of GTC on this enzyme. Determining the impact of GTE on body composition, BMD, and obesity-related hormones will advance the understanding of the potential health benefits of green tea catechins as well as the complex relationship between obesity and skeletal health.

III. Methods

A. Study Design

The Minnesota Green Tea Trial (MGTT) was a randomized, double-blinded, placebo-controlled study designed to examine the effect of GTE on breast cancer risk factors, including obesity. Details of the study design, eligibility criteria, randomization, blinding, study conduct, and patient flow through the trial have been previously published (Samavat, et al., under review). Briefly, postmenopausal women aged 50 to 70 years and classified as having high breast density (a breast cancer risk factor) were recruited from 2009 to 2013 at clinical centers in the Minneapolis-St. Paul metropolitan area. Baseline health status was assessed by standardized questionnaire (Appendix 1) and a blood sample taken at the initial screening clinic visit. Exclusionary criteria included: any history of breast cancer, proliferative breast disease, or ovarian cancer or any cancer diagnosis within 5 years; consumption of >7 alcoholic drinks per week; consumption of >1 cup per week of green tea; BMI < 25.0 or >40 kg/m^2; >4.6 kg weight change during the previous year; current use of menopausal hormone therapy (MHT) or use within past 6 months; use of methotrexate or Enbrel (etanercept); current smoking; history of breast augmentation; positive serology for Hepatitis B or C antibodies; or alanine aminotransferase higher than 1.5 times the upper limit of normal (defined as 60 U/L).

Of 937 women who completed the full study duration, 214 women classified as overweight (BMI = 25.0-29.9 kg/m^2) or obese (BMI = 30.0-40.0 kg/m^2) at the screening clinic visit were additionally consented for body composition analysis beginning in May 2013. Of these, 146 were randomized into the study (GTE: n=76; placebo: n = 70).

Fifteen women allocated to GTE and 10 allocated to placebo withdrew from participation; 121 completed the duration of the study (GTE: n = 61; placebo: n = 60).

B. Randomization and Blinding

Participants were randomly assigned to receive oral GTE or placebo in 4 capsules daily for 12 months. Randomization was performed by the Investigational Drug Services (IDS) pharmacy at University of Minnesota Medical Center-Fairview, who used a computer-generated permuted block randomization scheme with blocks of 8 stratified by COMT genotype activity: low (A/A), intermediate (A/G) or high (G/G). Participants and study staff were blind to treatment allocation throughout the trial. Sample size calculations were done using power analysis based on previous reports of GTE's effect on body fat parameters. The present study has greater than 80% statistical power to detect a 1.5% difference in body fat percentage between GTE and placebo groups. Institutional Review Board (IRB) approval was obtained at each clinical center. All participants provided written informed consent. This trial was registered at clinicaltrials.gov as NCT00917735.

C. COMT Genotyping

COMT genotype analysis was completed at the University of Minnesota Genomics Center. DNA was extracted from buffy coat samples by the Qiagen DNAeasy Blood and Tissue Kit method (Qiagen Inc., Gaithersburg, MD, USA). A TaqMan assay was developed for defining the COMT rs4680 polymorphism using a TaqMan PCR Core Reagent kit (Applied Biosystems, Foster City, CA). Cell lines with known COMT genotype were used as quality controls with each PCR run.

D. Study Supplement

Decaffeinated Green Tea Extract Catechin Complex and placebo capsules were supplied by Corban Laboratories (Eniva Nutraceutics, Plymouth, MN) in 8 batches. Mean daily catechin content was 1315 ± 116 mg/day (843 ± 44 mg as EGCG). Placebo capsules were identical in appearance to GTE and contained 816 mg maltodextrin, 808 mg cellulose, and 8 mg magnesium stearate (**Table 3.1**). GTE ingredients were analyzed by high-performance liquid chromatography (HPLC) (Rutgers University, Piscataway, NJ)

to demonstrate comparability with the stated catechin contents of the manufacturer. Participants consumed 4 capsules of GTE or placebo daily, and were advised to ingest 2 capsules in the morning hours and 2 in the evening to maintain circulating catechin concentrations throughout each day.

E. Anthropometry and Body Composition

Body weight was measured to the nearest 0.1 kg at baseline and months 3, 6, 9, and 12 using a stand-on digital scale (Scale-Tronix Inc., White Plains, NY). Standing height was assessed by wall-mounted stadiometer (Seca, Hanover, MD) to the nearest 0.1 cm at the screening clinic visit and month 12. BMI was calculated by dividing weight in kilograms by height in meters squared (kg/m^2).

DXA scans were completed using a GE Healthcare Lunar iDXA (GE Healthcare Lunar, Madison, WI, USA) and analyzed using Encore software version 13.6, revision 2. Total body fat was expressed as percent of total body mass and android and gynoid were expressed as percent of total body fat. Subcutaneous fat was determined using an algorithm and measurements of total abdominal thickness and the width of the subcutaneous fat layer along the lateral extent of the abdomen along with empirically derived geometric constants to estimate the subcutaneous fat in the android region. Visceral adipose tissue (VAT) was determined in the android region by subtracting subcutaneous fat from total fat. The android region was defined as the caudal limit placed at the top of the iliac crest and its height set to 20% of the distance from the top of the iliac crest to the base of the skull. The upper limit of the gynoid region was set below the iliac crest a distance 1.5 times the height of the android region. The lower limit was set a distance of 2 times the height of the android region. Central fat distribution was assessed by the android:gynoid fat ratio, calculated as android fat divided by gynoid fat.

Areal BMD was expressed as g/cm^2. T-scores were expressed in standard deviations using the peak bone mass from the manufacturer's reference population. Z-scores were measured as the deviation from the normal age- and sex-matched mean and SD. Osteoporosis was defined in accordance with the World Health Organization as BMD at any site greater than 2.5 standard deviations below the young adult mean and osteopenia as BMD 1.0-2.5 standard deviations below the young adult mean (260).

F. Obesity-associated Hormone and Glucose Homeostasis Analysis

Blood samples for obesity-associated hormone and glucose assessment were collected at baseline and month 12 after an overnight fast of >10 hours. Whole blood samples were separated into plasma and serum. Plasma leptin, adiponectin, and ghrelin were measured using radioimmunoassay kits manufactured by EMD Millipore (Billerica, MA) [inter-assay % coefficient of variation (% CV): leptin = 7.1%, adiponectin = 8.9%, ghrelin = 7.8%; intra-assay % CV: leptin = 6.6%; adiponectin = 7.4%, ghrelin = 5.5%]. Serum insulin was measured using a simultaneous one-step immunoenzymatic, chemiluminescent assay (Access Ultrasensitive Insulin assay, Quest Diagnostics, Wood Dale, IL, intra-assay % CV: 3-5.0%; inter-assay % CV = 3.9%). Serum glucose concentrations were measured using a hexokinase enzymatic reference method (Quest Diagnostics, Wood Dale, IL, monthly % CV = 1.4%). Homeostasis model assessment for insulin resistance (HOMA-IR) was calculated as fasting insulin (μIU/mL) x fasting glucose (mg/dL) / 405.

G. Dietary Assessment

Participants completed a food frequency questionnaire (FFQ) at baseline and month 12 for dietary intake assessment over the previous 12 months. The Diet History Questionnaire (DHQ) is a FFQ consisting of 124 food items and includes portion size and dietary supplement questions. The food list and nutrient database used with the DHQ are based on national dietary data (USDA's 1994-96 Continuing Survey of Food Intakes by Individuals, available from the USDA Food Surveys Research Group). FFQ data was analyzed using Diet*Calc software developed at the National Cancer Institute (NCI).

H. Physical Activity

Recreational physical activity was assessed at baseline and month 12 by a validated physical activity questionnaire (261), in which participants were asked about frequency and duration of several types of physical activity. Metabolic equivalent hours (MET-hours), defined as the ratio of work metabolic rate to a standard resting metabolic rate, were computed as the product of average hours per week of each activity multiplied by its MET-hour equivalent. All recorded activities were summed to obtain the total MET-hours of activity performed per week for a given participant. This sub-study used a

separate physical activity questionnaire from the parent study in order to gather more specific data than the original questionnaire, since changes in activity habits from baseline to the end of the intervention could have an impact on body composition. See **Appendix 2** for a sample questionnaire.

I. Statistical Analyses

Differences in baseline demographic and anthropometric characteristics between treatment groups at baseline were assessed by non-paired Student's *t*-test for continuous variables and Chi-square test for categorical variables. Two-way repeated measures ANOVA was used to compare BMI, adiposity measures and bone density variables between GTE and placebo groups and within group at baseline and at month 12. Change from baseline was calculated by subtracting the baseline from the month 12 values for each participant. Linear regression was used to compare the mean change from baseline in GTE and placebo groups after adjusting by baseline value, change in BMI, years since menopause and MHT use. The same analyses were used to compare adiposity measures and bone density variables between COMT genotype groups.

Obesity-associated hormones, glucose, and HOMA-IR were natural log-transformed and a repeated measures 2-way ANOVA was used to compare geometric means at baseline and month 12. The pairwise differences between groups were used to compute the *P*-values between and within treatment groups. Similar analyses were performed to compare geometric means between and within COMT genotype at baseline and month 12. Mean changes in obesity-associated hormones, glucose, and HOMA-IR at 12 months were calculated by subtracting the baseline value from the value at month 12 and analyzed using linear regression. The explanatory variables included treatment group, baseline value of the variable, change in BMI, COMT genotype, and all possible 2-way interactions. Main effects were kept in the model. Model reduction was considered using backward elimination for 2-way interactions having *P*-values > 0.05. Pearson's correlation was used to evaluate the association between leptin, adiponectin and insulin, and BMI, adiposity measures and bone density variables at baseline.

Data are presented as arithmetic or geometric means and standard errors of the mean (SEM) or 95% confidence intervals for continuous variables or as counts and

percentages for categorical variables. All analyses were performed using the Mixed, GLM and Freq procedures of SAS, version 9.3 (SAS, Inc.). Cook's distance was used to evaluate influential outliers and residual plots were used to evaluate model assumptions.

IV. Results

A. Participant Characteristics

Demographic and baseline characteristics were similar for both groups (**Tables**

Table 4.1). The mean age of the study sample was 60.4 years and most subjects were of non-Hispanic white race/ethnicity (94.2%). Mean BMI was 27.8 kg/m^2 and waist circumference was 91.5 cm. Approximately 50% of participants regularly consumed calcium supplements in both groups and only a small number of women were taking medication to inhibit bone resorption. Physical activity, total energy intake and intake of caffeine and macro- and micronutrients did not differ at baseline or at month 12. Prevalence for osteopenia (n = 3) was 2.5%. No participants met criteria for osteoporosis. Baseline characteristics did not differ between participants who completed the study and those who withdrew (data not shown).

B. Comparison between Groups for Body Composition and Adiposity Variables

Body composition and adiposity variables are presented in **Table 4.2**. Groups were similar at baseline for most body composition and adiposity variables, though participants in the GTE group had significantly higher android % fat (48.6 ± 0.8% vs. 45.5 ± 0.8%, $P = 0.01$) and android/gynoid ratio (1.06 ± 0.02 vs. 0.99 ± 0.02, $P = 0.01$). No significant differences in change from baseline to month 12 were seen in any parameter of body composition. Non-significant reductions from baseline in VAT mass (-0.01 ± 0.02 vs. 0.03 ± 0.02 kg, respectively; $P = 0.11$) and estimated VAT volume (-0.02 ± 0.02 vs. 0.02 ± 0.02 cm^3; $P = 0.18$) were observed in the GTE group as compared to placebo. Interactions were observed between treatment, time, and baseline BMI for gynoid % fat and tissue % fat. While the gynoid % fat increased from baseline to month 12 in the placebo group as baseline BMI increased, gynoid % fat decreased over time as baseline BMI increased in the GTE group (P for interaction = 0.02). Similarly, while

tissue % fat increased from baseline to month 12 in the placebo group as baseline BMI increased, the opposite occurred in the GTE group - as baseline BMI increased, tissue % fat decreased during the intervention (*P* for interaction = 0.04). Android:gynoid ratio did not change from baseline in either study group.

No treatment by genotype interactions were observed, so we conducted an exploratory analysis of baseline body composition variables by COMT genotype, independent of treatment group. A statistically significant difference in lean weight at baseline was observed between participants with the high-activity COMT genotype (n=34) as compared to the intermediate-activity group (n=46) (41.9 ± 0.82 kg vs. 39.1 ± 0.77 kg, respectively; *P* = 0.02). No differences were seen between genotype groups for any other body composition variable.

C. Comparison between Groups for Bone Mineral Density Variables

No differences were observed between or within groups when comparing change from baseline to month 12 in BMD, T-score, or Z-score (
Table 4.3). Mean T-score and Z-scores were positive in both groups at both time points. Results did not differ by COMT genotype.

D. Comparison of Obesity-associated Hormone Concentrations

Mean fasting concentrations for obesity-associated hormones and glucose homeostasis markers at baseline and month 12 are presented in **Table 4.4**. Concentrations did not differ between treatment group at either time point for any variable, aside from differences in baseline adiponectin (*P* = 0.05), and there were no significant differences in change from baseline for any variable. No interaction was observed between GTE and COMT genotype for change from baseline in these variables.

E. Associations between obesity-associated hormones and body composition variables

Table 4.5 reports Pearson correlations between baseline concentrations of leptin, adiponectin, and insulin and body composition and BMD measurements from the pooled group of all participants (n = 121). Fasting plasma leptin concentrations were positively correlated with BMI and body fat measurements and were negatively correlated with

tissue % lean (r = -0.270; $P < 0.001$) and Z-score (r = -0.314; $P < 0.001$). Concentration of adiponectin was positively correlated with gynoid % fat (r = 0.264; P = 0.004) and negatively correlated with VAT mass (r = -0.342; $P < 0.001$). Insulin concentration was positively correlated with BMI and body fat measurements and was negatively correlated with tissue % lean (r = -0.188; P = 0.04). Adiponectin and insulin concentrations were not correlated with BMD. These relationships remained similar at month 12.

V. Discussion

The results of the present study indicate that intake of 1315 mg green tea catechins daily (843 mg as EGCG) for one year did not affect overall adiposity, fat-free mass, or BMD in postmenopausal overweight or obese women. Similarly, GTE did not alter concentrations of obesity-associated hormones or glucose homeostasis markers as compared to placebo. However, reductions in visceral adiposity in GTE participants nearly reached significance and participants with higher baseline BMI randomized to GTE reduced tissue % fat to a greater degree than those randomized to placebo.

Epidemiologic evidence has demonstrated that individuals with the highest degree of tea consumption may have reduced risk for obesity-related diseases (262,263), and several randomized trials (typically ≤ 12 weeks in duration) have demonstrated that GTC supplementation may lead to modest decreases in body weight and adiposity. However, results are inconsistent and the clinical relevance of these reductions is a topic of debate (163,164). Further, most studies examining this relationship have included caffeine as part of the intervention, making it impossible to measure the independent effects of green tea catechins. While we did not see a change in BMI, total fat mass, or % body fat between treatment groups, trends toward reductions in total VAT mass and volume in the GTE group suggest that green tea catechins may inhibit accumulation of abdominal fat when consumed over the life span. These effects are notable, given that central adiposity is associated with higher risk of metabolic disorders, cardiovascular disease, and some forms of cancer (264). Though we are not able to extrapolate our results beyond the 12-month intervention period, our results are consistent with evidence from other randomized trials that have found an influential effect of green tea catechins on abdominal and visceral fat (158,177). In addition, our results show that participants with

higher baseline BMI randomized to GTE reduced tissue % fat to a greater extent over time as compared to those randomized to placebo, indicating that GTE supplementation may be especially beneficial for individuals with a higher degree of adiposity.

As previously stated, the effect of green tea catechins on body composition has not been consistent across all study populations and trial designs. Genetic variations may at least partially explain these differences in outcomes. The G to A polymorphism at position 4680 of the COMT gene is often cited as playing a potential role in sensitivity to GTC with respect to energy expenditure and fat oxidation. EGCG has been suggested to inhibit COMT, an enzyme responsible for metabolizing both green tea catechins and catecholamines such as norepinephrine (265). Reduced activity of COMT may lead to extended effects of these compounds, with resulting increases in SNS activity, fat oxidation, and energy expenditure. Additionally, the A/A form of the COMT enzyme is associated with a 3- to 4-fold reduction in enzymatic activity as compared to the G/G genotype (117), suggesting that those with the A/A form of COMT may have prolonged circulating concentrations of green tea catechins which would translate into prolonged physiologic effects. Since people with the high-activity form of COMT metabolize GTC and norepinephrine at a faster rate, it was hypothesized that GTE supplementation would be most beneficial in these individuals due to the inhibitory effects of GTC on the enzyme. However, the results of the present study do not indicate a modifying effect of COMT genotype on body composition variables. Indeed, there is little *in vivo* evidence to support a role of COMT in relation to clinically meaningful reductions in body weight and adiposity. Hodgson et al. (266), using targeted catecholamine profiling techniques, determined that GTE did not increase concentrations of norepinephrine, suggesting that GTE supplementation may not alter COMT activity. Further, Lorenz et al. (267). determined that administration of 750 mg EGCG did not impair the *in vivo* activity of COMT.

Epidemiologic studies have linked green tea consumption to increased bone density (195) and reduced risk for hip fractures (268). Biologic mechanisms are thought to involve the weak estrogenic effect of green tea catechins (269) and induction of osteoclast apoptosis by EGCG (189), therefore inhibiting bone resorption and leading to

increased BMD. We did not observe an effect of GTE on measures of BMD in this study population. This is in contrast to Shen et al. (198), who found that supplementation of 500 mg/d of green tea polyphenols (233 mg as EGCG) for 6 months increased bone-specific alkaline phosphatase (BAP), a glycoprotein found on the surface of osteoblasts that is indicative of bone biosynthesis, in postmenopausal women with osteopenia. However, since their trial design did not include diagnostic markers of bone health (i.e., T-score), it is difficult to determine the clinical relevance of these physiologic changes. The women in the present study had high T-scores and Z-scores in comparison to the respective reference populations, so it is possible that any favorable effect of GTE on bone density may be best observed in individuals with low bone density.

In addition to being a fat storage depot, adipose tissue secretes inflammatory markers and adipokines, such as leptin and adiponectin. Leptin concentrations are proportional to fat mass, while adiponectin concentrations are negatively correlated with degree of adiposity. Adiponectin is known to play important roles in energy homeostasis and insulin sensitivity. Recent research has shown that receptors for adiponectin are present on osteoblasts and may influence proliferation, differentiation, and the mineralization effects of these bone remodeling cells (270,271). However, correlational analysis has not proven a consistent positive association between adiponectin and BMD (272). The impact of leptin on bone metabolism has also been studied, but results remain controversial. However, we did not observe significant changes in obesity-associated hormone concentrations between treatment groups and these hormones did not correlate with bone-related end points. These results are in contrast to other studies that have shown increases in adiponectin concentrations and improvements in insulin sensitivity with GTE supplementation (215,253). This could be largely attributed to the lack of change in BMI and body fat mass in our study participants, as well as their general good health status – no participant had been previously diagnosed with diabetes or any other metabolic disorder. Hormone analysis and correlations to other end points were largely exploratory in this data set and we may not have had sufficient power to detect an effect of GTE on these variables.

Pearson correlations confirmed established positive associations between leptin and adiponectin with BMI and measurements of adiposity. No relationship was seen between adiponectin or insulin concentrations and BMD. Interestingly, we observed a negative correlation between leptin concentration and Z-score ($P < 0.0001$), though not with BMD or T-score. As Z-scores compare BMD measurements to a reference population of the same age, it is possible that age may have been a confounder in this association. However, this correlation is in agreement with Morcov et al., who observed that leptin was an independent predictor of bone mass in postmenopausal but not premenopausal women (273).

It is important to note that the GTE used in this study was decaffeinated (< 16 mg caffeine/day), therefore demonstrating the effect of green tea catechins independent of the well-understood effects of caffeine on fat oxidation and energy expenditure (274). While modest, non-significant reductions in VAT were observed, the overall lack of effect of decaffeinated GTE on body composition and adiposity aligns with the results of a previous randomized trial examining the effect of a 12-week caffeine-free green tea intervention on body weight in 38 obese postmenopausal subjects (141). This suggests that any beneficial effect of green tea on improvements in body weight and adiposity is largely due to its caffeine content or synergism between catechins and caffeine. The latter combination may have the greatest effect: a meta-analysis by Phung et al. (163) determined that in studies using interventions incorporating both green tea catechins and caffeine, a significant decrease in body weight, BMI, and waist circumference was observed as compared to individuals randomized to a caffeine-only placebo.

The MGTT is the largest and longest clinical trial investigating the impact of GTE on health outcomes in postmenopausal women, including body composition and bone health. We used the highest daily dosage of green tea catechins in comparison to other published articles of GTE interventions (1315 mg/d, 843 mg as EGCG) and have previously proven the overall safety of our intervention supplement (Dostal et al., in press, *Food and Chemical Toxicology*). An additional strength of the current study is our use of accurate methods of assessing regional adiposity, which eliminates intra- and inter-examiner variation in measurements as compared to manual measurements such as waist and hip

circumference. Limitations of the current study include the lack of measurements of resting energy expenditure and respiratory quotient, which would have allowed us to form relationships between the observed changes in central adiposity, energy expenditure, and substrate utilization. Lastly, our genotypic analysis was limited to just one polymorphism of one enzyme involved in green tea catechin metabolism. It is possible that other enzymes and physiologic pathways may play a role in green tea's effect on body composition.

VI. Conclusion

Daily consumption of decaffeinated GTE containing 843 mg EGCG for 12 months was not associated with changes in adiposity, BMD, or obesity-associated hormones. COMT genotype did not modify these results. However, GTE may be beneficial for reduction of visceral fat, as well as total body fat in individuals with higher BMI.

VII. Tables

Table 4.1. Baseline characteristics of study participants.

Characteristic	GTE (n = 61)	Placebo (n = 60)	P-value
Age at baseline, y	60.7 (0.60)	60.0 (0.65)	0.45
Race/ethnicity			0.72
Non-Hispanic white, n (%)	57 (93.4)	57 (95.0)	
African American, n (%)	2 (3.3)	2 (3.3)	
Other, n (%)	2 (3.3)	1 (1.7)	
Weight, kg	74.9 (1.12)	74.1 (1.25)	0.63
Height, cm	163.7 (0.01)	163.5 (0.01)	0.89
BMI, kg/m^2	27.9 (0.34)	27.6 (0.35)	0.53
COMT genotype, n (%)			0.49
Low (A/A)	20 (32.8)	21 (35.0)	
Intermediate (A/G)	21 (34.4)	25 (41.7)	
High (G/G)	20 (32.8)	14 (23.3)	
Years since menopause, y	9.8 (8.1, 11.8)	8.5 (7.0, 10.3)	0.31
MHT use (ever), n (%)			0.80
Yes	22 (36.1)	23 (38.3)	
No	39 (63.9)	37 (61.7)	
Length of MHT use, y	3.5 (1.9, 6.5)	2.9 (1.6, 5.4)	0.66
n	21	21	
Use of bone resorption inhibitors, n (%)			0.66
Yes	3 (4.9)	2 (3.3)	
No	57 (93.4)	57 (95.0)	
Use of calcium supplements, n (%)			0.65
Yes	30 (49.2)	27 (45.0)	
No	31 (50.8)	33 (55.0)	
Physical activity, MET-hr/week	13.8 (10.7, 17.7)	16.8 (13.1, 21.7)	0.27
Alcohol consumption, n (%)			0.78
Yes	51 (83.6)	49 (81.7)	
No	10 (16.4)	11 (18.3)	
Alcohol, drinks/week (drinkers only)	2.08 (1.54, 2.82)	2.07 (1.52, 2.82)	0.98
Energy intake, kcal/day	1384 (1262, 1517)	1426 (1300, 1564)	0.65
Caffeine, mg/day	141 (89, 222)	161 (102, 254)	0.68

Data reported as arithmetic mean (SEM) or geometric mean (95% confidence interval) for continuous variables or n (%) for categorical variables.

Abbreviations: BMI, body mass index; COMT, catechol-*O*-methyltransferase; GTE, green tea extract; MET-hr. metabolic equivalent hours; MHT, menopausal hormone therapy.

Table 4.2. Comparison of BMI and adiposity measures between GTE and placebo groups.

	GTE (n = 61)	Placebo (n = 60)	*P*-value
BMI, kg/m^2			
Baseline	28.0 ± 0.34	27.**6** ± 0.35	0.30
Month 12	27.9 ± 0.34	27.5 ± 0.35	0.37
Change from baseline	-0.13 ± 0.11	-0.05 ± 0.11	0.61
Total fat mass, kg			
Baseline	32.2 ± 0.8	30.9 ± 0.8	0.25
Month 12	31.7 ± 0.8	30.9 ± 0.8	0.45
Change from baseline	-0.30 ± 0.16	-0.12 ± 0.15	0.40
% Total body fat			
Baseline	42.5 ± 0.5	41.5 ± 0.5	0.21
Month 12	42.3 ± 0.5	41.4 ± 0.6	0.29
Change from baseline	-0.15 ± 0.17	-0.15 ± 0.16	0.99
VAT mass, kg			
Baseline	1.06 ± 0.07	0.88 ± 0.07	0.06
Month 12	1.04 ± 0.07	0.91 ± 0.07	0.17
Change from baseline	-0.01 ± 0.02	0.03 ± 0.02	0.11
Estimated VAT volume, cm^3			
Baseline	1.12 ± 0.07	0.93 ± 0.07	0.06
Month 12	1.10 ± 0.07	0.96 ± 0.07	0.17
Change from baseline	-0.01 ± 0.02	0.02 ± 0.02	0.18
Android % fat			
Baseline	48.6 ± 0.8	45.5 ± 0.8	0.01
Month 12	47.4 ± 0.9	45.4 ± 0.9	0.09
Change from baseline	-0.92 ± 0.43	-0.27 ± 0.43	0.29
Gynoid % fat[a]			
Baseline	45.6 ± 0.6	45.8 ± 0.6	0.80
Month 12	45.5 ± 0.6	45.7 ± 0.6	0.81
Change from baseline	-0.06 ± 0.18	-0.15 ± 0.17	0.71
Android/gynoid ratio			
Baseline	1.06 ± 0.02	0.99 ± 0.02	0.01
Month 12	1.05 ± 0.02	0.99 ± 0.02	0.03
Change from baseline	-0.009 ± 0.007	-0.003 ± 0.006	0.52
Lean weight, kg			
Baseline	40.6 ± 0.6	40.7 ± 0.6	0.99
Month 12	40.7 ± 0.5	40.7 ± 0.5	0.97
Change from baseline	0.10 ± 0.11	0.09 ± 0.11	0.91
Tissue % lean			
Baseline	54.4 ± 0.5	55.3 ± 0.5	0.18
Month 12	54.6 ± 0.5	55.5 ± 0.5	0.26
Change from baseline	0.19 ± 0.17	0.18 ± 0.16	0.95
Tissue % fat[a]			
Baseline	32.2 ± 0.8	30.9 ± 0.8	0.25
Month 12	31.7 ± 0.8	30.9 ± 0.8	0.45
Change from baseline	-0.30 ± 0.16	-0.12 ± 0.15	0.40

Data presented as arithmetic mean ± SEM. BMI change from baseline adjusted for baseline value, years since menopause, and MHT use. All subsequent change from baseline data adjusted for baseline value, change of BMI from baseline, years since menopause and MHT use.

[a]Statistically significant time x treatment interaction

Abbreviations: BMI, body mass index; GTE, green tea extract; MHT, menopausal hormone therapy; VAT, visceral adipose tissue.

Table 4.3. Comparison of bone density measurements between GTE and placebo.

	GTE (n = 61)	Placebo (n = 60)	*P*-value
BMD, g/cm^2			
Baseline	1.17 ± 0.01	1.14 ± 0.01	0.07
Month 12	1.17 ± 0.01	1.14 ± 0.01	0.09
Change from baseline	-0.006 ± 0.002	-0.003 ± 0.002	0.49
T-score			
Baseline	0.95 ± 0.12	0.62 ± 0.12	0.06
Month 12	0.88 ± 0.12	0.59 ± 0.12	0.09
Change from baseline	-0.07 ± 0.03	-0.03 ± 0.02	0.31
Z-score			
Baseline	1.37 ± 0.12	1.10 ± 0.12	0.11
Month 12	1.40 ± 0.12	1.12 ± 0.12	0.10
Change from baseline	0.02 ± 0.03	0.02 ± 0.03	0.99

Data presented as arithmetic mean ± SEM. Change from baseline data adjusted for, BMI change from baseline, years since menopause, baseline value of the variable, and menopausal hormone therapy use.

Abbreviations: BMD, bone mineral density; GTE, green tea extract.

Table 4.4. Mean concentrations of obesity-associated hormones, glucose, and HOMA-IR, by treatment group.

Variable	GTE (n=61)	Placebo (n=60)	P-value[1]
Insulin, µIU/mL			
Baseline	6.8 (6.0, 7.8)	5.9 (5.2, 6.7)	0.12
Month 12	7.5 (6.6, 8.5)	6.4 (5.7, 7.3)	0.09
Change from baseline	0.72 (0.12, 1.32)	0.50 (-0.11, 1.10)	0.61
Glucose, mg/dL			
Baseline	101.5 (98.3, 104.8)	99.0 (95.9, 102.3)	0.29
Month 12	97.4 (94.3, 100.6)	95.7 (92.6, 98.8)	0.45
Change from baseline[2]	-3.1 (-5.6, -0.69)	-4.32 (-6.78, -1.85)	0.56
HOMA-IR			
Baseline	1.71 (1.50, 1.96)	1.44 (1.27, 1.66)	0.09
Month 12	1.81 (1.57, 2.07)	1.52 (1.32, 1.74)	0.08
Change from baseline	0.13 (-0.3, 0.29)	0.07 (-0.09, 0.24)	0.61
Adiponectin, µg/mL			
Baseline	6.6 (5.5, 7.8)	8.4 (7.1, 10.0)	0.05
Month 12	6.9 (5.8, 8.2)	8.1 (6.8, 9.7)	0.21
Change from baseline	0.54 (-0.32, 1.41)	-0.05 (-0.93, 0.84)	0.35
Ghrelin, pg/mL			
Baseline	1061.9 (935.1, 1205.9)	1119.8 (985.0, 1273.0)	0.57
Month 12	1057.5 (931.2, 1201.0)	1194.6 (1050.9, 1358.1)	0.19
Change from baseline[3]	-31.1 (-119.2, 56.9)	84.2 (-5.98, 174.2)	0.16
Leptin, ng/mL			
Baseline	31.1 (27.2, 35.6)	30.9 (27.0, 35.3)	0.93
Month 12	30.4 (26.6, 34.8)	30.1 (26.3, 34.4)	0.90
Change from baseline	0.002 (-2.68, 2.68)	-0.59 (-3.33, 2.15)	0.76

Baseline and month 12 data presented as geometric mean (95% confidence interval). Change from baseline data presented as arithmetic mean (95% confidence interval).

[1]*P*-value for the comparison of the means between GTE and placebo. Within-group comparisons of baseline and month 12 values were not significant.

[2]Significant interaction of change of BMI and treatment.

[3]Significant interaction of baseline value and treatment.

Abbreviations: GTE, green tea extract; HOMA-IR, homeostatic model assessment for insulin resistance.

Table 4.5. Baseline Pearson correlations between leptin, adiponectin, and insulin concentrations with BMI, adiposity, and BMD.

	BMI	Total fat mass, kg	% Total body fat	VAT mass, kg	Android % fat	Gynoid % fat	A/G Ratio	Lean weight, kg	Tissue % lean	Tissue % fat	BMD (g/cm^2)	T-score	Z-score
Leptin, ng/mL	0.515[a]	0.508[a]	0.495[a]	0.386[a]	0.378[a]	0.159	0.007	0.037	-0.270[b]	0.486[a]	-0.089	-0.080	-0.314[a]
Adiponectin, ug/mL	-0.146	-0.080	0.060	-0.342[a]	-0.109	0.264[b]	-0.086	-0.125	-0.087	0.059	-0.143	-0.146	-0.056
Insulin, uIU/mL	0.427[a]	0.429[a]	0.322[a]	0.578[a]	0.39[a]	-0.023	0.034	0.185[b]	-0.188[b]	0.320[a]	0.052	0.059	-0.136

[a] $P < 0.0001$
[b] $P < 0.05$

Chapter 5 - Acute Postprandial Effects of Green Tea Extract Administration with a High-Carbohydrate Meal on Appetite-Associated Hormones, Glucose Homeostasis, and Satiety

I. Overview

Background: Green tea extract (GTE) may be involved in a favorable postprandial response to a high-carbohydrate meal. Suggested mechanisms include increases in insulin sensitivity and satiety. COMT genotype may modify these effects. **Objective:** We examined the acute effects of GTE supplementation on postprandial response to a high-carbohydrate meal through assessing appetite-associated hormone concentrations and glucose homeostasis markers in women who had consumed GTE (1315 mg total catechins/day, 843 mg as (-)-epigallocatechin-3-gallate [EGCG]) or placebo capsules for 11-12 months. **Methods:** Sixty Caucasian postmenopausal women (BMI $\geq$ 25.0 kg/m^2) were included in a randomized, double-blind feeding study. GTE capsules were consumed with a breakfast meal (665.4 kcal; 67.2% carbohydrate). Blood samples were drawn pre-meal, post-meal, and every 30 minutes for 4 h. Participants completed six satiety questionnaires (pre-meal, post-meal, and hourly). **Results:** Leptin, ghrelin, and adiponectin were not different between GTE and placebo at any time point; COMT genotype did not modify these results. Participants randomized to GTE with the high-activity form of the COMT enzyme (GTE-high COMT) had higher insulin concentrations at time 0, 0.5, and 1.0 h post-meal compared to all COMT groups randomized to placebo. Insulin remained higher in the GTE-high COMT group at 1.5, 2.0, and 2.5 h compared to Placebo-low COMT group ($P < 0.02$). GTE-high COMT had higher insulin concentrations at times 0, 0.5, 1.0, 1.5, and 2.0 h compared to the GTE-low COMT group ($P \leq 0.04$). AUC measurements of satiety were not different between GTE and placebo at any time. **Conclusions:** GTE supplementation and COMT do not alter the acute postprandial response of leptin, ghrelin, adiponectin, or satiety after consumption of a high-carbohydrate meal, but may be involved in the post-meal glycemic response.

II. Introduction

Overweight and obesity are major public health concerns with worldwide obesity rates having doubled since 1980 (275). Obesity is associated with several serious health conditions, including cardiovascular disease, diabetes, and some forms of cancer, including postmenopausal breast cancer (276-278). Studies have suggested that green tea and its high content of polyphenolic catechins may reduce risk for these diseases in part via beneficial effects on body weight, as green tea consumption has been shown to modestly reduce body weight and adiposity in many randomized trials (134,156,163,175). Among the catechins present in green tea, the four most prominent types are epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and (-)-epigallocatechin-3-gallate (EGCG). EGCG has been the most widely studied and is also thought to be the most bioactive catechin (154). However, these results have not been consistent across all study designs and populations, as several studies have shown no weight loss or weight maintenance effects with green tea supplementation (163,164) (Dostal et al., under review).

Numerous mechanisms have been proposed for the anti-obesity effects of green tea catechins (GTC), including increases in β-oxidation and thermogenesis (138,259,266) and reductions in adipocyte differentiation and proliferation, lipogenesis, and nutrient absorption (132,150,154,279). Another mechanism by which GTC may reduce body weight or promote weight maintenance is through modification of several hormones associated with energy balance, the postprandial glycemic response, and satiety. The gut-derived hormone ghrelin, as well as hormones derived from adipose tissue, including adiponectin and leptin, have been shown to be involved in appetite cues and satiety after ingestion of a meal, respectively. Insulin has also been associated with energy balance (280), and several randomized trials have demonstrated increased insulin sensitivity with consumption of green tea catechins (217,230,244). However, few randomized intervention studies examining the immediate postprandial effects of green tea catechins on these hormones have been conducted (130, 160) and results are overall inconclusive.

The proposed beneficial effects of GTC on body weight and adiposity may be further modulated by the catechol-*O*-methyltransferase (COMT) enzyme, one of the main

enzymes responsible for catechin degradation as well as metabolism of the catecholamines, including norepinephrine. The gene encoding COMT is polymorphic and its alleles correspond to different activity levels of the enzyme: A/A = homozygous low-activity, A/G = heterozygous intermediate-activity, and G/G = homozygous high-activity. A specific allele consisting of an amino acid change from valine to methionine at codon 108 of the soluble form of the enzyme/158 of the membrane-bound form reduces the thermostability of the enzyme and lowers its enzymatic activity by 3- to 4-fold (117,118). These differences in activity are thought to affect individual variation in metabolism of GTC, thus potentially influencing the biological effects of green tea consumption on weight loss and weight control. In addition, GTC have been shown to inhibit the action of COMT *in vitro*, which is significant given that COMT is also responsible for the metabolism of catecholamines including norepinephrine, a potent sympathetic nervous system stimulant. Reduced activity of COMT, therefore, may prolong the effects of catecholamines on increasing thermogenesis and satiety (157,281). The polymorphic nature of the COMT enzyme indicates that the low-activity form of the COMT enzyme may further potentiate these actions, resulting in even greater increases in thermogenesis and satiety and possibly translating into improved weight loss or weight maintenance.

The primary aim of the present study was to determine the effect of a decaffeinated green tea extract (GTE) containing 1315 mg total catechins/day (843 mg as EGCG) on postprandial concentrations of appetite-related hormones and blood glucose as well as measures of satiety in a population of healthy overweight and obese postmenopausal women at high risk for breast cancer. A secondary aim was to evaluate the modification of these effects by COMT genotype. We hypothesized that GTE supplementation would cause favorable changes in hormone concentrations and increase postprandial satiety, and that women with the low-activity genotype (A/A) would have a more favorable response to GTE consumption than those with the intermediate- (A/G) or high-activity (G/G) COMT genotypes, due to greater exposure to GTC.

III. Methods

A. Study Design

This acute postprandial feeding study was conducted in a subset of overweight and obese women enrolled in the Minnesota Green Tea Trial (MGTT), a phase II, randomized, double blind, placebo-controlled, intervention study which is described in detail elsewhere (Samavat, et al., under review). In the parent study, healthy postmenopausal women at high-risk of breast cancer due to increased mammographic density were randomized by COMT genotype into one of six groups: GTE, low COMT activity (A/A); GTE, intermediate COMT activity (A/G); GTE, high COMT activity (G/G); placebo, low COMT activity (A/A); placebo, intermediate COMT activity (A/G); or placebo, high COMT activity (G/G). The subjects consumed either four decaffeinated (< 16 mg caffeine/day) GTE capsules containing a total of 1315 ± 116 mg GTC (843 ± 44 mg as EGCG) or placebo capsules daily for 12 months to determine the effects of GTE exposure on a number of breast cancer biomarkers including mammographic density, reproductive hormones, oxidative stress, and insulin-like growth factor (IGF) axis proteins.

To evaluate the postprandial effects of GTE consumption following a standardized meal, 60 subjects (10 from each treatment/genotype group) were invited to participate in the postprandial study during month 11 or 12 of the 12-month parent study. The postprandial study was designed to assess the acute effects of GTE ingestion (independent of caffeine) and COMT genotype on energy balance-related measures. This research took place during a half-day clinic visit. For the half-day visit, subjects were instructed to adhere to their normal energy intake and to refrain from exercise and alcohol the day before the test day. They arrived at the research unit between 07:00 and 08:00 after a 10-hour fast. Baseline satiety questionnaires were completed and fasting blood drawn for assessment of the energy-related hormones. After a standardized high-carbohydrate breakfast (consisting of a bagel with cream cheese, orange juice, and low-fat, fruit-flavored yogurt or 2% milk), blood was drawn immediately post-meal and every 30 minutes over a period of 4 hours for evaluation of change in energy- and obesity-

related hormones. Satiety questionnaires were completed before the meal, immediately after consumption of the meal, and every hour thereafter for a total of six questionnaires.

B. Participant Recruitment and Eligibility Criteria

Participant eligibility, screening, and recruitment followed the same protocol as the parent study (Samavat, et al., under review). Inclusion criteria included healthy, non-smoking postmenopausal women aged 50-70 years who were classified as having "heterogeneously dense" or "extremely dense" breast tissue by a trained radiologist after a routine screening mammogram. Additional inclusion criteria included: no use of systemic menopausal hormone therapy (MHT) or chemopreventive agents, no history of breast cancer or proliferative breast disease, alcohol consumption < 7 drinks/week, stable weight in the past year (< 4.6 kg change), no positive serology for Hepatitis B or C antibodies or elevated concentrations of liver enzymes (defined as alanine aminotransferase (ALT) above 1.5 times the upper limit of normal, 60 U/L) at the screening clinic visit, and willingness to avoid green tea beverage consumption for the 12-month intervention period. Specific criteria for this sub-study included: overweight/obese (BMI: ≥25 to ≤40 kg/m^2), no use of weight loss medications, and no history of bariatric surgery.

Recruitment for the parent study took place through the Fairview and Park Nicollet hospital systems through identification of postmenopausal women at high risk of breast cancer due to high mammographic density (assessed by routine screening mammogram). Of 1075 women randomized into the parent study, 230 women classified as overweight (BMI = 25.0-29.9 kg/m^2) or obese (BMI = 30.0-40.0 kg/m^2) at the screening clinic visit were additionally invited to participate in this acute feeding study beginning in September 2012. Of these, 149 participants responded to the invitation, 50 of whom were excluded due to the need for equal numbers in each COMT genotype group. An additional 33 women declined to participate, and 5 did not participate for various other reasons. Ultimately, 61 participants were randomized into the study (GTE: n=30; placebo: n=31). One participant in the placebo group was excluded at the end of the half-day study due to not consuming the intervention product with the breakfast meal,

so the final sample size was 30 participants per treatment group (n=10 for each COMT genotype within each treatment group).

C. Randomization, Blinding, and Participant Consent

Randomization of subjects was performed by the University of Minnesota Medical Center-Fairview's Investigational Drug Service (IDS) pharmacy using a computer-generated permuted block randomization scheme with blocks of 8 stratified by COMT genotype activity: low (A/A), intermediate (A/G) or high (G/G), assuring both participants and investigators were blinded to the treatment of a subject. Institutional Review Board (IRB) approval was obtained at each clinical center. All participants provided additional written informed consent for this ancillary study (see **Appendix 3**). This trial was registered at clinicaltrials.gov as NCT00917735.

D. Determination of COMT Genotype

DNA was purified from buffy coats of peripheral blood samples using a PureGene Blood kit (Gentra Systems, Minneapolis, MN). A TaqMan assay was developed for determining the COMT G/A polymorphism using a TaqMan PCR Core Reagent kit (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. PCR amplification using ~10 ng of genomic DNA was performed in a thermal cycler (MWG Biotech, High Point, NC) with an initial step of 95°C for 10 min followed by 50 cycles of 95°C for 25 s and 62°C for 1 min. The fluorescence profile of each well was measured in an ABI 7900HT Sequence Detection System and the results analyzed with Sequence Detection Software (Applied Biosystems). Experimental samples were compared with 12 controls to identify the three genotypes at each locus (G/G, G/A, and A/A). Any samples that fell outside the parameters defined by the controls were identified as non-informative and retested.

E. Green Tea Extract Composition

This study used decaffeinated Green Tea Extract Catechin Complex (GTE) in capsule form, provided by Corban Laboratories, (Eniva Nutraceutics, Plymouth, MN). Mean daily catechin content was 1315 ± 116 mg/day (843 ± 44 mg as EGCG). Placebo capsules were identical in appearance to GTE and contained 816 mg maltodextrin, 808

mg cellulose, and 8 mg magnesium stearate (flow agent). See Table 3.1 for detailed information on the GTE composition. GTE ingredients were analyzed by high-performance liquid chromatography (HPLC) (Rutgers University, Piscataway, NJ) to demonstrate comparability with the stated catechin contents of the manufacturer. Participants were required to consume four capsules daily and were advised to ingest 2 capsules in the morning hours and 2 in the evening to maintain circulating catechin concentrations throughout each day, and to take the capsules with meals to reduce any potential gastrointestinal discomfort associated with consuming GTE in the fasted state. For this specific sub-study, participants were asked to consume the morning dose of GTE with the breakfast meal.

F. Demographics Data Collection

All participants completed a health history questionnaire (**Appendix 1**) upon entry into the parent study that included comprehensive data of demographics, lifestyle factors (physical activity, smoking history, and alcohol intake), and information about medical history, medication use (current and former), and full reproductive history.

G. Dietary Intake

To evaluate dietary intake participants were asked to record food intake on two assigned weekdays and one weekend day prior to the half-day visit. Recording errors were minimized by providing the subjects detailed instructions on how to keep accurate diet records (see **Appendix 3** for example). Subjects were encouraged to measure foods eaten using measuring spoons and cups whenever possible. Diet records were then reviewed for accuracy and completeness during the clinic visit by a Registered Dietitian Nutritionist and later analyzed for nutrient content using the Food Processor Diet Analysis and Fitness software, version 10.10 (ESHA Research, Salem, OR). The average of the three days was used to represent typical food and nutrient intake.

H. Test Meal Composition

The high-carbohydrate test meal consisted of a plain bagel (300 kcal, 59.4 g carbohydrate, 11.6 g protein, 1.8 g fat), 1 ounce cream cheese spread (84 kcal, 0.1 g carbohydrate, 2 g protein, 8.1 g fat), 8 ounces orange juice (122 kcal, 28.7 g

carbohydrate, 1.7 g protein, 0.3 g fat), and choice of 6 ounces low-fat fruit yogurt (183 kcal, 34.5 g carbohydrate, 7.4 g protein, 2.1 g fat) or one cup 2% milk (138 kcal, 13.5 g carbohydrate, 9.7 g protein, 4.9 g fat). Mean total energy content was 665.4 kcal; macronutrient content was as follows: carbohydrate = 113.3 g (67.2%); protein = 23.9 g (14.3%); and fat = 10.0 g (18.5%). **Appendix 3** details the dietary analysis of the test meals. Participants were instructed to take two GTE or placebo capsules with the test meal.

I. Biological Sample Analyses

Plasma adiponectin, leptin, and ghrelin were measured using radioimmunoassay kits manufactured by EMD Millipore (Billerica, MA) (inter-assay % coefficient of variation [% CV]: leptin = 9.8%, adiponectin = 8.6%, ghrelin = 6.2%; intra-assay % CV: leptin = 10.1%; adiponectin = 7.7%, ghrelin = 8.1%). Serum insulin was measured using a simultaneous one-step immunoenzymatic, chemiluminescent assay (Access Ultrasensitive Insulin assay, Quest Diagnostics, Wood Dale, IL, intra-assay % CV: 3-5.0%; inter-assay % CV = 3.9%). Serum glucose concentrations were measured using a hexokinase enzymatic reference method (Quest Diagnostics, Wood Dale, IL, monthly % CV = 1.4%).

J. Assessment of Satiety

To test satiety, we used a set of nine visual analogue scale (VAS) ratings associated with the standardized high-carbohydrate test meal. Participants were asked to answer each separate VAS question relating to hunger, fullness, desire to eat, prospective consumption, perceived satiety, contentedness, irritability, sleepiness, and mental alertness in a continuous linear scale from 0 to 100 millimeters, where 0 represented "not/none at all" and 100 represented "extremely/very" (example included in **Appendix 3**). These scores were then converted to centimeters. The VAS is considered to be a valid and reliable assessment tool for monitoring the effects of energy, palatability, and macronutrient manipulations on subjective ratings in appetite studies (155,282). .

K. Statistical Analyses

Power calculations were unable to be performed for the half-day postprandial sub-study, since there was no data available on these outcomes at that time. We chose 60 participants as a feasible number that is large in comparison to similar studies examining appetite-related hormones in response to a specific dietary component.

Demographic characteristics of participants at baseline were compared between treatments using a one-sample t-test for continuous variables. Natural logarithmic transformation was considered to normalize the distribution of these variables. Chi-square and Fisher exact tests were used to compare the distribution of categorical variables between treatments. Change in hormones (leptin, ghrelin, adiponectin, and insulin) and blood glucose over time was evaluated using linear regression with repeated measurements. Hormones and blood glucose were transformed using natural logarithms to normalize their distribution. The model included time, treatment, COMT genotype and the 2-way and 3-way interactions as explanatory variables. Interactions were excluded from the model if non-significant at P-value < 0.05, using backward elimination. When statistically significant differences were detected, a post hoc pairwise comparison across treatment and COMT genotype groups was performed. Results are expressed as means and 95% confidence interval. An unstructured working correlation matrix was fitted to model the correlation between time points within participants; this was chosen based on Akaike's information criterion. The area under the curve (AUC) for insulin and glucose was calculated using the trapezoidal rule and pairwise compared within genotypes between treatment groups using repeated measurements linear regression. The AUC of the satiety VAS at different time points before and after the meal was calculated for each participant and each VAS question using the trapezoid method. The AUC of each question and participant were used as dependent variables in a linear mixed model to evaluate the effect of treatment on the AUC of each question. Baseline VAS (pre-meal) was used as a random effect in the model. Model assumptions were evaluated using residual plots and Cook's distance greater than 0.5 was used to evaluate possible influential outliers for both hormones and VAS measures. Significance was set at $P \leq$

0.05 for all comparisons. All analyses were conducted using SAS system version 9.4 (The SAS system for Windows, 2005, Cary NC, SAS Institute, Inc.).

IV. Results

A. Baseline Characteristics

Participants randomized to GTE and placebo were similar with respect to baseline demographics and characteristics, as presented in **Table 5.1**. Groups differed in the distribution of level of education, in that the GTE group had a greater number of participants who had obtained education beyond high school ($P = 0.001$). Mean intake of macro- and micronutrients as assessed by 3-day diet records did not differ by treatment group.

B. Postprandial Hormone and Glucose Response

Postprandial concentrations of leptin, ghrelin, and adiponectin are presented in **Figure 5.1**. Baseline fasting concentrations of all variables were similar between treatment groups. These hormone concentrations were not different between GTE and placebo at any time point, and COMT genotype did not modify these results.

There was a statistically significant interaction between treatment and COMT genotype for insulin and glucose that varied across time (

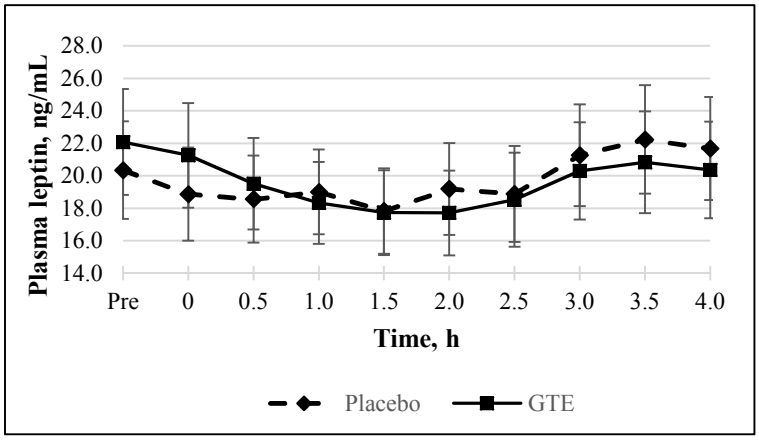


Figure 5.2). Participants randomized to GTE with the high-activity form of the COMT enzyme (GTE-high COMT) had significantly higher insulin concentrations at time 0 (post-meal), 0.5, and 1.0 h post-meal as compared to all COMT genotype groups randomized to placebo. Insulin concentrations remained significantly higher in the GTE-high COMT group 1.5, 2.0, and 2.5 h as compared to the Placebo-low COMT group ($P < 0.02$). The GTE-high COMT group also had higher insulin concentrations at time 0, 0.5, 1.0, 1.5, and 2.0 h after the meal as compared to the GTE-low COMT group ($P \leq 0.04$). No differences between treatment groups and COMT genotypes existed 3.0, 3.5, or 4.0 h after the meal. With respect to glucose, the GTE-high COMT group had significantly higher glucose concentrations at time 0 as compared to Placebo-high COMT ($P = 0.05$) and the GTE-low COMT group ($P = 0.004$). The GTE-low COMT group had higher serum glucose 3.5 h post-meal as compared to the GTE-high COMT and Placebo-low COMT groups ($P = 0.01$ and $P = 0.05$, respectively). Insulin AUC comparisons within genotypes between treatment groups are listed in Table 5.2. The insulin AUC for GTE-high COMT was significantly higher as compared to Placebo-high COMT at all time points, though the overall mean AUC comparison failed to reach significance ($P = 0.06$). Mean insulin AUCs between intermediate- and low-COMT groups did not differ at any time point, and glucose AUCs did not differ within any COMT genotype between treatment groups (data not shown).

C. Appetite Sensations

AUC measurements of satiety-related variables did not show any significant difference between treatments at baseline or over the 4-h time period between GTE and placebo groups (Table 5.3). COMT genotype did not modify these results.

V. Discussion

The results of the present study indicate that GTE supplementation does not alter the acute postprandial response of leptin, ghrelin, or adiponectin after consumption of a high-carbohydrate meal, and that COMT genotype does not modify this relationship. However, GTE supplementation and COMT genotype may both be involved in the post-meal glycemic response, as significant interactions were observed between COMT genotype

and glucose and insulin concentrations over the 4-h test period. No effect of GTE or COMT genotype was seen on measures of postprandial satiety.

To our knowledge, this is the first study to examine the effect of GTE supplementation on acute postprandial concentrations of the appetite-associated peptide hormones leptin, ghrelin, and adiponectin. Ghrelin is a peptide produced mainly in the stomach and has been shown to influence feeding behavior, energy metabolism, and gastrointestinal function (283). Ghrelin concentrations typically increase prior to a meal and decrease after feeding (284). Leptin exerts an anorectic effect resulting in reduced food intake (37) and increased energy expenditure (38). Circulating levels of leptin are proportional to fat mass (40); however, obese individuals have demonstrated a resistance to the protein's effects (41). Adiponectin modulates several metabolic processes including glucose regulation (48) and fatty acid metabolism (49), and also has centrally mediated effects on food intake and energy expenditure (54,55). Concentrations of adiponectin are negatively correlated with body fat percentage in adults (51). We did not find significant differences between treatment groups in these hormones prior to the meal or at any time point thereafter, indicating that GTE did not modify fasting concentrations of ghrelin, leptin, and adiponectin in these participants. This is consistent with our previous work, which demonstrated that GTE did not modify concentrations of these hormones over the MGTT's 12-month intervention period in larger samples of overweight and obese study participants (Chapters 3 and 4). We did observe a somewhat unexpected leptin response, in which plasma leptin concentrations decreased moderately from pre-meal to 1.5 h in both groups before increasing to the end of the 4 h period. Yet, these results correlate with other studies that have examined leptin concentrations in overweight and obese women after a high-carbohydrate meal (285,286), affirming that obesity is associated with an impaired postprandial leptin response. When correlated to the AUC analysis of satiety-related endpoints, satiety and prospective food intake were not differentially influenced by the effect of GTE on energy-related hormones. Together, the results of these studies indicate that any effect of GTE supplementation is not mediated through alteration of leptin, ghrelin, or adiponectin concentrations.

We observed a significant interaction between GTE, COMT genotype, and time, in which participants randomized to GTE with the high-activity (G/G) form of the COMT enzyme demonstrated increased postprandial insulin concentrations as compared to the Placebo-high COMT and GTE-low (A/A) COMT groups, despite similar glucose profiles at most time points. The significance of an increased insulin response to a high-carbohydrate meal in participants with the G/G genotype taking GTE is unclear, though it could be indicative of the need to secrete additional insulin to manage blood glucose concentrations over time, as compared to individuals with other forms of the COMT genotype and those not taking GTE. The sample size of this group was small, thus making it difficult to draw definitive conclusions. Yet, our results are in agreement with those of another study (130) that found greater postprandial insulin concentrations after consumption of GTE (836 mg catechins) in individuals with the G/G genotype as compared to those with the G/A or A/A COMT genotypes. Similarly, Kring, et al. (129), determined that there was an 11.6% increased frequency of the G/G COMT genotype in individuals with impaired glucose tolerance or type 2 diabetes. These studies seem to suggest that the high-activity form of the COMT enzyme is associated with increased risk for glycemia-related health conditions, and that GTE consumption may potentiate an exaggerated insulin response after a meal. These results coincide with our previous findings in a larger sample of postmenopausal overweight and obese women (n = 237) (Dostal, et al., under review), in which participants with the G/G form of COMT showed significantly higher insulin concentrations at month 12 as compared to those with the A/A genotype irrespective of treatment group (GTE and placebo groups combined); yet, among participants with baseline fasting insulin ≥10 μIU/mL, reductions in fasting insulin concentrations were seen in the GTE group over 12 months compared to both the placebo group and all participants with baseline insulin < 10 μIU/mL. It is plausible that GTE consumption may elicit a higher immediate postprandial insulin response, particularly in those with the G/G COMT genotype, while at the same time acting to reduce fasting insulin concentrations over time. Additional research is needed to determine the specific mechanisms of the COMT enzyme on insulin concentrations after administration of GTE and how this may affect short- and long-term glycemic response.

Early animal studies demonstrated reduced food intake with administration of green tea catechins (154,199), which generated interest in the idea that green tea may increase satiety. However, this effect has not been confirmed in human subjects. One study found that inclusion of 167 mg green tea catechins and 100 mg caffeine in a beverage containing 10 g soluble fiber created lower hunger and higher fullness ratings and was associated with the lowest energy intake in the next meal as compared to fiber-only (46 mg caffeine), isocaloric control beverage (no caffeine), and no beverage conditions (159). In contrast, Diepvens, et al. showed that women randomized to receive green tea (1125 mg catechins + 225 mg caffeine/day) for nearly 3 months with a low-energy diet became hungrier over time and showed increased prospective food consumption as compared to placebo (157). The authors suggested that this could be due to down-regulation of the leptin response through stimulation of the sympathetic nervous system by green tea, as leptin is known to reduce appetite. However, leptin concentrations were not measured in their study, so this conclusion could not be confirmed. Several other randomized trials examining the effect of GTC on acute measures of satiety (136,287) or long-term changes in energy intake (134,147) (Dostal, et al., under review) have shown null results, including the present study, in which we did not observe differences between treatment groups in any of the 9 questions related to hunger, satiation, and prospective food intake in the 4 hours following a high-carbohydrate breakfast meal. Taken together with the existing evidence, it is unlikely that oral GTE consumption is independently associated with increased satiety or reduction in voluntary food intake.

The results of this research contribute depth not only to the growing body of research on the effects of GTC on insulin sensitivity, but also to the association between COMT genotype and risk for glycemia-related health conditions. Our results also strongly indicate that GTE does not induce appetite inhibition or satiety, thus weakening the argument for these effects as mechanisms behind green tea's association with reductions in body weight and adiposity. However, our research has several limitations. We conducted this analysis after just one meal and were unable to compare each participant's response to that of a reference meal. There was no measure of hedonic

liking of the meal, which may have influenced satiety and prospective food intake. Standardization of the diets of participants 24 hours prior to the breakfast meal could have increased similarity of the glycemic response. Lastly, the sample size in this study may have been too small to yield significant results.

VI. Conclusion

These results indicate that supplementation of 1315 mg GTE per day for 12 months did not influence pre- or postprandial concentrations of appetite-associated hormones or measures of satiety after a high-carbohydrate breakfast meal. Yet, GTE and COMT genotype may have specific influences on post-meal insulin and glucose concentrations, and these findings warrant additional research to determine the specific impact of GTE on the postprandial glycemic response.

VII. Tables and Figures

Table 5.1. Baseline characteristics and mean 3-day dietary intake of study participants, by treatment group

	GTE (n=30)	Placebo (n=30)	P-value
Age, y	61.0 (59.2, 62.8)	60.8 (59.0, 62.6)	0.88
White, non-Hispanic, n (%)	29 (96.7)	30 (100)	1.00[a]
Weight, kg	74.2 (71.1, 77.4)	75.3 (72.1, 78.4)	0.64
Body mass index, kg/m^2	28.2 (27.1, 29.4)	28.3 (27.2, 29.5)	0.91
Waist-to-hip ratio	0.87 (0.83, 0.89)	0.85 (0.82, 0.87)	0.31
Years postmenopausal[a]	8.8 (6.3, 12.2)	7.8 (5.6, 10.8)	0.60
Type of menopause, n (%)			0.75
Natural	23 (76.7)	24 (80.0)	
Surgical	7 (23.3)	6 (20.0)	
Education, n (%)			0.001[a]
Masters/PhD/Professional	10 (33.3)	8 (26.7)	
College degree	8 (26.7)	15 (50.0)	
Some college	12 (40.0)	2 (6.7)	
High school or below	0	5 (16.7)	
Physical activity, MET-hr/week	39.5 (26.2, 52.7)	43.8 (30.6, 57.1)	0.64
Dietary variables			
Total energy, kcal	1761.9 (1589.7, 1934.0)	1849.9 (1677.8, 2022.0)	0.48
Protein, g	73.4 (65.7, 81.0)	78.6 (70.9, 86.2)	0.35
Total fat, g	67.5 (57.6, 77.4)	73.9 (64.0, 83.8)	0.37
Dietary cholesterol, mg	230.0 (189.6, 270.5)	237.8 (197.3, 278.2)	0.79
Saturated fat, g	23.4 (19.9, 26.9)	24.5 (21.0, 28.0)	0.66
MUFA[b], g	11.9 (9.7, 14.6)	13.4 (10.9, 16.4)	0.44
PUFA, g	7.0 (5.0, 9.0)	8.5 (6.5, 10.5)	0.31
Omega-3 FA[b], g	0.5 (0.4, 0.7)	0.6 (0.5, 0.8)	0.65
Omega-6 FA[b], g	1.2 (1.0, 1.5)	1.3 (1.1, 1.7)	0.38
Total carbohydrate, g	216.7 (192.1, 241.2)	215.6 (191.0, 240.1)	0.95
Dietary fiber, g	19.0 (16.8, 21.2)	20.6 (18.4, 22.9)	0.31
Soluble fiber, g	1.8 (1.4, 2.2)	1.6 (1.2, 2.0)	0.42
Alcohol[b], g	0.1 (0.0, 0.3)	0.1 (0.0, 0.4)	0.75
Caffeine[b], mg	36.8 (13.6, 99.9)	46.2 (17.0, 125.3)	0.75

Continuous data expressed as arithmetic mean (95% confidence interval) unless otherwise indicated by superscript. Categorical variables compared using Chi-square test and expressed as n (%).

[a]Fisher's exact test used for comparison.

[b]Data expressed as geometric mean (95% confidence interval).

Abbreviations: FA, fatty acid; GTE, green tea extract; MET-hr, metabolic equivalent hours; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

Table 5.2. Mean insulin area under the curves in participants after the ingestion of a meal with or without GTE, by COMT genotype (expressed in µIU/(mL-h))

	Placebo (n=30)			GTE (n=30)		
Time (h)	High (G/G)	Intermediate (G/A)	Low (A/A)	High (G/G)	Intermediate (G/A)	Low (A/A)
0-0.5	14.0 ± 4.9	14.6 ± 4.9	21.2 ± 4.9	35.4 ± 4.9*	25.4 ± 5.1	16.4 ± 4.6
0-1	31.9 ± 11.4	37.6 ± 11.4	51.1 ± 11.4	81.1 ± 11.4*	55.1 ± 12.0	45.0 ± 10.9
0-1.5	48.1 ± 18.5	58.9 ±18.5	70.5 ± 18.5	120.0 ± 18.5*	83.9 ± 19.5	73.3 ± 17.6
0-2	65.8 ± 25.8	79.0 ± 25.8	84.1 ± 25.8	155.9 ± 25.8*	120.1 ± 27.2	99.3 ± 24.6
0-2.5	82.9 ± 32.9	95.0 ± 32.9	96.1 ± 32.9	190.1 ± 32.9*	148.1 ± 34.7	123.7 ± 31.4
0-3	98.7 ± 38.7	105.6 ± 38.7	105.6 ± 38.7	216.3 ± 38.7*	167.6 ± 40.8	143.1 ± 36.9
0-3.5	109.1 ± 42.8	113.6 ± 42.8	112.5 ± 42.8	231.1 ± 42.8*	183.5 ± 45.1	156.3 ± 40.8
0-4	114.6 ± 45.7	119.7 ± 45.7	117.9 ± 45.7	241.4 ± 45.7	195.8 ± 48.2	165.5 ± 43.6

Data presented as arithmetic mean ± SEM. Time 0 indicates post-meal.
*Statistically significant difference between COMT genotypes, GTE vs. placebo ($P \leq 0.05$).

Table 5.3. Mean satiety and fullness AUCs after ingestion of a high-carbohydrate meal (expressed in cm/h).

Question	GTE (n=30)	Placebo (n=30)	*P*-value
1. Hunger	7.5 ± 2.2	9.1 ± 2.0	0.37
2. Fullness	24.7 ± 1.4	25.3 ± 1.5	0.77
3. Desire to eat	6.8 ± 1.8	7.6 ± 1.8	0.59
4. Prospective consumption	3.1 ± 2.5	4.6 ± 2.3	0.36
5. Satiety	26.5 ± 1.5	26.5 ± 1.5	0.98
6. Contentedness	22.4 ± 2.0	20.8 ± 2.2	0.33
7. Irritability	2.7 ± 0.8	2.3 ± 0.7	0.65
8. Sleepiness	10.1 ± 1.6	10.9 ± 1.4	0.57
9. Mental alertness	17.1 ± 2.1	19.8 ± 2.1	0.07

Data expressed as arithmetic mean ± SEM.

Figure 5.1. Mean change in postprandial adiponectin, leptin, and ghrelin concentrations, by treatment group.

Figure 5.1a. Adiponectin

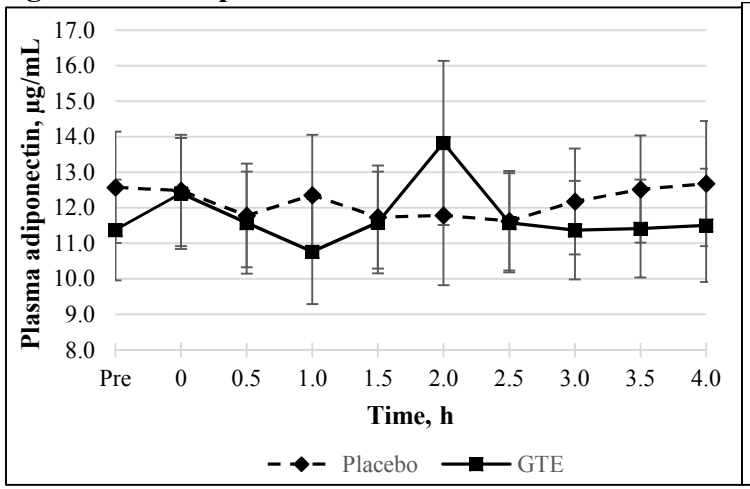


Figure 5.1c. Ghrelin

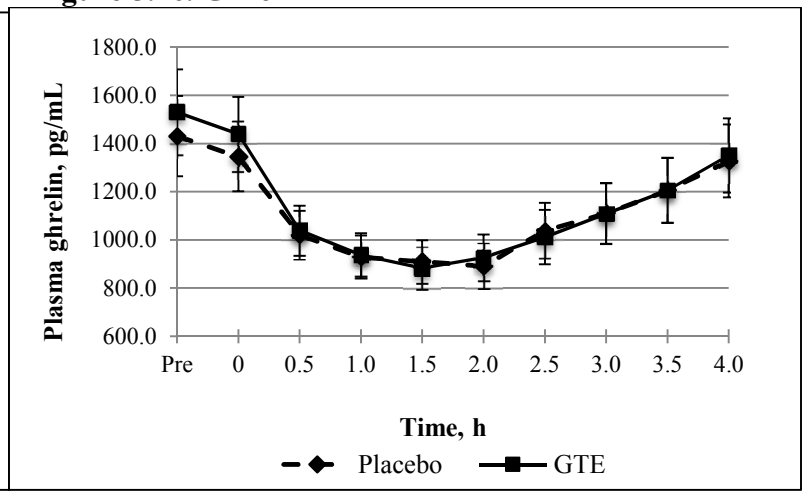


Figure 5.1b. Leptin

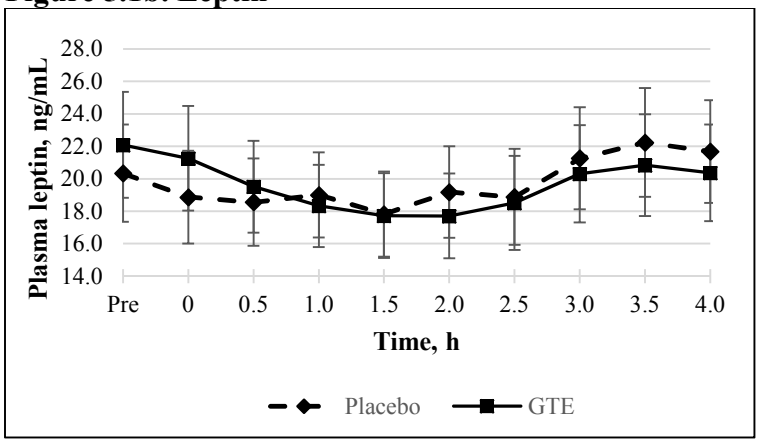


Figure 5.2 Mean change in postprandial insulin and glucose concentrations, by treatment and COMT genotype.

Figure 5.2a. Insulin

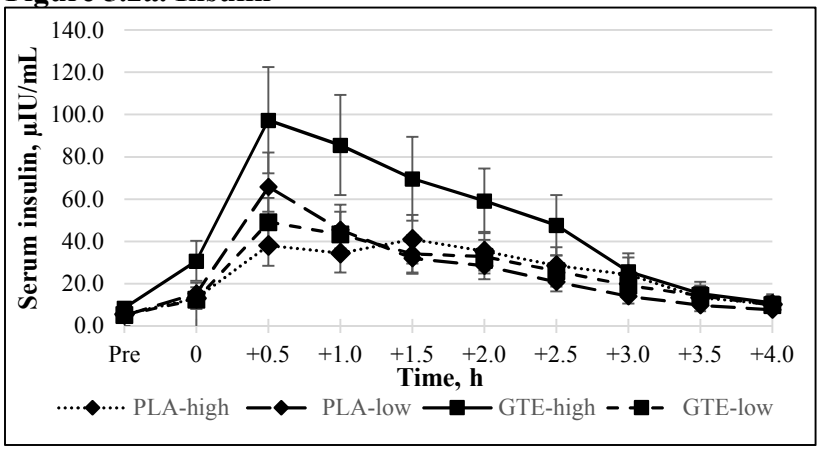
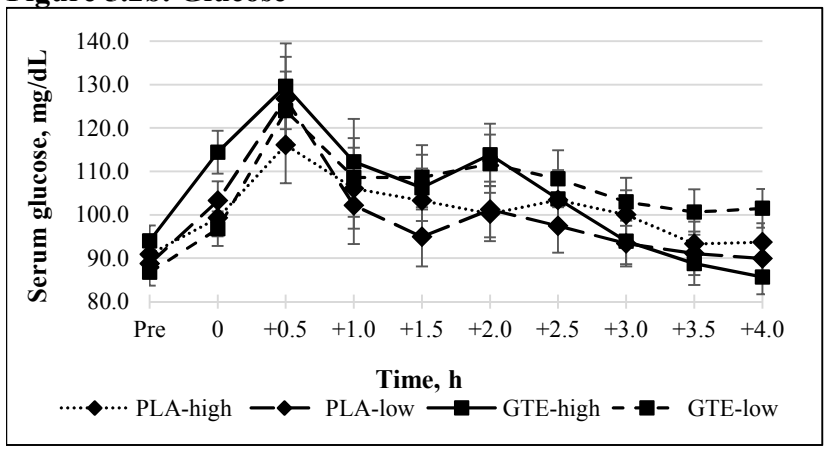


Figure 5.2b. Glucose



Chapter 6 - Dissertation Summary

The studies detailed in Chapters 3, 4, and 5 demonstrate that daily supplementation of 1315 mg decaffeinated GTE (843 mg as EGCG) was not associated with reductions in energy intake, body weight, BMI, or waist circumference over 12 months in overweight and obese postmenopausal women. Similarly, GTE was not associated with overall reductions in adiposity or improvements in BMD, though GTE may be more beneficial for body fat reduction in individuals with higher BMI. GTE did not alter leptin, adiponectin, or ghrelin concentrations over 12 months or during the acute postprandial phase, and did not influence satiety after consumption of a high-carbohydrate meal. However, GTE decreased fasting insulin concentrations after 12 months in individuals with elevated baseline fasting concentrations. The high-activity form of the COMT enzyme may be associated with elevations in insulin and reduction in adiponectin concentrations over time, which is a metabolic profile associated with increased risk for type 2 diabetes and the metabolic syndrome. In correlation with this, COMT genotype influenced the post-meal glycemic response in a small sample of overweight and obese women, in that participants with the high-activity COMT genotype randomized to GTE showed increased insulin release as compared to those randomized to placebo or those with the low-activity form of COMT.

These results suggest that daily supplementation of GTE may be beneficial for overweight and obese postmenopausal women with a higher degree of visceral adiposity and increased circulating insulin concentrations. Women with the high-activity form of COMT may see particular benefit. Therefore, GTE could be a safe and effective dietary supplement for those at risk for – or already diagnosed with - metabolic syndrome or type 2 diabetes. Since these conditions are strongly associated with increased risk for breast cancer, it is possible that breast cancer risk could be reduced through decreasing the incidence and prevalence of these metabolic derangements. Additional research remains to be done to confirm these findings in clinical high-risk populations, to determine possible interactions with medications used to treat type 2 diabetes, and to fully elucidate the influence of COMT on the chronic and acute glycemic response.

Bibliography

(1) American Cancer Society. Cancer Facts & Figures 2014. American Cancer Society 2014.

(2) American Cancer Society. What are the risk factors for breast cancer? 2015; Available at: http://www.cancer.org/cancer/breastcancer/detailedguide/breast-cancer-risk-factors. Accessed May/16, 2015.

(3) American Cancer Society. *Breast cancer: detailed guide.*. 2015; Available at: http://www.cancer.org/cancer/breastcancer/detailedguide/. Accessed May/16, 2015.

(4) Centers for Disease Control and Prevention. Breast Cancer Rates by Race and Ethnicity. 2014; Available at: http://www.cdc.gov/cancer/breast/statistics/race.htm. Accessed May/16, 2015.

(5) Wang AT, Vachon CM, Brandt KR, Ghosh K. Breast density and breast cancer risk: a practical review. Mayo Clin Proc 2014 Apr;89(4):548-557.

(6) Anderson KN, Schwab RB, Martinez ME. Reproductive risk factors and breast cancer subtypes: a review of the literature. Breast Cancer Res Treat 2014 Feb;144(1):1-10.

(7) Chlebowski RT, Anderson GL. Menopausal hormone therapy and breast cancer mortality: clinical implications. Ther Adv Drug Saf 2015 Apr;6(2):45-56.

(8) Gierisch JM, Coeytaux RR, Urrutia RP, Havrilesky LJ, Moorman PG, Lowery WJ, et al. Oral contraceptive use and risk of breast, cervical, colorectal, and endometrial cancers: a systematic review. Cancer Epidemiol Biomarkers Prev 2013 Nov;22(11):1931-1943.

(9) Rossi RE, Pericleous M, Mandair D, Whyand T, Caplin ME. The role of dietary factors in prevention and progression of breast cancer. Anticancer Res 2014 Dec;34(12):6861-6875.

(10) Joya X, Manzano C, Alvarez AT, Mercadal M, Torres F, Salat-Batlle J, et al. Transgenerational exposure to environmental tobacco smoke. Int J Environ Res Public Health 2014 Jul 16;11(7):7261-7274.

(11) National Center for Chronic Disease Prevention and Health Promotion (US) Office on Smoking and Health. 2014.

(12) Friedenreich CM. Physical activity and breast cancer: review of the epidemiologic evidence and biologic mechanisms. Recent Results Cancer Res 2011;188:125-139.

(13) Turner LB. A meta-analysis of fat intake, reproduction, and breast cancer risk: an evolutionary perspective. Am J Hum Biol 2011 Sep-Oct;23(5):601-608.

(14) Kim EH, Willett WC, Colditz GA, Hankinson SE, Stampfer MJ, Hunter DJ, et al. Dietary fat and risk of postmenopausal breast cancer in a 20-year follow-up. Am J Epidemiol 2006 Nov 15;164(10):990-997.

(15) Ahn J, Schatzkin A, Lacey JV,Jr, Albanes D, Ballard-Barbash R, Adams KF, et al. Adiposity, adult weight change, and postmenopausal breast cancer risk. Arch Intern Med 2007 Oct 22;167(19):2091-2102.

(16) Berstad P, Coates RJ, Bernstein L, Folger SG, Malone KE, Marchbanks PA, et al. A case-control study of body mass index and breast cancer risk in white and African-American women. Cancer Epidemiol Biomarkers Prev 2010 Jun;19(6):1532-1544.

(17) Eng SM, Gammon MD, Terry MB, Kushi LH, Teitelbaum SL, Britton JA, et al. Body size changes in relation to postmenopausal breast cancer among women on Long Island, New York. Am J Epidemiol 2005 Aug 1;162(3):229-237.

(18) Kawai M, Minami Y, Kuriyama S, Kakizaki M, Kakugawa Y, Nishino Y, et al. Adiposity, adult weight change and breast cancer risk in postmenopausal Japanese women: the Miyagi Cohort Study. Br J Cancer 2010 Oct 26;103(9):1443-1447.

(19) Lahmann PH, Schulz M, Hoffmann K, Boeing H, Tjonneland A, Olsen A, et al. Long-term weight change and breast cancer risk: the European prospective investigation into cancer and nutrition (EPIC). Br J Cancer 2005 Sep 5;93(5):582-589.

(20) Tehard B, Clavel-Chapelon F. Several anthropometric measurements and breast cancer risk: results of the E3N cohort study. Int J Obes (Lond) 2006 Jan;30(1):156-163.

(21) van den Brandt PA, Dirx MJ, Ronckers CM, van den Hoogen P, Goldbohm RA. Height, weight weight change, and postmenopausal breast cancer risk: The Netherlands Cohort Study. Cancer Causes Control 1997 Jan;8(1):39-47.

(22) Morimoto LM, White E, Chen Z, Chlebowski RT, Hays J, Kuller L, et al. Obesity, body size, and risk of postmenopausal breast cancer: the Women's Health Initiative (United States). Cancer Causes Control 2002 Oct;13(8):741-751.

(23) Feigelson HS, Jonas CR, Teras LR, Thun MJ, Calle EE. Weight gain, body mass index, hormone replacement therapy, and postmenopausal breast cancer in a large prospective study. Cancer Epidemiol Biomarkers Prev 2004 Feb;13(2):220-224.

(24) Demark-Wahnefried W, Campbell KL, Hayes SC. Weight management and its role in breast cancer rehabilitation. Cancer 2012 Apr 15;118(8 Suppl):2277-2287.

(25) Lorincz AM, Sukumar S. Molecular links between obesity and breast cancer. Endocr Relat Cancer 2006 Jun;13(2):279-292.

(26) Cleary MP, Grossmann ME. Minireview: Obesity and breast cancer: the estrogen connection. Endocrinology 2009 Jun;150(6):2537-2542.

(27) Schapira DV, Kumar NB, Lyman GH, Cox CE. Abdominal obesity and breast cancer risk. Ann Intern Med 1990 Feb 1;112(3):182-186.

(28) Harvie M, Howell A, Vierkant RA, Kumar N, Cerhan JR, Kelemen LE, et al. Association of gain and loss of weight before and after menopause with risk of postmenopausal breast cancer in the Iowa women's health study. Cancer Epidemiol Biomarkers Prev 2005 Mar;14(3):656-661.

(29) World Cancer Research Fund / American Institute for Cancer Research. Food, Nutrition, Physical Activity, and the Prevention of Cancer: a Global Perspective. AICR; Washington DC 2007.

(30) American Institute for Cancer Research. Breast Cancer. 2015; Available at: http://www.aicr.org/learn-more-about-cancer/breast-cancer/. Accessed May/16, 2015.

(31) Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of childhood and adult obesity in the United States, 2011-2012. JAMA 2014 Feb 26;311(8):806-814.

(32) Finkelstein EA, Trogdon JG, Cohen JW, Dietz W. Annual medical spending attributable to obesity: payer-and service-specific estimates. Health Aff (Millwood) 2009 Sep-Oct;28(5):w822-31.

(33) Grundy SM, Brewer HB,Jr, Cleeman JI, Smith SC,Jr, Lenfant C, American Heart Association, et al. Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. Circulation 2004 Jan 27;109(3):433-438.

(34) Fujioka S, Matsuzawa Y, Tokunaga K, Tarui S. Contribution of intra-abdominal fat accumulation to the impairment of glucose and lipid metabolism in human obesity. Metabolism 1987 Jan;36(1):54-59.

(35) Doyle SL, Donohoe CL, Lysaght J, Reynolds JV. Visceral obesity, metabolic syndrome, insulin resistance and cancer. Proc Nutr Soc 2012 Feb;71(1):181-189.

(36) Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature 1994 Dec 1;372(6505):425-432.

(37) Schwartz MW, Woods SC, Porte D,Jr, Seeley RJ, Baskin DG. Central nervous system control of food intake. Nature 2000 Apr 6;404(6778):661-671.

(38) Cottrell EC, Mercer JG. Leptin receptors. Handb Exp Pharmacol 2012;(209)(209):3-21.

(39) Sahu A. Evidence suggesting that galanin (GAL), melanin-concentrating hormone (MCH), neurotensin (NT), proopiomelanocortin (POMC) and neuropeptide Y (NPY) are targets of leptin signaling in the hypothalamus. Endocrinology 1998 Feb;139(2):795-798.

(40) Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N Engl J Med 1996 Feb 1;334(5):292-295.

(41) Munzberg H. Leptin-signaling pathways and leptin resistance. Forum Nutr 2010;63:123-132.

(42) Carroll PA, Healy L, Lysaght J, Boyle T, Reynolds JV, Kennedy MJ, et al. Influence of the metabolic syndrome on leptin and leptin receptor in breast cancer. Mol Carcinog 2011 Aug;50(8):643-651.

(43) Garofalo C, Koda M, Cascio S, Sulkowska M, Kanczuga-Koda L, Golaszewska J, et al. Increased expression of leptin and the leptin receptor as a marker of breast cancer progression: possible role of obesity-related stimuli. Clin Cancer Res 2006 Mar 1;12(5):1447-1453.

(44) Jeong YJ, Bong JG, Park SH, Choi JH, Oh HK. Expression of leptin, leptin receptor, adiponectin, and adiponectin receptor in ductal carcinoma in situ and invasive breast cancer. J Breast Cancer 2011 Jun;14(2):96-103.

(45) Rene Gonzalez R, Watters A, Xu Y, Singh UP, Mann DR, Rueda BR, et al. Leptin-signaling inhibition results in efficient anti-tumor activity in estrogen receptor positive or negative breast cancer. Breast Cancer Res 2009;11(3):R36.

(46) Babaei A, Zarkesh-Esfahani SH, Bahrami E, Ross RJ. Restricted leptin antagonism as a therapeutic approach to treatment of autoimmune diseases. Hormones (Athens) 2011 Jan-Mar;10(1):16-26.

(47) Ryan AS, Berman DM, Nicklas BJ, Sinha M, Gingerich RL, Meneilly GS, et al. Plasma adiponectin and leptin levels, body composition, and glucose utilization in adult women with wide ranges of age and obesity. Diabetes Care 2003 Aug;26(8):2383-2388.

(48) Ng KW. Regulation of glucose metabolism and the skeleton. Clin Endocrinol (Oxf) 2011 Aug;75(2):147-155.

(49) Kadowaki T, Yamauchi T, Kubota N, Hara K, Ueki K. Adiponectin and adiponectin receptors in obesity-linked insulin resistance. Novartis Found Symp 2007;286:164-76; discussion 176-82, 200-3.

(50) Swarbrick MM, Havel PJ. Physiological, pharmacological, and nutritional regulation of circulating adiponectin concentrations in humans. Metab Syndr Relat Disord 2008 Jun;6(2):87-102.

(51) Ryo M, Nakamura T, Kihara S, Kumada M, Shibazaki S, Takahashi M, et al. Adiponectin as a biomarker of the metabolic syndrome. Circ J 2004 Nov;68(11):975-981.

(52) Spranger J, Kroke A, Mohlig M, Bergmann MM, Ristow M, Boeing H, et al. Adiponectin and protection against type 2 diabetes mellitus. Lancet 2003 Jan 18;361(9353):226-228.

(53) Liu Y, Retnakaran R, Hanley A, Tungtrongchitr R, Shaw C, Sweeney G. Total and high molecular weight but not trimeric or hexameric forms of adiponectin correlate with markers of the metabolic syndrome and liver injury in Thai subjects. J Clin Endocrinol Metab 2007 Nov;92(11):4313-4318.

(54) Qi Y, Takahashi N, Hileman SM, Patel HR, Berg AH, Pajvani UB, et al. Adiponectin acts in the brain to decrease body weight. Nat Med 2004 May;10(5):524-529.

(55) Kadowaki T, Yamauchi T, Kubota N. The physiological and pathophysiological role of adiponectin and adiponectin receptors in the peripheral tissues and CNS. FEBS Lett 2008 Jan 9;582(1):74-80.

(56) Rose DP, Komninou D, Stephenson GD. Obesity, adipocytokines, and insulin resistance in breast cancer. Obes Rev 2004 Aug;5(3):153-165.

(57) Barb D, Williams CJ, Neuwirth AK, Mantzoros CS. Adiponectin in relation to malignancies: a review of existing basic research and clinical evidence. Am J Clin Nutr 2007 Sep;86(3):s858-66.

(58) Tworoger SS, Eliassen AH, Kelesidis T, Colditz GA, Willett WC, Mantzoros CS, et al. Plasma adiponectin concentrations and risk of incident breast cancer. J Clin Endocrinol Metab 2007 Apr;92(4):1510-1516.

(59) Cummings DE, Frayo RS, Marmonier C, Aubert R, Chapelot D. Plasma ghrelin levels and hunger scores in humans initiating meals voluntarily without time- and food-related cues. Am J Physiol Endocrinol Metab 2004 Aug;287(2):E297-304.

(60) Kamegai J, Tamura H, Shimizu T, Ishii S, Sugihara H, Wakabayashi I. Chronic central infusion of ghrelin increases hypothalamic neuropeptide Y and Agouti-related protein mRNA levels and body weight in rats. Diabetes 2001 Nov;50(11):2438-2443.

(61) Toshinai K, Date Y, Murakami N, Shimada M, Mondal MS, Shimbara T, et al. Ghrelin-induced food intake is mediated via the orexin pathway. Endocrinology 2003 Apr;144(4):1506-1512.

(62) Dietrich MO, Horvath TL. Feeding signals and brain circuitry. Eur J Neurosci 2009 Nov;30(9):1688-1696.

(63) Schmid DA, Held K, Ising M, Uhr M, Weikel JC, Steiger A. Ghrelin stimulates appetite, imagination of food, GH, ACTH, and cortisol, but does not affect leptin in normal controls. Neuropsychopharmacology 2005 Jun;30(6):1187-1192.

(64) Tassone F, Broglio F, Destefanis S, Rovere S, Benso A, Gottero C, et al. Neuroendocrine and metabolic effects of acute ghrelin administration in human obesity. J Clin Endocrinol Metab 2003 Nov;88(11):5478-5483.

(65) Hansen TK, Dall R, Hosoda H, Kojima M, Kangawa K, Christiansen JS, et al. Weight loss increases circulating levels of ghrelin in human obesity. Clin Endocrinol (Oxf) 2002 Feb;56(2):203-206.

(66) Jeffery PL, Murray RE, Yeh AH, McNamara JF, Duncan RP, Francis GD, et al. Expression and function of the ghrelin axis, including a novel preproghrelin isoform, in human breast cancer tissues and cell lines. Endocr Relat Cancer 2005 Dec;12(4):839-850.

(67) Dossus L, McKay JD, Canzian F, Wilkening S, Rinaldi S, Biessy C, et al. Polymorphisms of genes coding for ghrelin and its receptor in relation to anthropometry, circulating levels of IGF-I and IGFBP-3, and breast cancer risk: a case-control study nested within the European Prospective Investigation into Cancer and Nutrition (EPIC). Carcinogenesis 2008 Jul;29(7):1360-1366.

(68) Feigelson HS, Teras LR, Diver WR, Tang W, Patel AV, Stevens VL, et al. Genetic variation in candidate obesity genes ADRB2, ADRB3, GHRL, HSD11B1, IRS1, IRS2, and SHC1 and risk for breast cancer in the Cancer Prevention Study II. Breast Cancer Res 2008;10(4):R57.

(69) Gahete MD, Cordoba-Chacon J, Hergueta-Redondo M, Martinez-Fuentes AJ, Kineman RD, Moreno-Bueno G, et al. A novel human ghrelin variant (In1-ghrelin) and ghrelin-O-acyltransferase are overexpressed in breast cancer: potential pathophysiological relevance. PLoS One 2011;6(8):e23302.

(70) Woods SC, Lotter EC, McKay LD, Porte D,Jr. Chronic intracerebroventricular infusion of insulin reduces food intake and body weight of baboons. Nature 1979 Nov 29;282(5738):503-505.

(71) Menendez JA, Atrens DM. Insulin and the paraventricular hypothalamus: modulation of energy balance. Brain Res 1991 Aug 2;555(2):193-201.

(72) Bagdade JD, Bierman EL, Porte D,Jr. The significance of basal insulin levels in the evaluation of the insulin response to glucose in diabetic and nondiabetic subjects. J Clin Invest 1967 Oct;46(10):1549-1557.

(73) Stewart AJ, Johnson MD, May FE, Westley BR. Role of insulin-like growth factors and the type I insulin-like growth factor receptor in the estrogen-stimulated proliferation of human breast cancer cells. J Biol Chem 1990 Dec 5;265(34):21172-21178.

(74) Muniyappa R, Lee S, Chen H, Quon MJ. Current approaches for assessing insulin sensitivity and resistance in vivo: advantages, limitations, and appropriate usage. Am J Physiol Endocrinol Metab 2008 Jan;294(1):E15-26.

(75) Duggan C, Irwin ML, Xiao L, Henderson KD, Smith AW, Baumgartner RN, et al. Associations of insulin resistance and adiponectin with mortality in women with breast cancer. J Clin Oncol 2011 Jan 1;29(1):32-39.

(76) Emaus A, Veierod MB, Tretli S, Finstad SE, Selmer R, Furberg AS, et al. Metabolic profile, physical activity, and mortality in breast cancer patients. Breast Cancer Res Treat 2010 Jun;121(3):651-660.

(77) Irwin ML, Duggan C, Wang CY, Smith AW, McTiernan A, Baumgartner RN, et al. Fasting C-peptide levels and death resulting from all causes and breast cancer: the health, eating, activity, and lifestyle study. J Clin Oncol 2011 Jan 1;29(1):47-53.

(78) Pasanisi P, Berrino F, De Petris M, Venturelli E, Mastroianni A, Panico S. Metabolic syndrome as a prognostic factor for breast cancer recurrences. Int J Cancer 2006 Jul 1;119(1):236-238.

(79) Kabat GC, Kim M, Caan BJ, Chlebowski RT, Gunter MJ, Ho GY, et al. Repeated measures of serum glucose and insulin in relation to postmenopausal breast cancer. Int J Cancer 2009 Dec 1;125(11):2704-2710.

(80) Xue F, Michels KB. Diabetes, metabolic syndrome, and breast cancer: a review of the current evidence. Am J Clin Nutr 2007 Sep;86(3):s823-35.

(81) Del Giudice ME, Fantus IG, Ezzat S, McKeown-Eyssen G, Page D, Goodwin PJ. Insulin and related factors in premenopausal breast cancer risk. Breast Cancer Res Treat 1998 Jan;47(2):111-120.

(82) Lawlor DA, Smith GD, Ebrahim S. Hyperinsulinaemia and increased risk of breast cancer: findings from the British Women's Heart and Health Study. Cancer Causes Control 2004 Apr;15(3):267-275.

(83) Jernstrom H, Barrett-Connor E. Obesity, weight change, fasting insulin, proinsulin, C-peptide, and insulin-like growth factor-1 levels in women with and without breast

cancer: the Rancho Bernardo Study. J Womens Health Gend Based Med 1999 Dec;8(10):1265-1272.

(84) Mink PJ, Shahar E, Rosamond WD, Alberg AJ, Folsom AR. Serum insulin and glucose levels and breast cancer incidence: the atherosclerosis risk in communities study. Am J Epidemiol 2002 Aug 15;156(4):349-352.

(85) Muti P, Quattrin T, Grant BJ, Krogh V, Micheli A, Schunemann HJ, et al. Fasting glucose is a risk factor for breast cancer: a prospective study. Cancer Epidemiol Biomarkers Prev 2002 Nov;11(11):1361-1368.

(86) Esposito K, Chiodini P, Capuano A, Bellastella G, Maiorino MI, Rafaniello C, et al. Metabolic syndrome and postmenopausal breast cancer: systematic review and meta-analysis. Menopause 2013 Dec;20(12):1301-1309.

(87) Alissa EM, Alnahdi WA, Alama N, Ferns GA. Relationship between nutritional profile, measures of adiposity, and bone mineral density in postmenopausal Saudi women. J Am Coll Nutr 2014;33(3):206-214.

(88) Watts NB, GLOW investigators. Insights from the Global Longitudinal Study of Osteoporosis in Women (GLOW). Nat Rev Endocrinol 2014 Jul;10(7):412-422.

(89) Ong T, Sahota O, Tan W, Marshall L. A United Kingdom perspective on the relationship between body mass index (BMI) and bone health: a cross sectional analysis of data from the Nottingham Fracture Liaison Service. Bone 2014 Feb;59:207-210.

(90) Zhao LJ, Jiang H, Papasian CJ, Maulik D, Drees B, Hamilton J, et al. Correlation of obesity and osteoporosis: effect of fat mass on the determination of osteoporosis. J Bone Miner Res 2008 Jan;23(1):17-29.

(91) Fu X, Ma X, Lu H, He W, Wang Z, Zhu S. Associations of fat mass and fat distribution with bone mineral density in pre- and postmenopausal Chinese women. Osteoporos Int 2011 Jan;22(1):113-119.

(92) Suzuki N, Yano T, Nakazawa N, Yoshikawa H, Taketani Y. A possible role of estrone produced in adipose tissues in modulating postmenopausal bone density. Maturitas 1995 Jun;22(1):9-12.

(93) Thomas T, Gori F, Khosla S, Jensen MD, Burguera B, Riggs BL. Leptin acts on human marrow stromal cells to enhance differentiation to osteoblasts and to inhibit differentiation to adipocytes. Endocrinology 1999 Apr;140(4):1630-1638.

(94) Key TJ, Appleby PN, Reeves GK, Roddam A, Dorgan JF, Longcope C, et al. Body mass index, serum sex hormones, and breast cancer risk in postmenopausal women. J Natl Cancer Inst 2003 Aug 20;95(16):1218-1226.

(95) Zhao LJ, Liu YJ, Liu PY, Hamilton J, Recker RR, Deng HW. Relationship of obesity with osteoporosis. J Clin Endocrinol Metab 2007 May;92(5):1640-1646.

(96) Ellis KJ. Human body composition: in vivo methods. Physiol Rev 2000 Apr;80(2):649-680.

(97) Rothney MP, Brychta RJ, Schaefer EV, Chen KY, Skarulis MC. Body composition measured by dual-energy X-ray absorptiometry half-body scans in obese adults. Obesity (Silver Spring) 2009 Jun;17(6):1281-1286.

(98) Lustgarten MS, Fielding RA. Assessment of analytical methods used to measure changes in body composition in the elderly and recommendations for their use in phase II clinical trials. J Nutr Health Aging 2011 May;15(5):368-375.

(99) Sang S, Lambert JD, Ho CT, Yang CS. The chemistry and biotransformation of tea constituents. Pharmacol Res 2011 Aug;64(2):87-99.

(100) Inoue-Choi M, Yuan JM, Yang CS, Van Den Berg DJ, Lee MJ, Gao YT, et al. Genetic Association Between the COMT Genotype and Urinary Levels of Tea Polyphenols and Their Metabolites among Daily Green Tea Drinkers. Int J Mol Epidemiol Genet 2010;1(2):114-123.

(101) Balentine DA, Wiseman SA, Bouwens LC. The chemistry of tea flavonoids. Crit Rev Food Sci Nutr 1997 Dec;37(8):693-704.

(102) Wiseman SA, Balentine DA, Frei B. Antioxidants in tea. Crit Rev Food Sci Nutr 1997 Dec;37(8):705-718.

(103) Khan N, Mukhtar H. Tea polyphenols for health promotion. Life Sci 2007 Jul 26;81(7):519-533.

(104) Bigelow RL, Cardelli JA. The green tea catechins, (-)-Epigallocatechin-3-gallate (EGCG) and (-)-Epicatechin-3-gallate (ECG), inhibit HGF/Met signaling in immortalized and tumorigenic breast epithelial cells. Oncogene 2006 Mar 23;25(13):1922-1930.

(105) Chin JM, Merves ML, Goldberger BA, Sampson-Cone A, Cone EJ. Caffeine content of brewed teas. J Anal Toxicol 2008 Oct;32(8):702-704.

(106) Balentine DA, Wiseman SA, Bouwens LC. The chemistry of tea flavonoids. Crit Rev Food Sci Nutr 1997 Dec;37(8):693-704.

(107) Suganuma M, Okabe S, Oniyama M, Tada Y, Ito H, Fujiki H. Wide distribution of [3H](-)-epigallocatechin gallate, a cancer preventive tea polyphenol, in mouse tissue. Carcinogenesis 1998 Oct;19(10):1771-1776.

(108) Swezey RR, Aldridge DE, LeValley SE, Crowell JA, Hara Y, Green CE. Absorption, tissue distribution and elimination of 4-[(3)h]-epigallocatechin gallate in beagle dogs. Int J Toxicol 2003 May-Jun;22(3):187-193.

(109) Clifford MN, van der Hooft JJ, Crozier A. Human studies on the absorption, distribution, metabolism, and excretion of tea polyphenols. Am J Clin Nutr 2013 Dec;98(6 Suppl):1619S-1630S.

(110) Lambert JD, Sang S, Yang CS. Biotransformation of green tea polyphenols and the biological activities of those metabolites. Mol Pharm 2007 Nov-Dec;4(6):819-825.

(111) Chow HH, Cai Y, Alberts DS, Hakim I, Dorr R, Shahi F, et al. Phase I pharmacokinetic study of tea polyphenols following single-dose administration of epigallocatechin gallate and polyphenon E. Cancer Epidemiol Biomarkers Prev 2001 Jan;10(1):53-58.

(112) Kida K, Suzuki M, Matsumoto N, Nanjo F, Hara Y. Identification of biliary metabolites of (-)-epigallocatechin gallate in rats. J Agric Food Chem 2000 Sep;48(9):4151-4155.

(113) Lu H, Meng X, Yang CS. Enzymology of methylation of tea catechins and inhibition of catechol-O-methyltransferase by (-)-epigallocatechin gallate. Drug Metab Dispos 2003 May;31(5):572-579.

(114) Lautala P, Ulmanen I, Taskinen J. Molecular mechanisms controlling the rate and specificity of catechol O-methylation by human soluble catechol O-methyltransferase. Mol Pharmacol 2001 Feb;59(2):393-402.

(115) Zhu BT, Patel UK, Cai MX, Conney AH. O-Methylation of tea polyphenols catalyzed by human placental cytosolic catechol-O-methyltransferase. Drug Metab Dispos 2000 Sep;28(9):1024-1030.

(116) Crespy V, Nancoz N, Oliveira M, Hau J, Courtet-Compondu MC, Williamson G. Glucuronidation of the green tea catechins, (-)-epigallocatechin-3-gallate and (-)-epicatechin-3-gallate, by rat hepatic and intestinal microsomes. Free Radic Res 2004 Sep;38(9):1025-1031.

(117) Dawling S, Roodi N, Mernaugh RL, Wang X, Parl FF. Catechol-O-methyltransferase (COMT)-mediated metabolism of catechol estrogens: comparison of wild-type and variant COMT isoforms. Cancer Res 2001 Sep 15;61(18):6716-6722.

(118) Syvanen AC, Tilgmann C, Rinne J, Ulmanen I. Genetic polymorphism of catechol-O-methyltransferase (COMT): correlation of genotype with individual variation of S-COMT activity and comparison of the allele frequencies in the normal population and parkinsonian patients in Finland. Pharmacogenetics 1997 Feb;7(1):65-71.

(119) Wu AH, Tseng CC, Van Den Berg D, Yu MC. Tea intake, COMT genotype, and breast cancer in Asian-American women. Cancer Res 2003 Nov 1;63(21):7526-7529.

(120) Wen W, Cai Q, Shu XO, Cheng JR, Parl F, Pierce L, et al. Cytochrome P450 1B1 and catechol-O-methyltransferase genetic polymorphisms and breast cancer risk in Chinese women: results from the shanghai breast cancer study and a meta-analysis. Cancer Epidemiol Biomarkers Prev 2005 Feb;14(2):329-335.

(121) Peterson NB, Trentham-Dietz A, Garcia-Closas M, Newcomb PA, Titus-Ernstoff L, Huang Y, et al. Association of COMT haplotypes and breast cancer risk in caucasian women. Anticancer Res 2010 Jan;30(1):217-220.

(122) Goodman JE, Jensen LT, He P, Yager JD. Characterization of human soluble high and low activity catechol-O-methyltransferase catalyzed catechol estrogen methylation. Pharmacogenetics 2002 Oct;12(7):517-528.

(123) Mitrunen K, Jourenkova N, Kataja V, Eskelinen M, Kosma VM, Benhamou S, et al. Polymorphic catechol-O-methyltransferase gene and breast cancer risk. Cancer Epidemiol Biomarkers Prev 2001 Jun;10(6):635-640.

(124) Thompson PA, Shields PG, Freudenheim JL, Stone A, Vena JE, Marshall JR, et al. Genetic polymorphisms in catechol-O-methyltransferase, menopausal status, and breast cancer risk. Cancer Res 1998 May 15;58(10):2107-2110.

(125) Qin X, Peng Q, Qin A, Chen Z, Lin L, Deng Y, et al. Association of COMT Val158Met polymorphism and breast cancer risk: an updated meta-analysis. Diagn Pathol 2012 Oct 8;7:136-1596-7-136.

(126) Karhunen T, Tilgmann C, Ulmanen I, Julkunen I, Panula P. Distribution of catechol-O-methyltransferase enzyme in rat tissues. J Histochem Cytochem 1994 Aug;42(8):1079-1090.

(127) Wang JP, Liu IM, Tzeng TF, Cheng JT. Decrease in catechol-O-methyltransferase activity in the liver of streptozotocin-induced diabetic rats. Clin Exp Pharmacol Physiol 2002 May-Jun;29(5-6):419-422.

(128) Gerozissis K. Brain insulin, energy and glucose homeostasis; genes, environment and metabolic pathologies. Eur J Pharmacol 2008 May 6;585(1):38-49.

(129) Kring SI, Werge T, Holst C, Toubro S, Astrup A, Hansen T, et al. Polymorphisms of serotonin receptor 2A and 2C genes and COMT in relation to obesity and type 2 diabetes. PLoS One 2009 Aug 19;4(8):e6696.

(130) Miller RJ, Jackson KG, Dadd T, Mayes AE, Brown AL, Minihane AM. The impact of the catechol-O-methyltransferase genotype on the acute responsiveness of vascular reactivity to a green tea extract. Br J Nutr 2011 Apr;105(8):1138-1144.

(131) Brown AL, Lane J, Holyoak C, Nicol B, Mayes AE, Dadd T. Health effects of green tea catechins in overweight and obese men: a randomised controlled cross-over trial. Br J Nutr 2011 Dec;106(12):1880-1889.

(132) Rains TM, Agarwal S, Maki KC. Antiobesity effects of green tea catechins: a mechanistic review. J Nutr Biochem 2011 Jan;22(1):1-7.

(133) Dulloo AG, Seydoux J, Girardier L. Potentiation of the thermogenic antiobesity effects of ephedrine by dietary methylxanthines: adenosine antagonism or phosphodiesterase inhibition? Metabolism 1992 Nov;41(11):1233-1241.

(134) Auvichayapat P, Prapochanung M, Tunkamnerdthai O, Sripanidkulchai BO, Auvichayapat N, Thinkhamrop B, et al. Effectiveness of green tea on weight reduction in obese Thais: A randomized, controlled trial. Physiol Behav 2008 Feb 27;93(3):486-491.

(135) Rudelle S, Ferruzzi MG, Cristiani I, Moulin J, Mace K, Acheson KJ, et al. Effect of a thermogenic beverage on 24-hour energy metabolism in humans. Obesity (Silver Spring) 2007 Feb;15(2):349-355.

(136) Belza A, Toubro S, Astrup A. The effect of caffeine, green tea and tyrosine on thermogenesis and energy intake. Eur J Clin Nutr 2009 Jan;63(1):57-64.

(137) Berube-Parent S, Pelletier C, Dore J, Tremblay A. Effects of encapsulated green tea and Guarana extracts containing a mixture of epigallocatechin-3-gallate and caffeine on 24 h energy expenditure and fat oxidation in men. Br J Nutr 2005 Sep;94(3):432-436.

(138) Dulloo AG, Duret C, Rohrer D, Girardier L, Mensi N, Fathi M, et al. Efficacy of a green tea extract rich in catechin polyphenols and caffeine in increasing 24-h energy expenditure and fat oxidation in humans. Am J Clin Nutr 1999 Dec;70(6):1040-1045.

(139) Westerterp-Plantenga MS, Lejeune MP, Kovacs EM. Body weight loss and weight maintenance in relation to habitual caffeine intake and green tea supplementation. Obes Res 2005 Jul;13(7):1195-1204.

(140) Takeshita Mea. Effects of long-term consumption of tea catechins-enriched beverage with no caffeine on body composition in humans. Jpn Pharmacol Ther 2008;36:767-776.

(141) Hill AM, Coates AM, Buckley JD, Ross R, Thielecke F, Howe PR. Can EGCG reduce abdominal fat in obese subjects? J Am Coll Nutr 2007 Aug;26(4):396S-402S.

(142) Tentolouris N, Liatis S, Katsilambros N. Sympathetic system activity in obesity and metabolic syndrome. Ann N Y Acad Sci 2006 Nov;1083:129-152.

(143) Klaus S, Pultz S, Thone-Reineke C, Wolfram S. Epigallocatechin gallate attenuates diet-induced obesity in mice by decreasing energy absorption and increasing fat oxidation. Int J Obes (Lond) 2005 Jun;29(6):615-623.

(144) Murase T, Nagasawa A, Suzuki J, Hase T, Tokimitsu I. Beneficial effects of tea catechins on diet-induced obesity: stimulation of lipid catabolism in the liver. Int J Obes Relat Metab Disord 2002 Nov;26(11):1459-1464.

(145) McClave SA, Lowen CC, Kleber MJ, McConnell JW, Jung LY, Goldsmith LJ. Clinical use of the respiratory quotient obtained from indirect calorimetry. JPEN J Parenter Enteral Nutr 2003 Jan-Feb;27(1):21-26.

(146) Mielgo-Ayuso J, Barrenechea L, Alcorta P, Larrarte E, Margareto J, Labayen I. Effects of dietary supplementation with epigallocatechin-3-gallate on weight loss, energy homeostasis, cardiometabolic risk factors and liver function in obese women: randomised, double-blind, placebo-controlled clinical trial. Br J Nutr 2014 Apr 14;111(7):1263-1271.

(147) Maki KC, Reeves MS, Farmer M, Yasunaga K, Matsuo N, Katsuragi Y, et al. Green tea catechin consumption enhances exercise-induced abdominal fat loss in overweight and obese adults. J Nutr 2009 Feb;139(2):264-270.

(148) Janssens PL, Hursel R, Westerterp-Plantenga MS. Long-term green tea extract supplementation does not affect fat absorption, resting energy expenditure, and body composition in adults. J Nutr 2015 May;145(5):864-870.

(149) Chan PT, Fong WP, Cheung YL, Huang Y, Ho WK, Chen ZY. Jasmine green tea epicatechins are hypolipidemic in hamsters (Mesocricetus auratus) fed a high fat diet. J Nutr 1999 Jun;129(6):1094-1101.

(150) Xu Y, Zhang M, Wu T, Dai S, Xu J, Zhou Z. The anti-obesity effect of green tea polysaccharides, polyphenols and caffeine in rats fed with a high-fat diet. Food Funct 2015 Jan;6(1):297-304.

(151) Zhong L, Furne JK, Levitt MD. An extract of black, green, and mulberry teas causes malabsorption of carbohydrate but not of triacylglycerol in healthy volunteers. Am J Clin Nutr 2006 Sep;84(3):551-555.

(152) Walkowiak J, Bajerska J, Kargulewicz A, Lisowska A, Siedlerski G, Szczapa T, et al. Single dose of green tea extract decreases lipid digestion and absorption from a test meal in humans. Acta Biochim Pol 2013;60(3):481-483.

(153) Kamphuis MM, Mela DJ, Westerterp-Plantenga MS. Diacylglycerols affect substrate oxidation and appetite in humans. Am J Clin Nutr 2003 May;77(5):1133-1139.

(154) Kao YH, Hiipakka RA, Liao S. Modulation of endocrine systems and food intake by green tea epigallocatechin gallate. Endocrinology 2000 Mar;141(3):980-987.

(155) Flint A, Raben A, Blundell JE, Astrup A. Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. Int J Obes Relat Metab Disord 2000 Jan;24(1):38-48.

(156) Hursel R, Viechtbauer W, Westerterp-Plantenga MS. The effects of green tea on weight loss and weight maintenance: a meta-analysis. Int J Obes (Lond) 2009 Sep;33(9):956-961.

(157) Diepvens K, Kovacs EM, Nijs IM, Vogels N, Westerterp-Plantenga MS. Effect of green tea on resting energy expenditure and substrate oxidation during weight loss in overweight females. Br J Nutr 2005 Dec;94(6):1026-1034.

(158) Wang H, Wen Y, Du Y, Yan X, Guo H, Rycroft JA, et al. Effects of catechin enriched green tea on body composition. Obesity (Silver Spring) 2010 Apr;18(4):773-779.

(159) Carter BE, Drewnowski A. Beverages containing soluble fiber, caffeine, and green tea catechins suppress hunger and lead to less energy consumption at the next meal. Appetite 2012 Dec;59(3):755-761.

(160) Josic J, Olsson AT, Wickeberg J, Lindstedt S, Hlebowicz J. Does green tea affect postprandial glucose, insulin and satiety in healthy subjects: a randomized controlled trial. Nutr J 2010 Nov 30;9:63.

(161) Wu CH, Lu FH, Chang CS, Chang TC, Wang RH, Chang CJ. Relationship among habitual tea consumption, percent body fat, and body fat distribution. Obes Res 2003 Sep;11(9):1088-1095.

(162) Hughes LA, Arts IC, Ambergen T, Brants HA, Dagnelie PC, Goldbohm RA, et al. Higher dietary flavone, flavonol, and catechin intakes are associated with less of an increase in BMI over time in women: a longitudinal analysis from the Netherlands Cohort Study. Am J Clin Nutr 2008 Nov;88(5):1341-1352.

(163) Phung OJ, Baker WL, Matthews LJ, Lanosa M, Thorne A, Coleman CI. Effect of green tea catechins with or without caffeine on anthropometric measures: a systematic review and meta-analysis. Am J Clin Nutr 2010 Jan;91(1):73-81.

(164) Jurgens TM, Whelan AM, Killian L, Doucette S, Kirk S, Foy E. Green tea for weight loss and weight maintenance in overweight or obese adults. Cochrane Database Syst Rev 2012 Dec 12;12:CD008650.

(165) Baladia E, Basulto J, Manera M, Martinez R, Calbet D. Effect of green tea or green tea extract consumption on body weight and body composition; systematic review and meta-analysis. Nutr Hosp 2014 Mar 1;29(3):479-490.

(166) Kozuma K, Chikamam A., Hishino E, Kataoka K, Mori K, Hase T, et al. Effect of intake of a beverage containing 540 mg catechins on the body composition of obese women and men. Prog Med 2005;25:185–197.

(167) Tsuchida T, Hakura H, Nakamura H. Reduction of body fat in humans by long-term ingestion of catechins. Progr Med 2002;22:2189-203.

(168) Nagao T, Hase T, Tokimitsu I. A green tea extract high in catechins reduces body fat and cardiovascular risks in humans. Obesity (Silver Spring) 2007 Jun;15(6):1473-1483.

(169) Hase T, Komine Y, Meguro S, Takeda Y, Takahashi H, Matsui Y, et al. Anti-obesity effects of tea catechins in humans. J Oleo Sci 2001;50:599–605.

(170) Hsu CH, Tsai TH, Kao YH, Hwang KC, Tseng TY, Chou P. Effect of green tea extract on obese women: a randomized, double-blind, placebo-controlled clinical trial. Clin Nutr 2008 Jun;27(3):363-370.

(171) Wang M, Wen Y, Du Y, Yan X, Wei GH, Rycroft J, et al. The effects of 90 days consumption of a high-catechin green tea beverage on body weight. Eur J Clin Nutr 2009 (Submitted).

(172) Kovacs EM, Lejeune MP, Nijs I, Westerterp-Plantenga MS. Effects of green tea on weight maintenance after body-weight loss. Br J Nutr 2004 Mar;91(3):431-437.

(173) Palmatier MA, Kang AM, Kidd KK. Global variation in the frequencies of functionally different catechol-O-methyltransferase alleles. Biol Psychiatry 1999 Aug 15;46(4):557-567.

(174) Frank J, George TW, Lodge JK, Rodriguez-Mateos AM, Spencer JP, Minihane AM, et al. Daily consumption of an aqueous green tea extract supplement does not impair liver function or alter cardiovascular disease risk biomarkers in healthy men. J Nutr 2009 Jan;139(1):58-62.

(175) Nagao T, Meguro S, Hase T, Otsuka K, Komikado M, Tokimitsu I, et al. A catechin-rich beverage improves obesity and blood glucose control in patients with type 2 diabetes. Obesity (Silver Spring) 2009 Feb;17(2):310-317.

(176) Matsuyama T, Tanaka Y, Kamimaki I, Nagao T, Tokimitsu I. Catechin safely improved higher levels of fatness, blood pressure, and cholesterol in children. Obesity (Silver Spring) 2008 Jun;16(6):1338-1348.

(177) Nagao T, Hase T, Tokimitsu I. A green tea extract high in catechins reduces body fat and cardiovascular risks in humans. Obesity (Silver Spring) 2007 Jun;15(6):1473-1483.

(178) Nagao T, Komine Y, Soga S, Meguro S, Hase T, Tanaka Y, et al. Ingestion of a tea rich in catechins leads to a reduction in body fat and malondialdehyde-modified LDL in men. Am J Clin Nutr 2005 Jan;81(1):122-129.

(179) Chan CC, Koo MW, Ng EH, Tang OS, Yeung WS, Ho PC. Effects of Chinese green tea on weight, and hormonal and biochemical profiles in obese patients with polycystic ovary syndrome--a randomized placebo-controlled trial. J Soc Gynecol Investig 2006 Jan;13(1):63-68.

(180) Fukino Y, Shimbo M, Aoki N, Okubo T, Iso H. Randomized controlled trial for an effect of green tea consumption on insulin resistance and inflammation markers. J Nutr Sci Vitaminol (Tokyo) 2005 Oct;51(5):335-342.

(181) Maron DJ, Lu GP, Cai NS, Wu ZG, Li YH, Chen H, et al. Cholesterol-lowering effect of a theaflavin-enriched green tea extract: a randomized controlled trial. Arch Intern Med 2003 Jun 23;163(12):1448-1453.

(182) Kajimoto O, Kajimoto Y, Yabune M, Nakamura T, Kotani K, Suzuki Y, et al. Tea catechins with a galloyl moiety reduce body weight and fat. J Health Sci 2005;51(2):161-71.

(183) Kataoka K, Takashima S, Shibata E, Hoshino E. Body fat reduction by the long term intake of catechins and the effects of physical activity. Progress in Medicine 2004;24(12):3358-70.

(184) Suzuki Y, Nozawa A, Miyamoto S, Sagesaka Y, Azuma M, Kajimoto Y, et al. Reduction of visceral fat in overweight female volunteers by long-term ingestion of tea catechins with a galloyl moiety: a randomised double-blind placebo-controlled study. Japanese Pharmacology and Therapeutics 2009;37(6):521-7.

(185) Takase H, Nagao T, Otsuka K, Meguro S, Komikado M, Tokimitsu I. Effects of long-term ingestion of tea catechins on visceral fat accumulation and metabolic syndrome risk in women with abdominal obesity. Japanese Pharmacology and Therapeutics 2008;36(3):237-45.

(186) Takashima S, Kataoka K, Shibata E, Hoshino E. The long term intake of catechins improves lipid catabolism during exercise. Progress in Medicine 2004;24(12):3371-9.

(187) Hursel R, Viechtbauer W, Westerterp-Plantenga MS. The effects of green tea on weight loss and weight maintenance: a meta-analysis. Int J Obes (Lond) 2009 Sep;33(9):956-961.

(188) Chen CH, Ho ML, Chang JK, Hung SH, Wang GJ. Green tea catechin enhances osteogenesis in a bone marrow mesenchymal stem cell line. Osteoporos Int 2005 Dec;16(12):2039-2045.

(189) Nakagawa H, Wachi M, Woo JT, Kato M, Kasai S, Takahashi F, et al. Fenton reaction is primarily involved in a mechanism of (-)-epigallocatechin-3-gallate to induce osteoclastic cell death. Biochem Biophys Res Commun 2002 Mar 22;292(1):94-101.

(190) Chen CH, Kang L, Lin RW, Fu YC, Lin YS, Chang JK, et al. (-)-Epigallocatechin-3-gallate improves bone microarchitecture in ovariectomized rats. Menopause 2013 Jun;20(6):687-694.

(191) Song D, Gan M, Zou J, Zhu X, Shi Q, Zhao H, et al. Effect of (-)-epigallocatechin-3-gallate in preventing bone loss in ovariectomized rats and possible mechanisms. Int J Clin Exp Med 2014 Nov 15;7(11):4183-4190.

(192) Chen Z, Pettinger MB, Ritenbaugh C, LaCroix AZ, Robbins J, Caan BJ, et al. Habitual tea consumption and risk of osteoporosis: a prospective study in the women's health initiative observational cohort. Am J Epidemiol 2003 Oct 15;158(8):772-781.

(193) Hegarty VM, May HM, Khaw KT. Tea drinking and bone mineral density in older women. Am J Clin Nutr 2000 Apr;71(4):1003-1007.

(194) Devine A, Hodgson JM, Dick IM, Prince RL. Tea drinking is associated with benefits on bone density in older women. Am J Clin Nutr 2007 Oct;86(4):1243-1247.

(195) Muraki S, Yamamoto S, Ishibashi H, Oka H, Yoshimura N, Kawaguchi H, et al. Diet and lifestyle associated with increased bone mineral density: cross-sectional study of Japanese elderly women at an osteoporosis outpatient clinic. J Orthop Sci 2007 Jul;12(4):317-320.

(196) Johnell O, Gullberg B, Kanis JA, Allander E, Elffors L, Dequeker J, et al. Risk factors for hip fracture in European women: the MEDOS Study. Mediterranean Osteoporosis Study. J Bone Miner Res 1995 Nov;10(11):1802-1815.

(197) Hamdi Kara I, Aydin S, Gemalmaz A, Akturk Z, Yaman H, Bozdemir N, et al. Habitual tea drinking and bone mineral density in postmenopausal Turkish women: investigation of prevalence of postmenopausal osteoporosis in Turkey (IPPOT Study). Int J Vitam Nutr Res 2007 Nov;77(6):389-397.

(198) Shen CL, Chyu MC, Yeh JK, Zhang Y, Pence BC, Felton CK, et al. Effect of green tea and Tai Chi on bone health in postmenopausal osteopenic women: a 6-month randomized placebo-controlled trial. Osteoporos Int 2012 May;23(5):1541-1552.

(199) Sayama K, Lin S, Zheng G, Oguni I. Effects of green tea on growth, food utilization and lipid metabolism in mice. In Vivo 2000 Jul-Aug;14(4):481-484.

(200) Wolfram S, Raederstorff D, Wang Y, Teixeira SR, Elste V, Weber P. TEAVIGO (epigallocatechin gallate) supplementation prevents obesity in rodents by reducing adipose tissue mass. Ann Nutr Metab 2005 Jan-Feb;49(1):54-63.

(201) Shen CL, Cao JJ, Dagda RY, Chanjaplammootil S, Lu C, Chyu MC, et al. Green tea polyphenols benefits body composition and improves bone quality in long-term high-fat diet-induced obese rats. Nutr Res 2012 Jun;32(6):448-457.

(202) Basu A, Du M, Sanchez K, Leyva MJ, Betts NM, Blevins S, et al. Green tea minimally affects biomarkers of inflammation in obese subjects with metabolic syndrome. Nutrition 2011 Feb;27(2):206-213.

(203) Hursel R, Westerterp-Plantenga MS. Green tea catechin plus caffeine supplementation to a high-protein diet has no additional effect on body weight maintenance after weight loss. Am J Clin Nutr 2009 Mar;89(3):822-830.

(204) Diepvens K, Kovacs EM, Vogels N, Westerterp-Plantenga MS. Metabolic effects of green tea and of phases of weight loss. Physiol Behav 2006 Jan 30;87(1):185-191.

(205) Cho SY, Park PJ, Shin HJ, Kim YK, Shin DW, Shin ES, et al. (-)-Catechin suppresses expression of Kruppel-like factor 7 and increases expression and secretion of adiponectin protein in 3T3-L1 cells. Am J Physiol Endocrinol Metab 2007 Apr;292(4):E1166-72.

(206) Shimada M, Mochizuki K, Sakurai N, Goda T. Dietary supplementation with epigallocatechin gallate elevates levels of circulating adiponectin in non-obese type-2 diabetic Goto-Kakizaki rats. Biosci Biotechnol Biochem 2007 Aug;71(8):2079-2082.

(207) Potenza MA, Marasciulo FL, Tarquinio M, Tiravanti E, Colantuono G, Federici A, et al. EGCG, a green tea polyphenol, improves endothelial function and insulin sensitivity, reduces blood pressure, and protects against myocardial I/R injury in SHR. Am J Physiol Endocrinol Metab 2007 May;292(5):E1378-87.

(208) Imatoh T, Tanihara S, Miyazaki M, Momose Y, Uryu Y, Une H. Coffee consumption but not green tea consumption is associated with adiponectin levels in Japanese males. Eur J Nutr 2011 Jun;50(4):279-284.

(209) Rebello SA, Chen CH, Naidoo N, Xu W, Lee J, Chia KS, et al. Coffee and tea consumption in relation to inflammation and basal glucose metabolism in a multi-ethnic Asian population: a cross-sectional study. Nutr J 2011 Jun 2;10:61-2891-10-61.

(210) Pham NM, Nanri A, Yasuda K, Kurotani K, Kuwahara K, Akter S, et al. Habitual consumption of coffee and green tea in relation to serum adipokines: a cross-sectional study. Eur J Nutr 2015 Mar;54(2):205-214.

(211) Wu AH, Yu MC, Stanczyk FZ, Tseng CC, Pike MC. Anthropometric, dietary, and hormonal correlates of serum adiponectin in Asian American women. Nutr Cancer 2011;63(4):549-557.

(212) Gaudet MM, Falk RT, Gierach GL, Lacey JV,Jr, Graubard BI, Dorgan JF, et al. Do adipokines underlie the association between known risk factors and breast cancer among a cohort of United States women? Cancer Epidemiol 2010 Oct;34(5):580-586.

(213) Cust AE, Stocks T, Lukanova A, Lundin E, Hallmans G, Kaaks R, et al. The influence of overweight and insulin resistance on breast cancer risk and tumour stage at diagnosis: a prospective study. Breast Cancer Res Treat 2009 Feb;113(3):567-576.

(214) Ryu OH, Lee J, Lee KW, Kim HY, Seo JA, Kim SG, et al. Effects of green tea consumption on inflammation, insulin resistance and pulse wave velocity in type 2 diabetes patients. Diabetes Res Clin Pract 2006 Mar;71(3):356-358.

(215) Wu AH, Spicer D, Stanczyk FZ, Tseng CC, Yang CS, Pike MC. Effect of 2-month controlled green tea intervention on lipoprotein cholesterol, glucose, and hormone levels in healthy postmenopausal women. Cancer Prev Res (Phila) 2012 Mar;5(3):393-402.

(216) Liu CY, Huang CJ, Huang LH, Chen IJ, Chiu JP, Hsu CH. Effects of green tea extract on insulin resistance and glucagon-like peptide 1 in patients with type 2 diabetes and lipid abnormalities: a randomized, double-blinded, and placebo-controlled trial. PLoS One 2014 Mar 10;9(3):e91163.

(217) Hsu CH, Liao YL, Lin SC, Tsai TH, Huang CJ, Chou P. Does supplementation with green tea extract improve insulin resistance in obese type 2 diabetics? A randomized, double-blind, and placebo-controlled clinical trial. Altern Med Rev 2011 Jun;16(2):157-163.

(218) Collins QF, Liu HY, Pi J, Liu Z, Quon MJ, Cao W. Epigallocatechin-3-gallate (EGCG), a green tea polyphenol, suppresses hepatic gluconeogenesis through 5'-AMP-activated protein kinase. J Biol Chem 2007 Oct 12;282(41):30143-30149.

(219) Shimizu M, Kobayashi Y, Suzuki M, Satsu H, Miyamoto Y. Regulation of intestinal glucose transport by tea catechins. Biofactors 2000;13(1-4):61-65.

(220) Sakurai N, Mochizuki K, Kameji H, Shimada M, Goda T. (-)-Epigallocatechin gallate enhances the expression of genes related to insulin sensitivity and adipocyte differentiation in 3T3-L1 adipocytes at an early stage of differentiation. Nutrition 2009 Oct;25(10):1047-1056.

(221) Han MK. Epigallocatechin gallate, a constituent of green tea, suppresses cytokine-induced pancreatic beta-cell damage. Exp Mol Med 2003 Apr 30;35(2):136-139.

(222) Iso H, Date C, Wakai K, Fukui M, Tamakoshi A, JACC Study Group. The relationship between green tea and total caffeine intake and risk for self-reported type 2 diabetes among Japanese adults. Ann Intern Med 2006 Apr 18;144(8):554-562.

(223) Maruyama K, Iso H, Sasaki S, Fukino Y. The Association between Concentrations of Green Tea and Blood Glucose Levels. J Clin Biochem Nutr 2009 Jan;44(1):41-45.

(224) Thielecke F, Boschmann M. The potential role of green tea catechins in the prevention of the metabolic syndrome - a review. Phytochemistry 2009 Jan;70(1):11-24.

(225) Sae-tan S, Grove KA, Lambert JD. Weight control and prevention of metabolic syndrome by green tea. Pharmacol Res 2011 Aug;64(2):146-154.

(226) Brown AL, Lane J, Coverly J, Stocks J, Jackson S, Stephen A, et al. Effects of dietary supplementation with the green tea polyphenol epigallocatechin-3-gallate on insulin resistance and associated metabolic risk factors: randomized controlled trial. Br J Nutr 2009 Mar;101(6):886-894.

(227) Venables MC, Hulston CJ, Cox HR, Jeukendrup AE. Green tea extract ingestion, fat oxidation, and glucose tolerance in healthy humans. Am J Clin Nutr 2008 Mar;87(3):778-784.

(228) Stendell-Hollis NR, Thomson CA, Thompson PA, Bea JW, Cussler EC, Hakim IA. Green tea improves metabolic biomarkers, not weight or body composition: a pilot study in overweight breast cancer survivors. J Hum Nutr Diet 2010 Dec;23(6):590-600.

(229) Di Pierro F, Menghi AB, Barreca A, Lucarelli M, Calandrelli A. Greenselect Phytosome as an adjunct to a low-calorie diet for treatment of obesity: a clinical trial. Altern Med Rev 2009 Jun;14(2):154-160.

(230) Bogdanski P, Suliburska J, Szulinska M, Stepien M, Pupek-Musialik D, Jablecka A. Green tea extract reduces blood pressure, inflammatory biomarkers, and oxidative stress and improves parameters associated with insulin resistance in obese, hypertensive patients. Nutr Res 2012 Jun;32(6):421-427.

(231) Vona-Davis L, Howard-McNatt M, Rose DP. Adiposity, type 2 diabetes and the metabolic syndrome in breast cancer. Obes Rev 2007 Sep;8(5):395-408.

(232) Shrubsole MJ, Lu W, Chen Z, Shu XO, Zheng Y, Dai Q, et al. Drinking green tea modestly reduces breast cancer risk. J Nutr 2009 Feb;139(2):310-316.

(233) Zhang M, Holman CD, Huang JP, Xie X. Green tea and the prevention of breast cancer: a case-control study in Southeast China. Carcinogenesis 2007 May;28(5):1074-1078.

(234) Zamora-Ros R, Forouhi NG, Sharp SJ, Gonzalez CA, Buijsse B, Guevara M, et al. Dietary intakes of individual flavanols and flavonols are inversely associated with incident type 2 diabetes in European populations. J Nutr 2014 Mar;144(3):335-343.

(235) Basu A, Sanchez K, Leyva MJ, Wu M, Betts NM, Aston CE, et al. Green tea supplementation affects body weight, lipids, and lipid peroxidation in obese subjects with metabolic syndrome. J Am Coll Nutr 2010 Feb;29(1):31-40.

(236) Wolfram S, Wang Y, Thielecke F. Anti-obesity effects of green tea: from bedside to bench. Mol Nutr Food Res 2006 Feb;50(2):176-187.

(237) Hursel R, Westerterp-Plantenga MS. Catechin- and caffeine-rich teas for control of body weight in humans. Am J Clin Nutr 2013 Dec;98(6 Suppl):1682S-1693S.

(238) Hodgson AB, Randell RK, Jeukendrup AE. The effect of green tea extract on fat oxidation at rest and during exercise: evidence of efficacy and proposed mechanisms. Adv Nutr 2013 Mar 1;4(2):129-140.

(239) Bhagwat S, Haytowitz DB, Holden JM. USDA Database for the Flavonoid Content of Selected Foods. Washington, DC: US Department of Agriculture 2014;3.

(240) Sarma DN, Barrett ML, Chavez ML, Gardiner P, Ko R, Mahady GB, et al. Safety of green tea extracts : a systematic review by the US Pharmacopeia. Drug Saf 2008;31(6):469-484.

(241) Mazzanti G, Menniti-Ippolito F, Moro PA, Cassetti F, Raschetti R, Santuccio C, et al. Hepatotoxicity from green tea: a review of the literature and two unpublished cases. Eur J Clin Pharmacol 2009 Apr;65(4):331-341.

(242) National Cancer Institute. Common Terminology Criteria for Adverse Events v4.0. NCI, NIH, DHHS May 29, 2009;NIH publication # 09-7473.

(243) Wing RR, Lang W, Wadden TA, Safford M, Knowler WC, Bertoni AG, et al. Benefits of modest weight loss in improving cardiovascular risk factors in overweight and obese individuals with type 2 diabetes. Diabetes Care 2011 Jul;34(7):1481-1486.

(244) Narotzki B, Reznick AZ, Navot-Mintzer D, Dagan B, Levy Y. Green tea and vitamin E enhance exercise-induced benefits in body composition, glucose homeostasis, and antioxidant status in elderly men and women. J Am Coll Nutr 2013;32(1):31-40.

(245) Ortsater H, Grankvist N, Wolfram S, Kuehn N, Sjoholm A. Diet supplementation with green tea extract epigallocatechin gallate prevents progression to glucose intolerance in db/db mice. Nutr Metab (Lond) 2012 Feb 14;9:11-7075-9-11.

(246) Anderson RA, Polansky MM. Tea enhances insulin activity. J Agric Food Chem 2002 Nov 20;50(24):7182-7186.

(247) Yang J, Mao QX, Xu HX, Ma X, Zeng CY. Tea consumption and risk of type 2 diabetes mellitus: a systematic review and meta-analysis update. BMJ Open 2014 Jul 22;4(7):e005632-2014-005632.

(248) Shehzad A, Iqbal W, Shehzad O, Lee YS. Adiponectin: regulation of its production and its role in human diseases. Hormones (Athens) 2012 Jan-Mar;11(1):8-20.

(249) Mather KJ, Goldberg RB. Clinical use of adiponectin as a marker of metabolic dysregulation. Best Pract Res Clin Endocrinol Metab 2014 Jan;28(1):107-117.

(250) Wannamethee SG, Tchernova J, Whincup P, Lowe GD, Rumley A, Brown K, et al. Associations of adiponectin with metabolic and vascular risk parameters in the British Regional Heart Study reveal stronger links to insulin resistance-related than to coronory heart disease risk-related parameters. Int J Obes (Lond) 2007 Jul;31(7):1089-1098.

(251) Gao H, Fall T, van Dam RM, Flyvbjerg A, Zethelius B, Ingelsson E, et al. Evidence of a causal relationship between adiponectin levels and insulin sensitivity: a Mendelian randomization study. Diabetes 2013 Apr;62(4):1338-1344.

(252) Barnes PM, Bloom B, Nahin RL. Complementary and alternative medicine use among adults and children: United States, 2007. Natl Health Stat Report 2008 Dec 10;(12)(12):1-23.

(253) Liu K, Zhou R, Wang B, Chen K, Shi LY, Zhu JD, et al. Effect of green tea on glucose control and insulin sensitivity: a meta-analysis of 17 randomized controlled trials. Am J Clin Nutr 2013 Aug;98(2):340-348.

(254) Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 2014 Aug 30;384(9945):766-781.

(255) Metz JA, Stern JS, Kris-Etherton P, Reusser ME, Morris CD, Hatton DC, et al. A randomized trial of improved weight loss with a prepared meal plan in overweight and

obese patients: impact on cardiovascular risk reduction. Arch Intern Med 2000 Jul 24;160(14):2150-2158.

(256) Hamman RF, Wing RR, Edelstein SL, Lachin JM, Bray GA, Delahanty L, et al. Effect of weight loss with lifestyle intervention on risk of diabetes. Diabetes Care 2006 Sep;29(9):2102-2107.

(257) Westerterp-Plantenga MS. Green tea catechins, caffeine and body-weight regulation. Physiol Behav 2010 Apr 26;100(1):42-46.

(258) Alvehus M, Buren J, Sjostrom M, Goedecke J, Olsson T. The human visceral fat depot has a unique inflammatory profile. Obesity (Silver Spring) 2010 May;18(5):879-883.

(259) Dulloo AG, Seydoux J, Girardier L, Chantre P, Vandermander J. Green tea and thermogenesis: interactions between catechin-polyphenols, caffeine and sympathetic activity. Int J Obes Relat Metab Disord 2000 Feb;24(2):252-258.

(260) Kanis JA. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis: synopsis of a WHO report. WHO Study Group. Osteoporos Int 1994 Nov;4(6):368-381.

(261) Kriska AM. Modifiable Activity Questionnaire. Med Sci Sports Med 2002(29):S73-S78.

(262) Kokubo Y, Iso H, Saito I, Yamagishi K, Yatsuya H, Ishihara J, et al. The impact of green tea and coffee consumption on the reduced risk of stroke incidence in Japanese population: the Japan public health center-based study cohort. Stroke 2013 May;44(5):1369-1374.

(263) Yuan JM. Cancer prevention by green tea: evidence from epidemiologic studies. Am J Clin Nutr 2013 Dec;98(6 Suppl):1676S-1681S.

(264) Folsom AR, Kushi LH, Anderson KE, Mink PJ, Olson JE, Hong CP, et al. Associations of general and abdominal obesity with multiple health outcomes in older women: the Iowa Women's Health Study. Arch Intern Med 2000 Jul 24;160(14):2117-2128.

(265) Chen D, Wang CY, Lambert JD, Ai N, Welsh WJ, Yang CS. Inhibition of human liver catechol-O-methyltransferase by tea catechins and their metabolites: structure-activity relationship and molecular-modeling studies. Biochem Pharmacol 2005 May 15;69(10):1523-1531.

(266) Hodgson AB, Randell RK, Boon N, Garczarek U, Mela DJ, Jeukendrup AE, et al. Metabolic response to green tea extract during rest and moderate-intensity exercise. J Nutr Biochem 2013 Jan;24(1):325-334.

(267) Lorenz M, Paul F, Moobed M, Baumann G, Zimmermann BF, Stangl K, et al. The activity of catechol-O-methyltransferase (COMT) is not impaired by high doses of epigallocatechin-3-gallate (EGCG) in vivo. Eur J Pharmacol 2014 Oct 5;740:645-651.

(268) Suzuki T, Yoshida H, Hashimoto T, Yoshimura N, Fujiwara S, Fukunaga M, et al. Case-control study of risk factors for hip fractures in the Japanese elderly by a Mediterranean Osteoporosis Study (MEDOS) questionnaire. Bone 1997 Nov;21(5):461-467.

(269) Miksicek RJ. Commonly occurring plant flavonoids have estrogenic activity. Mol Pharmacol 1993 Jul;44(1):37-43.

(270) Williams GA, Wang Y, Callon KE, Watson M, Lin JM, Lam JB, et al. In vitro and in vivo effects of adiponectin on bone. Endocrinology 2009 Aug;150(8):3603-3610.

(271) Oshima K, Nampei A, Matsuda M, Iwaki M, Fukuhara A, Hashimoto J, et al. Adiponectin increases bone mass by suppressing osteoclast and activating osteoblast. Biochem Biophys Res Commun 2005 Jun 3;331(2):520-526.

(272) Mohiti-Ardekani J, Soleymani-Salehabadi H, Owlia MB, Mohiti A. Relationships between serum adipocyte hormones (adiponectin, leptin, resistin), bone mineral density and bone metabolic markers in osteoporosis patients. J Bone Miner Metab 2014 Jul;32(4):400-404.

(273) Morcov C, Vulpoi C, Branisteanu D. Correlation between adiponectin, leptin, insulin growth factor-1 and bone mineral density in pre and postmenopausal women. Rev Med Chir Soc Med Nat Iasi 2012 Jul-Sep;116(3):785-789.

(274) Astrup A, Toubro S, Cannon S, Hein P, Breum L, Madsen J. Caffeine: a double-blind, placebo-controlled study of its thermogenic, metabolic, and cardiovascular effects in healthy volunteers. Am J Clin Nutr 1990 May;51(5):759-767.

(275) World Health Organization. Obesity and overweight fact sheet.
 . January 2015.

(276) Pan SY, Johnson KC, Ugnat AM, Wen SW, Mao Y, Canadian Cancer Registries Epidemiology Research Group. Association of obesity and cancer risk in Canada. Am J Epidemiol 2004 Feb 1;159(3):259-268.

(277) Pichard C, Plu-Bureau G, Neves-E Castro M, Gompel A. Insulin resistance, obesity and breast cancer risk. Maturitas 2008 May 20;60(1):19-30.

(278) La Vecchia C, Giordano SH, Hortobagyi GN, Chabner B. Overweight, obesity, diabetes, and risk of breast cancer: interlocking pieces of the puzzle. Oncologist 2011;16(6):726-729.

(279) Yang X, Yin L, Li T, Chen Z. Green tea extracts reduce adipogenesis by decreasing expression of transcription factors C/EBPalpha and PPARgamma. Int J Clin Exp Med 2014 Dec 15;7(12):4906-4914.

(280) Schloegl H, Percik R, Horstmann A, Villringer A, Stumvoll M. Peptide hormones regulating appetite--focus on neuroimaging studies in humans. Diabetes Metab Res Rev 2011 Feb;27(2):104-112.

(281) Kovacs EM, Mela DJ. Metabolically active functional food ingredients for weight control. Obes Rev 2006 Feb;7(1):59-78.

(282) Hill AJ, Rogers PJ, Blundell JE. Techniques for the experimental measurement of human eating behaviour and food intake: a practical guide. Int J Obes Relat Metab Disord 1995 Jun;19(6):361-375.

(283) Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, et al. A role for ghrelin in the central regulation of feeding. Nature 2001 Jan 11;409(6817):194-198.

(284) Ariyasu H, Takaya K, Tagami T, Ogawa Y, Hosoda K, Akamizu T, et al. Stomach is a major source of circulating ghrelin, and feeding state determines plasma ghrelin-like immunoreactivity levels in humans. J Clin Endocrinol Metab 2001 Oct;86(10):4753-4758.

(285) Romon M, Lebel P, Fruchart JC, Dallongeville J. Postprandial leptin response to carbohydrate and fat meals in obese women. J Am Coll Nutr 2003 Jun;22(3):247-251.

(286) Poretsky L, Lesser M, Brillon D. Lack of postprandial leptin peaks in patients with type 2 diabetes mellitus. Diabetes Obes Metab 2001 Apr;3(2):105-111.

(287) Gregersen NT, Bitz C, Krog-Mikkelsen I, Hels O, Kovacs EM, Rycroft JA, et al. Effect of moderate intakes of different tea catechins and caffeine on acute measures of energy metabolism under sedentary conditions. Br J Nutr 2009 Oct;102(8):1187-1194.

Appendix 1. MGTT Recruitment Materials and Health History Questionnaire

I. MGTT Recruitment Letter

Dear Ms. _____,

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.

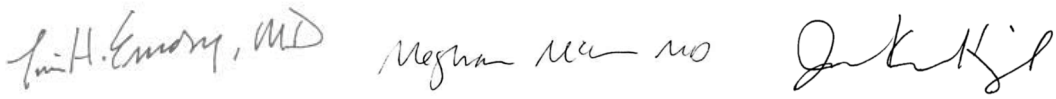
We're looking for healthy postmenopausal women whose normal mammograms show that they have relatively dense breasts. Your recent normal mammogram showed that you have relatively dense breasts, making you eligible to participate in medical research at the University of Minnesota. Reducing breast density 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

II. Telephone Screening Questionnaire

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**

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 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 had ovarian cancer? _____

9. Have you been diagnosed with any other form of cancer in the last 5 years? _____

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 yes, what are they?

    _____
    _____
    _____

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

    _____
    _____

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

- Diabetes? _____
- Hyperthyroidism? _____
- Uncontrolled high blood pressure (hypertension)? _____

- Crohn's or Ulcerative Colitis

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

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

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

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

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

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

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

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

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

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

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

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

~OR~

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

_____

_____

Thank you again, and have a great day.

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

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

Would you like to hear more information? _____

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

Would you like to hear more information? _____

I will give you a brief description of the study. If you would like to participate, we can schedule an orientation session to explain the study in more detail and answer all the questions you may have. As part of a clinical trial, you will be randomly assigned to

consume either a green tea extract or placebo capsule twice a day for one year. During one year, biological markers from your blood and urine associated with breast cancer risk will be evaluated throughout fourteen clinic visits at the Human Nutrition Research Clinic (HNRC) of the University of Minnesota. Each clinic visit can take 0.5-1.5 hours. During each clinic visit, your weight, blood pressure, heart rate, and respiratory rate will be measured. Your waist and hip circumferences will also be measured at the second and last (fourteenth) clinic visits. Each clinic visit involves drawing a little more than 1 teaspoon to 4 tablespoons blood depending on the clinic visit. For 3 visits, at the month 0, month 6, and month 12, you will need to have been fasting since 10 pm the night before your clinic visit. You will collect your entire 24 hour urine at the baseline, month 6, and 12 and will bring collected urine in provided jugs to the HRNC for the clinic visits 2, 8, and 14.You will also complete a food frequency questionnaire, which is a survey about your eating habits that takes approximately 60 minutes. Your first routine mammogram will be compared with the mammogram that you are normally scheduled for during the following year, at the end of study. Finally, you will be asked to avoid drinking green tea during the study period so we will be able to assess the exact effect of consumed green tea extract.

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

Circle one of each of the following:

Status: **Preferred contact:**

Orientation session:

ELIGIBLE **EMAIL**

_____/_____/_____

INELIGIBLE **PHONE**

_____

III. MGTT 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 6^{th} and 12^{th} month, collect all urine for 24 hours in jugs that will be provided.
3. Go to the University of Minnesota Medical Center (UMMC)/Fairview Breast Center, Fairview Southdale Breast Center, or Fairview Maple Grove Breast Center for your routine annual mammogram at the end of the study.
4. Allow a portion of the blood drawn at the first clinical blood draw (about 1 tablespoon) to be used for DNA analysis. DNA will be isolated from your blood sample and stored. We will then analyze the gene variations, which will allow us to determine if these gene variations influence your response to green tea extract consumption.
5. Keep your body weight stable during the study, and do not participate in any weight loss or weight gain studies or programs.
6. Consume four capsules per day for one year, containing either green tea extract or placebo, as decided by the researchers on a full stomach only, two in the morning and two in the afternoon.
7. Refrain from drinking more than one cup of green tea per week while participating in the study.
8. Refrain from drinking more than 7 alcoholic beverages per week while participating in the study.

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

- Body weight
- Height

- Waist and hip circumferences
- Blood pressure, heart rate, respiratory rate and body temperature
- Completing a Food Frequency 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 glucose over prolonged periods of time), C-peptide (a factor useful in assessing insulin function and secretion), HDL-Cholesterol, LDL-C, Total-C, TG (lipid factors), oxidized LDL-C (a risk factor for heart disease), hsCRP, IL-1β, IL-6, IL-8, TNF-α (proteins in blood involved in immune system regulation), prolactin (a hormone that affects growth of the mammary glands), adiponectin (a protein that regulates glucose and lipids metabolism), osteocalcin, pyridinolines, osteoprotegerin, CTX and NTX (biomarkers for bone metabolism), ghrelin and leptin (hormones involved in appetite and weight regulation), catechins (green tea bioactive compounds), HBsAg, anti-HBc, anti-HCV (markers for hepatitis B and C), assessing DNA repair capacity and specific changes in the following genes that are related to metabolism of the green tea bioactive compounds: COMT, GSTM1, GSTT1, UGT, SULT, IGF-1, IGFBP-3, PIK3CB and HSD3B1
- Urine collection (two spot urines at the clinic in 10% of the subjects and three 24-hour complete collections for all subjects) for measurement of creatinine (a muscle metabolite), estrogens and catechins
- Mammogram to evaluate the changes in your breast density from baseline visit to the end of the study. Breast density changes measured by the mammograms will be calculated by aid of a computer program.

Detailed Description of Clinic Visits:

Clinic visit 1

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

Clinic visit 2

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

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

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

Clinic visit 5
This clinic visit takes place approximately one month after clinic visit 4. A study staff member will weigh you and measure your blood pressure, body temperature and heart rate while you are resting. The trained medical professional will draw 5mL of blood (about one teaspoon), and you will provide a urine sample. You will be asked to bring your empty or partially empty bottles of your capsules and study log to this visit. At the end of this visit, you will be given your next 3-month supply of capsules and materials to complete your 24 hour urine collection for clinic visit 8 in several months. This visit will take approximately 30 minutes.

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

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

Clinic visit 8
This visit takes place at the HNRC approximately one month after your clinic visit 7. You

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

Clinic visit 9
This visit takes place at the HNRC approximately three months after your clinic visit 8. This visit repeats the tests and measurements taken in clinic visit 5. A study staff member will weigh you, measure your blood pressure, body temperature and heart rate while you are resting. A trained medical professional will draw 5mL of blood (about one teaspoon). At this visit, you will also provide a urine sample. You will be asked to bring your empty or partially empty bottles of your capsules and study log. At the end of this visit, you will be given your last 3 month supply of capsules and materials to complete your 24 hour urine collection for clinic visit 10 in several months. This visit will take approximately 30 minutes.

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

Specific procedures to be performed:

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

Menopause-Specific Quality of Life Questionnaire
At clinic visits 2, 8, and 10 you will answer questions regarding your experience of certain physical, psychosocial, and sexual symptoms over the previous week. These questions are designed to assess your quality of life in association with your menopausal

experience. The required time to complete this questionnaire will be less than 15 minutes.

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

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

Risks of Being in the Study:
Participating in this study has the following risks:

First, liver toxicity has been seen in a few subjects who used green tea extract as a weight reduction aid. The risk of toxicity from taking manufactured green tea extracts has been estimated to be about 1 case out of 83,812 treatments, although no toxicity has been reported in any clinical trials performed to date. To be cautious, we will measure liver enzymes 9 times throughout our study for possible toxicity and tolerance at each visit at the HNRC or a Fairview clinic. If your liver enzymes are elevated, you will be informed and released from the study.

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

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

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

Lastly, screening mammography is the best way to detect early breast cancers. You are currently getting your mammograms approximately every 12 months. If you participate in this study, your mammogram may be delayed by at most 3-4 months. Some experts (U.S. Preventive Services Task Force, 2009) have suggested that this type of delay has little effect on the benefits of mammography. If you wish to have your regular

mammogram on a yearly basis, we will ask you to have a second limited view research mammogram after you have been on the study for 1 year. The additional limited view mammogram would be at no cost to you.

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

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

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

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

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

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

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

Confidentiality:
The information provided by you and the information taken from the measurements of your body will be held strictly confidential and used for the purposes of research only. The HNRC, whose staff has completed the federally required training with regard to confidentiality of health information in research, will maintain medical records with your name on them for the purposes of scheduling and billing procedures only. Any/all medical information gathered, test results, lab samples will NOT have your name on them. Instead, they will be labeled with a study ID number only.

Laboratory results and other test results will not be included in the medical record. Your name will be associated with your study ID number on one list, to be kept in a locked file cabinet. Your study ID number will appear on all other study records. Representatives of the University of Minnesota or the National Institutes of Health may be given access to your records to assure that the study is conducted properly.

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

With regard to your blood and urine samples:

- We will send samples of your blood with only a code number on it to the University of Southern California (USC) to analyze it for biomarkers of breast cancer called IGF-1, binding proteins for IGF-1, as well as reproductive hormones. USC will be paid to do these tests .We will NOT tell USC researchers your name or give them any identifying information about you. Any excess blood will be destroyed when researchers have completed these tests.

- We will send samples of your blood and urine with a code number on it to Rutgers University to analyze it for plasma and urine catechin levels. Rutgers University will be paid to do these tests. We will NOT tell Rutgers University researchers your name or give them any identifying information about you. Any excess blood and urine will be destroyed when researchers have completed with these tests.

- We will store any remaining blood and urine in a freezer in the Food Science and Nutrition Building at the University of Minnesota (St. Paul campus). The vials will have your study ID on them and the date on which the blood was drawn. Your name will NOT be stored with your blood. We will store these vials for up to 5 years after the entire study is over. The freezer in which they are stored is kept behind a locked door. The only people who have access to this freezer are paid research staff members who have completed the federally required training with regard to confidentiality of health information in research. The purpose for storing these samples is to enable us to conduct additional tests regarding green tea health effects. The principal investigator will maintain ownership of these samples while they are stored. Samples will be destroyed within five years after the completion of the study. You will not receive any results from future tests conducted with these stored samples.

- USC and Rutgers University labs do NOT have access to your name. There is one confidential list and file that links your ID to your name. These files will be kept in the locked file cabinet as described above. The only people who will have

access to this list are the principal investigator (Dr. Kurzer) and her research staff, who have completed the federally required training with regard to confidentiality of health information in research.

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

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

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

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

Contacts and Questions:
The primary researcher conducting this study is Mindy Kurzer, Ph.D. If you have questions, you may contact her at the Department of Food Science and Nutrition, University of Minnesota, 1334 Eckles Ave., St. Paul, MN 55108. Phone: (612) 624-9789; email: mkurzer@umn.edu. You may also contact the study coordinators in the Department of Food Science and Nutrition, University of Minnesota, 1334 Eckles Ave., St. Paul, MN 55108. Phone: (612) 624-3412; email: greentea@umn.edu.

If you have any questions or concerns regarding the study and would like to talk to someone other than the researcher(s), you are encouraged to contact the Fairview Research Helpline at telephone number (612)672-7692 or toll-free at (866) 508-6961. You may also contact this office in writing or in person at University of Minnesota Medical Center/Fairview Riverside Campus, 2200 Riverside Avenue, Minneapolis, MN

55454.

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

_____
Signature of Participant Date

_____
Name of Participant (printed)

_____
Street Address City State Zip code

_____
Signature of Person Obtaining Consent Date
You will be given a copy of this form for your records.

IV. 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)

4.

   A) How many days per week do you exercise?
   _____

   B) How intense is your exercise:

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

5. 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)	

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

   A) If yes, please describe _____
   _____

7. Are you a regular blood donor? YES (1) NO (2)

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

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

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

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

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

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

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

12. 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 first- , or second-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)	Y / N	Y / N	Y / N

(11)			
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.

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?
YES (1) NO (2)
(homeopathy, acupuncture, meditation, massage, etc.)

A) If yes, please describe.

_____

_____

OB/GYN History

29. At approximately what age did you first get your period? _____

30. At approximately what age 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) What age 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 an estimated age. _____

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 how long (years)?_____

B) At approximately what age did you begin? _____

C) At approximately what age 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. What year was your first child born? _____

40. What year 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, how many months 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:

_____
_____

Appendix 2. Supplementary Materials for Body Composition Study (Chapter 4)

I. Consent Form

CONSENT FORM

Study Title: Green Tea Consumption and Reduction of Body Fat

Your Body Mass Index (BMI) of ≥ 25 kg/m^2 has qualified you to participate in a sub-study of the Green Tea and Breast Cancer Risk Reduction Study. The purpose of this study is to determine the effect of green tea consumption on body fat, as a percentage of total body weight and total body fat mass.

Procedures:

We anticipate that we will screen up to 300 women to find the required 130 participants to complete this portion of the study. Each woman will undergo two dual-energy X-ray absorptiometry (DXA) scans. A DXA scan is a painless, non-invasive procedure that is used to evaluate bone density and can also be used to determine total body composition and fat content with a high degree of accuracy. The first DXA scan will be completed at the beginning of your participation in the study, to determine your baseline level of body fat. The second scan will be completed at the end of your 12 months of participation in conjunction with your final clinic visit. Completing scans at baseline and at the end of the study will allow us to determine if there were any changes to body fat mass and/or body fat percentage during the time that participants were consuming the green tea or placebo supplement.

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

1. Go to the Delaware Clinical Research Unit (DCRU), located on the East Bank of the University of Minnesota in Minneapolis, MN, twice during a 12-month time period to complete DXA scans.
2. Comply with all guidelines detailed in the original consent form for the Green Tea Study, including not actively attempting to gain or lose weight during the study and not participating in any weight loss or weight gain studies or programs.

Specific Procedures to be Performed:

- On the day of the exam you may eat normally. You should wear loose, comfortable clothing, and you will be asked to remove some or all of your clothes and wear a gown during the exam. You may also be asked to remove jewelry, removable dental appliances, eyeglasses, and any metal objects or clothing that might interfere with the x-ray images.
- Inform DCRU personnel if you have recently had a barium examination or have been injected with a contrast material for a computed tomography (CT) scan or radioisotope scan. You may have to wait 30 days before undergoing a DXA test.

- You must hold very still and may be asked to keep from breathing for a few seconds while the x-ray picture is taken to reduce the possibility of a blurred image. The DXA scan is usually completed within 10 minutes.
- Plan to be at the DCRU for a total of 30 minutes.

Risks of Being in the Study:

DXA scans involve exposure to minimal amounts of ionizing radiation. The total of 2 scans over the duration of the one-year study results in a cumulative ionizing radiation exposure (0.20 mrems) that is far less than that of one chest x-ray (40 mrems), and which is well within acceptable limits and poses minimal risk to subjects.

Benefits of Being in the Study

You will receive a written report detailing the results of your baseline and end-of-study DXA scans at the end of your participation. There may be no change in body fat as a result of participation in this study.

Costs

No charges will be made for visits to the DCRU while you are a participant in this study.

Compensation

You will receive financial compensation of up to $50 for study participation: $20 for completing the first DXA scan and $30 for completing the end-of-study DXA.

Care in the Case of Injury

In the event that this research activity 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 ordinary manner to you or your insurance company. If you think that you have suffered a research related injury, let the study physicians know right away.

Dr. Carolyn Torkelson, MD: (612) 625-8718

Dr. Mindy Kurzer: (612) 624-9789 Study Coordinators: (612) 624-3412

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 DCRU, 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 and test results will NOT have your name on them. Instead, they will be labeled with a study ID number only.

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.

Your study records will be kept private, in locked storage according to HIPAA standards. None of your information will 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.

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.

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. The procedure to withdraw is to call 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.

You may also contact the Fairview Research Helpline Office:

Phone: (612)672-7690, or E-mail: research@fairview.org

Fairview Research Administration, 2433 Energy Park Drive, St. Paul, MN 55108.

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.

II. Physical Activity Questionnaire (Body Composition Study, Chapter 4)

STUDY ID: _____ DATE: _____

Modifiable Activity Questionnaire

1. Please circle all activities listed below that you have done more than 10 times in the past year.

01 Jogging (outdoor, treadmill)	15 Football/Soccer	28 Stair Master
02 Swimming (laps, snorkeling)	16 Racquetball/Handball/Squash	29 Fencing
03 Bicycling (indoor, outdoor)	17 Horseback riding	30 Hiking
04 Softball/Baseball	18 Hunting	31 Tennis
05 Volley Ball	19 Fishing	32 Golf
06 Bowling	20 Aerobic Dance/Step Aerobic	33 Canoeing
07 Basketball	21 Water Aerobics	34 Water skiing
08 Skating	22 Dancing (Square, Line, Ballroom)	35 Jumping Rope
09 Martial Arts (karate, judo)	23 Gardening or Yard work	36 Snow skiing (X-country,Nordic Trk)
10 Tai chi	24 Badminton	37 Snow skiing (Downhill)
11 Calisthenics/Toning exercises	25 Strength/Weight training	38 Snow shoeing
12 Wood Chopping	26 Rock Climbing	39 Yoga
13 Water/coal hauling	27 Scuba Diving	40 Other

14 Walking for exercise (outdoor, indoor at mall or fitness center, treadmill)

List each activity that you circled in the "Activity" box below, check the months you did each activity over the past year (12 months) and then estimate the average amount of time spent in that activity.

Activity	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	Average # of Times Per Month	Average # of Minutes Each Time

2. In general, how many HOURS per DAY do you usually spend watching television or on a computer doing *non-work related* activity? _____ hours

3. In general, how many HOURS per NIGHT do you usually sleep? _____ hours

Appendix 3. Supplementary Materials for Postprandial Feeding Study (Chapter 5)

I. Consent Form

CONSENT FORM

Study Title: Green Tea Consumption and Postprandial Response of Energy-Related Hormones Sub-Study

Your Body Mass Index (BMI) of ≥ 25 kg/m^2 and catechol-O-methyltransferase (*COMT*) genotype has qualified you to participate in a sub-study of the Green Tea and Breast Cancer Risk Reduction Study. The purpose of this sub-study is to determine the effect of green tea consumption on energy balance through assessment of hormones related to satiety and energy balance after consumption of a standardized meal. Participation in this sub-study will allow us to better understand the mechanisms by which green tea consumption may alter energy balance and appetite-related hormones, which in turn may lower the risk of breast cancer as well as other obesity-related chronic diseases. Dr. Mindy Kurzer at the University of Minnesota is conducting this study. Participation in this sub-study is optional and will not affect your participation in the main study.

Procedures:

We anticipate that we will screen up to 300 women to find the required 150 participants to complete this portion of the study. Participants in this sub-study will first complete food diaries and satiety questionnaires. You will have a catheter placed by a certified phlebotomist or Registered Nurse in order to obtain blood samples before consumption of a standardized meal. Blood samples will be obtained immediately after consumption of the standardized meal and every 30 minutes for four hours for a total of 10 blood samples. This half-day clinic visit will occur in the last two months of the Green Tea Study (during months 11 or 12).

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

3. Go to the Delaware Clinical Research Unit (DCRU), located at: 717 Delaware Street on the East Bank of the University of Minnesota in Minneapolis, MN, or the Human Nutrition Research Clinic (HNRC), located at 1134 Eckles Avenue on the St. Paul Campus of the University of Minnesota in St. Paul, MN once during month 11 or 12 of the Green Tea Study.
4. Comply with all guidelines detailed in the original consent form for the Green Tea Study, including not actively attempting to gain or lose weight during the study and not participating in any weight loss or weight gain studies or programs.

Specific Procedures to be Performed:

- During the week prior to the half-day visit, participants will record food intake on two assigned weekdays and one weekend day prior to the visit. Diet records will be reviewed during the clinic visit and later analyzed for nutrient content.

- Participants will be instructed to adhere to their normal energy intake and to refrain from exercise and alcohol the day before the test day.
- Participants will arrive at the DCRU or HNRC between 7:00 AM and 8:00 AM in a fasted state (no food or beverages, other than water, for 12 hours prior to arriving at the clinic).
- Baseline satiety questionnaires will be completed, a certified phlebotomist or Registered Nurse will place a catheter in the antecubital region of the arm, and fasting blood will be drawn for assessment of energy-related hormones.
- Participants will consume a standardized breakfast consisting of a bagel with cream cheese, orange slices or orange juice, and 2% milk or low-fat yogurt. Participants will be required to eat all of the breakfast.
- Blood will be drawn immediately after consumption of the breakfast and every 30 minutes over a period of 4 hours (for a total of 10 blood samples) for evaluation of change in the outcomes of interest.
- Participants will complete additional satiety questionnaires approximately 2 and 4 hours after eating the standardized breakfast.

Risks of Being in the Study:

Food diary collections involve inconvenience, but no risk. There is a small risk of infection and bruising at the site where the catheter is placed. The risk is minimal as all needles and equipment are sterilized and trained phlebotomists, registered nurses and certified medical assistants perform the procedures. You may also feel some pain, dizziness, and faintness lasting a few seconds upon insertion of the catheter used to draw blood.

Benefits of Being in the Study

There may be no direct reduction of breast cancer risk or body weight as a result of participation in this study.

Costs

No charges will be made for the visit to the DCRU while you are a participant in this study.

Compensation

You will receive financial compensation of $100 for study participation.

Care in the Case of Injury

In the event that this research activity 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 ordinary manner to you or your insurance company. If you think that you have suffered a research related injury, let the study physicians know right away.

Dr. Carolyn Torkelson, MD: (612) 625-8718
Dr. Mindy Kurzer: (612) 624-9789
Study Coordinators: (612) 624-3412

Confidentiality

The information provided by you and the information taken from analyses of your blood will be held strictly confidential and used for the purposes of research only. The DCRU staff has completed the federally required training with regard to confidentiality of health information in research. Any/all medical information gathered and test results will NOT have your name on them. Instead, they will be labeled with a study ID number only.

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.

Your study records will be kept private, in locked storage according to HIPAA standards. None of your information will 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.

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.

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. The procedure to withdraw is to call 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.

You may also contact the Fairview Research Helpline Office:

Phone: (612)672-7690, or E-mail: research@fairview.org

Fairview Research Administration, 2433 Energy Park Drive, St. Paul, MN 55108.

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.

II. Three-day Diet Record Instructions

INSTRUCTIONS TO RECORD DIETARY INTAKE

Use the forms provided to record all food and beverages (including water) consumed on two weekdays and one weekend day during the week prior to your half-day visit. We recommend filling it out each time you eat. Don't trust your memory.

Instructions:

1. **Record the time and type of meal** (e.g. breakfast, lunch, afternoon snack) when the food or beverage is consumed.
2. **Record every item consumed.** Please be as specific as possible to aid with computer entry and food substitutions if necessary. This means including all snacks, beverages, and any condiments added to food before, during, or after preparation such as mustard, ketchup, mayonnaise, butter, margarine, salt, oils added in cooking, dressings on salads, jam, jelly, cream, and sugar.
    - **Record all beverages consumed, including water.** Be sure to note if beverages such as coffee, tea and soda/pop are caffeinated and/or sweetened. Also, if consuming a mixed alcohol drink, remember to include a portion of both the alcohol and the mix (soda, juice, etc.)
    - **Record vitamin, mineral, and/or herbal supplements taken.**
3. **Record the amount of each item consumed.** It is important to record the amounts as accurately as possible, in household measurements (cups, teaspoons, and tablespoons) or by weight (grams and ounces for solids, fluid ounces for liquids). You may find the information on food labels helpful for converting amounts consumed to estimated measurement (i.e. a potato chip label may tell you that 16 chips is approximately one ounce). Do not simply record the amount listed as one serving on your food record; instead, record the actual amount you consumed.

4. **For complex mixture foods** (e.g. casserole, salad), record each ingredient separately, if possible. This will help you in the analysis if your item is not listed (e.g. tuna casserole).

5. **Record additional information,** such as the method of food preparation, brand of food, etc.

Below are examples of diet records. Note the type of information that is recorded. Please make sure that you collect all information on your foods eaten.

Diet Intake Record Example **Date:** MM/DD/YYYY

TIME	MEAL	FOOD CONSUMED	AMOUNT	TYPE & BRAND	HOW PREPARED
7 am	Breakfast	Special K cereal	1 cup	Kellogg's	
		Milk – skim	1 cup	Cub	
		Coffee	1 cup	Folgers	
		Cream	1 tbsp	Cub	
		Sugar	1 tbsp		
12 pm	Lunch	Whole wheat bread	2 slices	100% whole	Toasted
		Diet Coke	20 oz	wheat	On ice
		Water	12 oz	Pepperidge farm	
3 pm	Afternoon Snack	Blueberry muffin	200 g		
		Coffee	8 oz		
		Cream on top	1 oz		
		Water	4 oz		
7 pm	Dinner	Chicken breast	16 oz		Grilled
		Guacamole	2 oz		
		Sour cream	1 oz	Land O' Lakes	
		Flour tortilla	3 oz	Old Mexico	
		Corn chips	8 oz	Frito Lay	
		Water	16 oz		
		Ice cream-vanilla	1 cup	Kemps	
8:30 pm	Evening snack	Apple	1 medium	Honeycrisp	

Diet Intake Record Day 1/2/3 **Date:** **Weekday or Weekend**
(Please circle one)

TIME	MEAL	FOOD CONSUMED	AMOUNT	TYPE & BRAND	HOW PREPARED

III. Nutrient Analysis of High-Carbohydrate Test Meal

Green Tea Diet

Description:

Category: **Created By: ,**

Date Created: September 10, 2012 Date Last Used: September 10, 201 Date Modified: May 07, 2015

Breakfast

Food Item Description	Food Code	Amount	Measure	Weight (g)	Energy (kcal)	Protein (g)
Bagels, plain, toasted, enriched, with calcium propionate (includes onion, poppy, sesame)	18002	104.00	gram	104.00	299.52	11.59
Cheese spread, cream cheese base	43276	1.00	1 oz	28.35	83.63	2.01
Orange juice, chilled, includes from concentrate	09209	8.00	1 fl oz	248.80	121.91	1.69
Yogurt, fruit, low fat, 9 grams protein per 8 ounce	01120	185.00	gram	185.00	183.15	7.36

Diet Analysis

Nutrients	Energy (kcal)	Protein (g)	Total lipid (fat) (g)	Carbohydrate, by difference (g)	Sugars, total (g)	Vitamin A, RE (mcg_RE)	Vitamin E (mg_ATE)	Water (g)
Target Amount								
Diet Amount	688.21	22.65	12.32	123.58	62.20	0.00*	0.00*	402.29
Breakfast Amount	688.21	22.65	12.32	123.58	62.20	0.00*	0.00*	402.29

Calculated Nutrients

Nutrients	Protein (%)	Fat (%)	Carbohydrate (%)
Diet Amount	13.02	15.94	71.04
Breakfast Amount	13.02	15.94	71.04

Green Tea Diet

Description:

Category: **Created By: ,**

Date Created: September 10, 2012 Date Last Used: September 10, 201 Date Modified: May 07, 2015

Breakfast

Food Item Description	Food Code	Amount	Measure	Weight (g)	Energy (kcal)	Protein (g)
Bagels, plain, toasted, enriched, with calcium propionate (includes onion, poppy, sesame)	18002	104.00	gram	104.00	299.52	11.59
Cheese spread, cream cheese base	43276	1.00	1 oz	28.35	83.63	2.01
Orange juice, chilled, includes from concentrate	09209	8.00	1 fl oz	248.80	121.91	1.69
Milk, reduced fat, fluid, 2% milkfat, protein fortified, with added vitamin A and vitamin D	01081	1.00	1 cup	246.00	137.76	9.72

Diet Analysis

Nutrients	Energy (kcal)	Protein (g)	Total lipid (fat) (g)	Carbohydrate, by difference (g)	Sugars, total (g)	Vitamin A, RE (mcg_RE)	Vitamin E (mg_ATE)	Water (g)
Target Amount								
Diet Amount	642.82	25.01	15.07	102.60	40.66	0.00*	0.00*	478.76
Breakfast Amount	642.82	25.01	15.07	102.60	40.66	0.00*	0.00*	478.76

Calculated Nutrients

Nutrients	Protein (%)	Fat (%)	Carbohydrate (%)
Diet Amount	15.48	20.99	63.53
Breakfast Amount	15.48	20.99	63.53

IV. Visual Analog Scale for Satiety Assessment

V2 August 2012

Visual Analogue Rating Scales (Subjective Appetite, Mood & Mental Alertness)

Instructions for administering VAS to participants:

Paper VAS Questionnaires: 'Please answer the following questions by marking with a vertical line on each horizontal scale the position which best represents how you are feeling at this moment in time. Regard the ends of each line as indicating the most extreme sensations you have ever felt.'

1. How hungry do you feel now?

Not at all Hungry _____ Extremely Hungry

2. How full do you feel now?

Not at all Full _____ Extremely Full

3. How strong is your desire to eat now?

Not at all Strong _____ Very Strong

4. How much food do you think you could eat now?

None at all _____ A Very Large Amount

5. How satiated do you feel now?

Not at all Satiated _____ Extremely Satiated

6. How contented do you feel now?

Not at all Contented _____ Extremely Contented

7. How irritable do you feel now?

Not at all Irritable _____ Extremely Irritable

8. How sleepy do you feel now?

Not at all Sleepy _____ Extremely Sleepy

9. How mentally alert do you feel now?

Not at all Alert _____ Extremely Alert