**CLINICAL TRIAL OF HIGH-DOSE GREEN TEA EXTRACT ON HEPATOCELLULAR INJURY IN POSTMENOPAUSAL WOMEN IN THE UNITED STATES**

by

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Submitted to the Graduate Faculty of

Graduate School of Public Health in partial fulfillment

of the requirements for the degree of

Master of Public Health

University of Pittsburgh

2016

**ABSTRACT**

UNIVERSITY OF PITTSBURGH

GRADUATE SCHOOL OF PUBLIC HEALTH

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on

April 27, 2016

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**CLINICAL TRIAL OF HIGH-DOSE GREEN TEA EXTRACT ON HEPATOCELLULAR INJURY IN POSTMENOPAUSAL WOMEN IN THE UNITED STATES**

Zheming Yu, MPH

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**Objective:** To evaluate the liver toxicity effect of oral supplementation of a concentrated green tea extract (GTE) in postmenopausal women in the United States.

**Methods:** In a clinical trial, 999 postmenopausal women were randomly assigned to either placebo arm or treatment arm receiving a daily dose of 1315 mg total tea catechins (including 843 mg epigallocatechin gallate) for 12 months. Baseline information on demographic characteristics, dietary intake and medication use were collected. Alanine transaminase (ALT) and aspartate transaminase (AST) as liver function tests were quantified in plasma collected from study participants at baseline, monthly for the first 6 months, and every 3 months for the last 6 months. A linear mixed-effect model was used to evaluate the effect of GTE intake on plasma ALT and AST levels, respectively. An unconditional logistic regression model was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) of developing abnormal liver function test (ALT > 60 U/L or AST >35 U/L) during the 12-month study period.

**Results:** Overall, GTE supplements significantly increased ALT by 4.9 U/L (95% CI =3.8-6.0 U/L, P < 0.0001) and AST by 3.6 U/L (95% CI=2.7-4.5 U/L, P < 0.0001) during 12-month study period. After randomization, 48 participants taking GTE and 11 participants taking placebo capsules had at least once ALT greater 60 U/L. Among these 59 participants, GTE group had 383.4% increase of ALT over baseline compared to 76.7% increase of ALT in placebo group at the third month. Women in the GTE arm had a statistically significant increased odds of having abnormal ALT (OR =4.1, 95% CI= 2.1-8.2) and abnormal AST (OR=2.8, 95% CI = 1.8-4.3). Increased body mass index, alcohol consumption, and current use of antibiotics and non-steroidal anti-inflammatory drugs significantly aggravated the effect of GTE on ALT and/or AST elevations.

**Conclusion:** High dose of GTE intake significantly induced elevation of liver transaminases. With increasing global consumption of green tea, the potential liver toxicity effect of high-dose GTE as a dietary supplement has public health significance. Future studies are warranted to elucidate the potential biological mechanism of GTE in inducing liver injury.

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# Introduction

Tea, obtained from the plant *Camellia Sinensis*, is the second most popular beverage in the world. Several types of tea are produced by different postharvest processing methods. Green tea comes from fresh tea leaves steamed immediately after harvest with minimal enzymatic oxidation. Water-extractable components from green tea contain about 30-40% tea catechins by tea leaf dry weight, with epigallocatechin gallate (EGCG) comprising on average two-thirds of total tea catechins (1). A large body of evidence across the world has shown potential benefits of tea catechins, especially EGCG, across the world. The anti-oxidative characteristics of tea catechins were observed to have a beneficial influence on body weight reduction among obese individuals (2). Green tea- associated-reduction of mortality caused by cardiovascular disease was reported in a prospective Japanese cohort study (3). There were also cohort studies supporting that tea catechins functioning as chemopreventive agents may prevent against gastric, esophageal (4) and colon cancers (5).

With an increasing public awareness of tea’s abundant health benefits, US adults - with equal number of males and females - have begun to consume larger amounts of tea catechins by using dietary tea supplements (6). Nevertheless, the safety of these tea supplements has gained wide attention due to the increasing number of reports on associated adverse hepatic reactions.

There were 53 reported hepatic adverse events associated with consuming dietary tea supplements during the past 15 years (7-9). Among these 53 cases, 44 cases were women, the types of liver damage for 50 subjects were hepatocellular injury (i.e., increased liver enzymes), and 28 subjects had consumed other medications, including both synthetic and herbal drugs. These reviews and reports suggested a potential causal relationship between green tea supplements and liver damage, which also suggested that the interaction between use of green tea extracts and other medications at the same time was a major concern.

*Catechol-O-methyltransferase (COMT)* is involved in the metabolic pathway of tea catechins’ degeneration. In particular, genetic variability of *COMT* may have an impact on catechins’ degeneration. A *COMT* polymorphism at condon 108/158 (rs4680), resulting in a guanine (G) to adenine (A) transition, further decreases the enzymatic activity among individuals with homozygous low-activity (A/A) genotype (10, 11). These individuals metabolize tea catechins more slowly than individuals with high-activity (G/G) genotype, which may lead to longer and greater exposure to catechins. High urinary levels of epigallocatechin (EGC), used as a biomarker of green tea intake, were found to be associated with increased risk of developing hepatocellular carcinoma in individuals with positive serology for hepatitis B surface antigen (12).

Despite case reports and observational epidemiological studies, there has been no well controlled clinical trial investigating the relationship between green tea supplements and liver injury. Utilizing the resources of the Minnesota Green Tea Trial (MGTT), a randomized, placebo-controlled, double-blinded phase II clinical trial with a primary aim to investigate the effect of concentrated green tea extract (GTE) supplementation for 12 months on biomarkers of breast cancer (13), we conducted a comprehensive analysis for the effect of oral daily dose of 1315 mg total tea catechin on hepatocellular injury. In addition, we evaluate the potential modifying effect of *COMT* genotypes, lifestyles factors and medication use on the association between GTE consumption and hepatocellular injury.

# Subjects and METHODS

## study sUBJECTS and recruitment

The design of the MGTT has been described in detail previously (13). In brief, MGTT was a randomized, double-blinded, placebo-controlled phase II clinical trial that was designed to investigate the modifying effects of oral intake of GTE on mammographic density, circulating reproductive hormones and circulating insulin-like growth factor axis proteins in postmenopausal women. This clinical trial was approved by the Institutional Review Boards of the University of Minnesota, each of the participating clinical centers, and the University of Pittsburgh. All participants provided written informed consents.

The inclusion and exclusion criteria were described previously (13). Briefly, postmenopausal women 50-70 years old with heterogeneously or extremely dense breasts otherwise in good health were eligible for the clinical trial. Women with any of the following characteristics at baseline were ineligible for MGTT: 1) tested positive for serological status of hepatitis B surface antigen or antibodies to hepatitis C virus; 2) baseline alanine aminotransferase (ALT) higher than 1.5 times the upper limit of normal (ULN) (defined in this study as 60 U/L); 3) any history of cancer, proliferative breast disease, breast augmentation; 4) body mass index (BMI) below 18.5 or above 40 kg/m2 or weight change more than 10 pounds during the previous 12 months; 5) current or recent (within 6 months) use of hormone replacement therapy or anti-inflammatory agents such as methotrexate and Enbrel; 6) current smoker or regular consumption of 7 or more alcoholic beverages per week; and 7) regular consumption of 1 or more cups of green tea per week. A total of 1075 eligible subjects were recruited from 2009 to 2013 at 8 clinical centers in the Minneapolis-St. Paul metropolitan area; 538 were randomly assigned to the GTE intervention group and 537 to the placebo group (**Figure 1**).

Participants were eligible for the present analysis of GTE on liver toxicity if their baseline blood ALT was less than or equal to 60 U/L and aspartate transaminase (AST) less than or equal to 35 U/L and they had at least one follow-up liver function test of ALT and AST after the baseline test. Among the 1075 women randomized, 3 participants (all in GTE group) were excluded due to missing baseline hepatic tests, 51 participants (22 in the GTE and 29 in the placebo group) without hepatic tests at any follow-up visit were also excluded, and other 22 participants (8 in the GTE and 12 in the placebo group) were excluded for the present analysis due to their elevated ALT (> 60 U/L) or AST (> 35 U/L) tests at the baseline because we defined ALT greater than 60 U/L or AST greater than 35 U/L as being abnormal. The present analysis included 999 women (505 in the GTE and 494 in the placebo group).

## Questionnaire Data, BIospecimens and Biomarker data collection

At the baseline visit, each participant completed an in-depth health history questionnaire for demographics, lifestyle factors, medical history, medication use, and reproductive history. The status of alcohol use were collected at the baseline by asking whether the patients consumed alcohol chronically. If the answer was positive, the subject was defined as a current drinker and the amounts of drinks per week were recorded. One alcoholic drink was quantified as 12 fluid ounces (355 ml) of regular beer, 5 fluid ounces (148 ml) of wine, or 1.5 fluid ounces (44 ml) of liquor. Similarly, the current user of a specific medication, including aspirin, non-aspirin nonsteroidal anti-inflammatory drugs (NSAIDs), acetaminophen, antibiotics, anti-viral drugs and statin, were recorded as using the specific drug during the past 6 months.

We used the Diet History Questionnaire I (DHQI), developed and validated by the National Cancer Institute (NCI), for our study. The DHQI included 124 food items and inquiries about the food intake during the past 12 months with portion size, dietary supplements. Average daily food and nutrient intake were estimated using NCI DietCalc software. Anthropometric measurements including weight, height, and waist and hip circumferences were taken for all participants by trained clinic staff. Weight was measured to the nearest 0.1 kg using a digital scale at screening, baseline and every three months. Standing height was determined to the nearest 0.1 cm at baseline and month 12 with a wall-mounted stadiometer. BMI was calculated as weight in kilograms divided by squared height in meters (kg/m2).

Whole blood samples were collected by a trained nurse or phlebotomist via venipuncture at the Human Nutrition Research Clinic at the University of Minnesota St. Paul Campus. Serum and plasma were separated from whole blood by centrifugation at 3000 rpm for 10 minutes, measured into 1.5 mL aliquots and stored at -70 degrees Celsius until analysis. Frozen samples were sent in batches to Quest Diagnostics for measurement of a standard lipid panel. Batches were prepared by arranging participants into GTE-placebo pairs, and all samples from a given study participant were bundled together with a counterpart and analyzed in the same lab batch. Total cholesterol and triglycerides were measured in serum using the Beckman Olympus AU5400 chemistry analyzer at baseline, month 6 and month 12.

Buffy coat was collected by removing plasma from whole blood and adding 0.5 ml of 0.9% sodium chloride to each 0.5 ml aliquot, and then stored at -70 degrees Celsius until analysis. DNA was extracted from buffy coat samples by the Qiagen DNAeasy Blood and Tissue Kit method (Qiagen Inc., Gaithersburg, MD, US). *COMT* genotype analysis was completed at the University of Minnesota Genomics Center. A TaqMan assay was developed for defining the *COMT* G/A polymorphism by 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.

## study Interventions

Green Tea Extract Catechin Complex (Corban complex GTB, referred to as GTE) and placebo capsules were provided by Corban Laboratories (Eniva Nutraceutics, Plymouth, MN). All capsules were stored at ambient temperature and moisture conditions. Eight batches of GTE were produced for the clinical trial. The catechin contents for each batch was quantified using the high-performance liquid chromatography technology at the laboratory in Rutgers University (Chung S. Yang). Mean (± standard deviation, SD) total catechins of each GTE capsule was 328.8±28.9 mg, including 210.7±11.0 mg EGCG, 50.6±18.5 mg epicatechin gallate (ECG), 26.7±29.7 mg EGC, and 26.8±5.9 mg epicatechin (EC). Placebo capsules, identical to the GTE capsules in appearance, contained 816 mg maltodextrin (50%), 808 mg cellulose (49.5%), and 8 mg magnesium stearate (0.5%), but no tea catechins. Both capsules contained 3.9 mg caffeine.

Participants were instructed to take two GTE or placebo capsules, twice daily with breakfast and dinner for 12 months. That was four capsules a day, which contained an average (± SD) of 1315 ±115.4 mg total catechins (including 843±44.1 mg EGCG, 202±74.0 mg ECG, 106±118.8 mg EGC, 107±23.4 mg EC), and 15.8 mg caffeine. The daily dose of total catechins or EGCG was equivalent to approximately five cups (8-ounce or 240 ml per cup) of brewed green tea (14).

## HEPATIC Laboratory measurements

During the first 2 years of the study, participants came to the clinic monthly for ALT monitoring. Because very few women developed elevated ALT, especially after month 6 of entry into the study, we did not require study subjects, who were recruited in the trial in the last 3 years, to have clinical visits at months 7, 8, 10, and 11. As a result, only 24% of total study participants who completed the study had hepatic panel results at months 7, 8, 10, and 11 clinic visits.

Blood was draw into serum separator tubes with clot activator and gel for serum at the HNRC. Samples were centrifuged as described above and then sent to Quest Diagnostics for hepatic panel measurement. Hepatic panel results were available within 2 business days after the laboratory analysis. For the present analysis, a study participant with at least once ALT greater than 60 U/L or AST greater than 35 U/L at any clinic visits after randomization was defined as having a hepatocellular injury event. A participant found to have ALT levels at 1.5-5 times ULN (90-300 U/L) elevation was asked to refrain from taking study product for 14 days and a hepatic panel re-test was conducted. If the re-test ALT value was within acceptable range (below 1.5 times ULN or < 90 U/L), the participant was put back on study product. If the re-test ALT remained above 1.5 but below 5 times ULN, the participant was asked to complete another liver function re-test after additional 14 days. The participant was tested every two weeks until ALT levels returned to below 1.5 times ULN, at which time she was asked to resume taking study capsules. After retaking GTE or placebo capsules, the participant would be permanently withdrawal from the study if her ALT level was above 1.5 time ULN again. Any ALT elevation above 5.0 times ULN (> 300 U/L) was considered a severe adverse event. In such an event, the participant was permanently taken off from the study product but asked to continue in the study. Participants were re-tested for hepatic panels at 14-day intervals until their ALT level returned to within normal range.

## Statistical analysis

The distributions of demographic characteristics (age, race, level of education, BMI, smoking and alcohol status), dietary intake (vitamin C, vitamin E and et al.), and use of medication (antibiotics, antiviral drugs, NSAIDs, acetaminophen, statin and et al.) were compared between the GTE and placebo groups. Independent Student’s t-tests or Wilcoxon rank sum test were employed for comparisons between the two groups of continuous variables in normal or non-normal distributions, respectively. Pearson test was used for comparing the frequencies of categorical or nominal variables between the two groups.

Differences in the plasma concentrations of liver enzymes (ALT and AST) between the GTE and placebo groups from the baseline to each of the follow-up liver function tests after randomization were assessed using the generalized linear mixed effect model with controlling for age, level of education, BMI, and smoking status (never versus. former smokers) at baseline. The changes of liver enzymes over time were estimated by least squares means and their 95% confidence interval (CI).

We also investigated a potential modifying effect of dietary nutrients (e.g. vitamin C, vitamin E), levels of BMI (normal, overweight, and obese), alcohol consumption, plasma lipid levels (cholesterol and triglyceride), use of medications and *COMT* genotypes (*HH, HL* and *LL*) on the relationship between GTE consumption and hepatocellular injury. The same generalized linear mixed effect model was employed with an additional interaction term between a modifying factor of interest and the treatment assignment (GTE or placebo).

Analyses were also performed to evaluate the effect of GTE supplementation on the risk of developing hepatocellular injury defined as above the ULN of ALT and AST, respectively. The unconditional logistic regression method was employed to estimate the odds ratios (ORs) and their 95% CIs of developing hepatocellular injury due to the supplementation of GTE, controlling for age, level of education, BMI, and smoking status at the baseline. The same modifiers described in the above mixed effected models were also tested in the logistic regression models.

Statistical computing was conducted using SAS version 9.4 (SAS Inc., Cary, NC). All *P* values were two-sided. *P* values <0.05 were considered being statistically significant.

# Results

The distributions of baseline characteristics among study participants in the GTE and placebo groups are shown in **Table 1**. The mean ages (SD) of women in the GTE and placebo groups were 59.9 (5.0) and 59.6 (5.1) years, respectively. More than 97% of study participants were Caucasians. No difference was found in the distributions of BMI, level of education, smoking status, alcohol consumption status, current use of medications, dietary intake of vitamin C and other nutrients, plasma levels of total cholesterol, and *COMT* genotypes. Total triglyceride concentration was about 9-10 mg/dl lower in the GTE group than triglyceride in the placebo group (*P*=0.005).

The mean levels of ALT at baseline were comparable between women in GTE (17.9 U/L) and placebo groups (17.3 U/L) whereas women in the placebo had a slightly higher AST (20.2 U/L) than those in the GTE group (19.6 U/L) (*P* = 0.023) (**Supplemental Table 1**). Use of statin was associated with significantly higher baseline levels of ALT and AST in both GTE and placebo groups (both *P* < 0.001). In the placebo group, women with BMI ≥ 30 kg/m2 or serum triglyceride ≥ 150 mg/dl had significantly elevated ALT whereas those of current use of non-aspirin NSAIDs showed a lower ALT at baseline (all *P* < 0.05). We did not observe similar differences in ALT at the baseline among women in the GTE group. The difference in baseline ALT levels between the GTE and placebo groups reached statistical significance for women with BMI ≥30 kg/m2, non-drinking of alcohol, non-users of non-aspirin NSAIDs or acetaminophen. Baseline level of AST was slightly reduced in obese women or current drinkers of alcohol (*P*s < 0.05) in placebo group. A reduced baseline level of AST was observed in the GTE for women with normal weight (BMI < 25 kg/m2), current drinkers of alcohol, lower intake of dietary vitamin C (below median), lower serum triglyceride (<150 mg/dl), non-users of acetaminophen or statin (**Supplemental Table 1).**

The supplementation of GTE significantly increased plasma ALT and AST levels, where ALT increased by 4.9 U/L (28.5% over baseline level) and AST increased by 3.6 U/L (18.2% over baseline level) (both *Ps* < 0.0001). The largest increase in ALT was observed between months 2 and 3 after the initial supplementation of GTE. At month 3, the mean ALT was 90.3 U/L among women (n = 47) in GTE group who had at least one abnormal ALT test (>60 U/L) during the entire study period (**Figure 2b**), which was 383.4% over their mean baseline ALT (**Figure 2a**). Among women in the placebo group, the corresponding ALT was 46.1 U/L, which was 76.7% over their mean baseline ALT level. The decrease in ALT after month 3 in the GTE group was the result of the temporarily stopping taking GTE by women with ALT >1.5 - <5 times ULN and permanent withdrawals of women whose ALT was greater than 5 times ULN. A similar pattern of AST enzyme changes was over the course of GTE supplementation. At month 3, subjects with at least once AST greater than 35 U/L (ULN) had a 120.9% increase of AST over their baseline level and an average 45.9 U/L of AST, as compared to women in the placebo group who had 29.7% increase over their baseline AST and an average 30.2 U/L of AST (**Figure 3**). After randomization, 19 participants stopped taking study products (GTE or placebo capsules) because of persistent ALT elevation or ALT greater than 5.0 times ULN (300 U/L), and 11 of them dropped off the study before month 6 (**Supplemental table 3**). The hepatic transaminases levels of the rest participants were steady without obvious fluctuation.

The effects of GTE supplementation on increasing ALT and AST were different among women with different characteristics at baseline (**Table 2**). The effect of GTE supplementation on the elevation of liver enzymes (both ALT and AST) was stronger in obese women (BMI ≥30 kg/m2) or current use of antibiotics (both *Ps* for interaction < 0.05). In contrast, the GTE supplementation had a smaller effect on ALT elevation for current drinkers of alcoholic beverages or current users of non-aspirin NSAIDs. Participants with different *COMT* activity did not show significant differences in hepatic transaminase changes after intake of GTE (**Supplemental table 2**). There was no statistically significant modifying effect of dietary intake of vitamin C, plasma total cholesterol and triglyceride, or use of aspirin, acetaminophen, statin or antiviral drugs on the GTE’s effect on elevation of ALT or AST (**Table 2**).

The effects of GTE supplementation on the risk of developing hepatocellular injury (defined as ALT or AST > ULN) are shown in **Table 3**. Overall, the supplementation of GTE resulted in 4.1-fold increased risk of developing abnormal ALT (95% CI = 2.1-8.1) and 2.8-fold increased risk of developing abnormal AST (95% CI = 1.8-4.3) compared with the placebo group. The ORs of having abnormal ALT were even greater among non-drinkers of alcohol (OR = 9.3, 95% CI = 1.2-75.4), current non-users of NSAIDs (OR = 7.4, 95% CI = 1.6-33.7), or current users of antibiotics (OR = 9.3, 95% CI = 1.1-77.7) for GTE supplementation compared with their counterparts in the placebo group. The ORs of having abnormal AST were greater among current non-users of NSAIDs (OR = 5.7, 95% CI = 2.3-14.3), but less among non-drinkers of alcohol (OR = 1.8, 95% CI = 0.8-4.2), and current users of antibiotics (OR = 2.2, 95% CI = 0.8- 5.9) for GTE supplementation compared with placebo group counterparts.

# discussion

The present analysis has demonstrated the daily oral intake of 4 GTE capsules containing 1315 mg total catechins including 843 mg EGCG for approximately 90 days resulted in significant increase in ALT by 46.9% and AST by 26% among all participants. Women in the GTE group experienced a significant 4.1 times higher likelihood for developing abnormal ALT elevation and 2.8 times for abnormal AST elevation during the 12-month intervention period compared with those in the placebo group. To my knowledge, this is the first randomized clinical trial showing GTE supplementations-associated hepatic injury.

Overall, our data support previous case reports on hepatic adverse events after taking commercial GTE, and also show that the duration of GTE supplements intake may also contribute to occurrence of liver toxicity. The onset of hepatic adverse reaction was within 3 months in 70% of the reported cases. Among subjects who consumed the green tea supplement, the mean ( SE) latent time was 179.1 58.9 days corresponding to the amount of polyphenols varying between 186 and 1395 mg (7, 8). In the present study, 8 subjects began to have ALT greater than 90 U/L (1.5ULN) and stopped taking study capsules because of adverse hepatic events after taking 1315 mg catechins daily for three months. At the third month, the GTE subjects with at least one ALT greater than 60 U/L had an average 90.3 U/L ALT, which was 383.4% increase over the baseline. The drop of ALT and AST levels in the two figures after month 3 was partially due to temporary stoppage from taking GTE by participants with ALT at 1.5-5.0 ULN or those who were permanently withdrawal from the study by ALT greater than 5.0 times ULN. On the contrary, twenty healthy female and male subjects aged greater than 30 years old recruited in a phase I pharmacokinetic study consuming a single dose of 800mg EGCG or polyphenon E did not show any adverse hepatic event (15). The same subjects consumed 800mg EGCG or polyphenon E once daily for 4 weeks. No liver toxicity cases were reported after the 4-week catechin intervention (16). A longer intervention period might be required to detect liver toxicity events among high dose consumers of green tea or green tea-based supplements.

Abundant evidence supported anti-oxidative healthy benefits from catechins, however catechins as pro-oxidants may contribute to collapse the mitochondrial membrane potential, generate reactive oxygen species (ROS) and deplete glutathione (GSH) (17). Tea catechin was administrated to rat hepatic cells, and induced the collapse of mitochondrial membrane potential and then cell apoptosis. Total intracellular ROS was increased and GSH was depleted. The excess accumulation of ROS may alter intracellular protein, increase cell membrane permeability, and induce apoptosis (18). GSH-depleted cells were susceptible to DNA oxidation and further cell damage (19). In the rat model, EGCG related ROS formation was biphasic, indicating that low dose EGCG decreased ROS production, while high dose EGCG increased ROS generation (20). Accompanying elevation of oxidative stress markers, mice given a single oral dose of 1500mg/kg EGCG showed plasma ALT elevated by 8- and 108- fold after 24 and 48h, as compared to their placebo counterparts (21).

Obesity and its complications, including dyslipidemia, inflammation, oxidative stress, are strong metabolic risk factors for developing fatty liver disease (22, 23). The increasing prevalence of obesity resulted in higher proportion of nonalcoholic fatty liver disease among chronic liver disease in US (24). Based on its anti-oxidative benefits, green tea supplement is prevalent as a mean for weight loss (25-27). However, high dose of tea catechins (e.g. ≈800mg EGCG) as pro-oxidative agents may enhance liver injury in obese subjects by exacerbating ROS production, accumulation and succeeding damages (15, 21). Our study did observe that overweight (BMI = 25-<30 kg/m2) and obese (BMI 30 kg/m2) participants had higher levels of ALT at baseline than women with normal weight (BMI <25 kg/m2). The high dose catechins aggravated hepatocellular injury in obese subjects, as compared to subjects with normal weight. A recent longitudinal study in Mexican adults reported that weight gain increased the risk of developing elevated ALT (OR= 4.1, 95% CI 2.2-7.6), defined as ALT greater than 40 U/L, as compared to the participants maintaining the normal weight from 2004 to 2013 (28).

Alcohol is one of the most common causes of chronic liver disease in the US (29). The production of ROS from ethanol metabolic process is involved in mitochondrial damage and further liver toxicity (30). Diverse anti-oxidants can protect against hepatocellular injury from ROS damage, such as vitamins E and C(31, 32), ademetionine, and silymarin (33). The present study also observed the protective effect of GTE supplements against alcohol induced hepatocellular damages. Several potential mechanisms have been proposed to explain the protective effects of GTE (34-38). Catechins, agents with both anti-oxidative and pro-oxidative effects, are involved with the 63 kDa laminin receptor stimulation, which further down-regulate the Toll-like receptor 2 and 4 (34-36). Toll-like receptor 4 signaling pathway is associated with lipopolysaccharide-induced stimulation of inflammatory cytokines and Kupffer cell, which further induced alcoholic liver disease (34). Thus, catechins may protect from alcoholic liver disease via the reduction of lipopolysaccharide and inflammatory cytokines.

Statins, antimicrobial agents and NSAIDs are most critical causes of drug-induced liver injury (39). Our observation indicated that subjects using statins were associated with significantly increased baseline hepatic aminotransferases in both placebo and GTE groups. These results are consistent with the previous studies. Among patients with acute liver failure in the US from 1998 to 2007, six hepatotoxic cases (4.5%, 6 out of 133 were reported to be related with use of statins (40). A systemic review concluded that use of statin increased the risk of liver enzyme elevation (OR = 1.5, 95% CI = 1.5-1.6), though the definition of raised liver enzymes was heterogeneous among different studies (41).

Our present study observed that the supplementation of GTE significantly decreased ALT for current users of non-aspirin NSAIDs. One possible hypothesis is that increased concentration of these drugs may increase susceptibility to hepatocellular toxicity. EGCG is a competitive inhibitor against human hepatic CYP2B6, CYP2C8, and CYP3A, which may increase the concentration of drugs metabolized by the same hepatic metabolic enzymes and induce drug interactions (42). Another hypothesis for hepatotoxicity from NSAIDs is from inhibition of cyclo-oxygenase-2, and consequently down-regulation of the synthesis of prostaglandin E2. The prostanoids play important roles in protection against bile acid-induced apoptosis via upregulation of the anti-apoptotic mitochondrial protein Bcl-2 (43). NSAIDs thus contribute to oxidative stress, increase susceptibility of mitochondrial damages and liver cell apoptosis (44). In a mice study, tea polyphenols were associated with normalization of cyclooxygenase and Bcl-2 activity, and protection from NSAIDs- induced hepatotoxicity (45).

An important strength of the current study is the longitudinal data collection of liver enzymes with several time points over 12 months. Multiple measurements over 12 months are better than a single measurement to reduce effects of intra-individual variabilities on total liver enzymes change. MGTT is a randomized, placebo-controlled, and double-blinded clinical trial, having a large study population, high compliance to treatment and standardized GTE intervention. Encapsulated GTE provided a standardized quantity of tea catechins. Urinary concentrations of EGC and EC were measured to assess compliance. At baseline, urinary levels of EGC and EC were similar between GTE and placebo groups. However, after 12-month intervention, GTE group had significantly higher urinary levels of EGC (6.8 versus 0.4 nmol/mg creatinine) and EG (15.5 versus 0.6 nmol/mg creatinine) as compared to the placebo group (both *P* <0.0001) (13). Additionally, we used levels of both plasma aminotransferases, ALT and AST, to identify GTE-induced liver injury in the current study. Both ALT and AST had significant increases after the administration of GTE supplements, which reflected hepatotoxicity. However, since AST does not only exist in liver cells, its increase may also reflect damage in other organs. The ORs of abnormal ALT and AST elevation did not have similar change directions between non-users and users of vitamin C, aspirin and antibiotics, as well as non-drinkers and drinkers of alcohol (**Table 3**).

A limitation of the study is that all subjects were predominant women and 97.69% were Caucasian. Lack of diversity in sex and race/ethnicity may limit the generalization of these results to the whole population. DHQI collects food intake information about the previous 12 months, and then average daily nutrient intakes at the baseline are estimated by NCI DietCalc software. This self-reported data collected at the baseline visit in the current study provided steady estimation of nutrient intakes for our trial duration. In contrast, current medication use was collected by an in-depth health survey only at the baseline visit. The duration of co-administration of these medications and GTE were unknown. The 3-5 U/L change of aminotransferase levels after 12-month GTE intervention were statistically significant, but perhaps not clinically significant. In clinical practices, a small aminotransferase elevation cannot determine hepatotoxicity, and the magnitude of elevation is not associated with the severity of liver damage. Mild hepatic aminotransferase elevation (< 5 times ULN) is a common phenomenon in the daily clinical practice. Repeated hepatic enzyme tests along with clinical guided screen tests should be firstly implemented for excluding top-ranked conditions, such as hepatitis B and C infection, and chronic alcohol consumption (46). Moderate (5-10 times ULN) and marked (>10 times ULN) aminotransferase elevations are more likely to be shown in acute liver damage cases, varying with the course of the injury. Additional examinations, such as liver biopsy would be necessary for diagnosis confirmation and assessment of liver damage severity (47). However, it is still meaningful that our findings of strong association between aminotransferase elevation and long-term high dose tea catechins could contribute to attention on tea supplements use among subjects with complicated conditions. Subjects with risk factors, such as alcohol consumption and other medication intakes, may be susceptible to moderate or marked aminotransferase elevations.

# CONCLUSION

There remains uncertainty regarding the safety of long-term green tea supplementation use. This study emphasizes the importance of research evaluating the association between GTE supplementation and liver injury. Our current study demonstrates that elevations of plasma liver enzymes are associated with the use of GTE, and are aggravated by co-administrated medications. To the best of our knowledge, no prior study has used multiple measurements of plasma liver enzymes to examine their relationship with GTE use over a 12-month. Since the consumption of dietary green tea supplementation is increasingly globally, it has clinical and public health implications to monitor liver enzyme levels for high-dose long-term green tea or tea supplement users in the future.

# Acknowledgement

I would like to thank my academic advisor Dr. Jian-Min Yuan, for his mentorship I have received during my graduate years. I sincerely appreciate for the learning and practicing opportunities provided by Dr. Yuan. Without his guidance and persistent help this master project would not have been possible. I am also thankful for valuable comments from my committee members Dr. Lesley Butler and Dr. Thomas Kensler.

I would also like to thank the principal investigator of the Green Tea Study, Dr. Mindy Kurzer, Professor and Director of Healthy Foods, Healthy Lives Institute, University of Minnesota, who offered me the opportunity to be involved in such a great study. I acknowledge Dr. Hamed Samavat, Dr. Allison Dostal, and all staffs in the Department of Food Science and Nutrition, University of Minnesota, for their great work in the Green Tea Study. I appreciate their detailed explanations and supports when I had misunderstandings about the data.

My sincere thanks also goes to Renwei Wang who gave me supports in analysis and interpretation of data. Finally, I would especially like to express my very profound gratitude to my parents for their continuous supports and encouragements throughout my years of study.

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Table 1 Distributions of characteristics at baseline among study participants in the green tea extract and placebo group, The Minnesota Green Tea Trial, 2009-2015

|  |  |  |  |
| --- | --- | --- | --- |
| **Characteristics a** | **Placebo** | **GTE** | ***Pb*** |
| **No. of total subjects, n (%)** | **494 (100)c** | **505 (100)c** |  |
| Age at baseline (year), mean (SD) | 59.6(5.1) | 59.9(5.0) | 0.444 |
| Caucasian race, N (%)b | 477(97.2) | 494(98.2) | 0.266 |
| Level of education, N (%) |  |  |  |
| High school or below | 37(7.5) | 30(6.0) | 0.181 |
| Some College | 84(17.1) | 108(21.5) |  |
| College Graduate | 212(43.2) | 224(44.5) |  |
| Postgraduate/Professional Degree | 158(32.2) | 141(28.0) |  |
| Body Mass Index (kg/m2), mean (SD) | 25.1(3.8) | 25.2(3.7) | 0.843 |
| <25, n (%) | 271 (54.9) | 277 (54.8) | 0.274 |
| 25- <30, n (%) | 175 (35.4) | 164 (32.5) |  |
| ≥30, n (%) | 48 (9.7) | 64 (12.7) |  |
| Former smokers, n (%) | 156(31.6) | 157(31.1) | 0.777 |
| No. years of quitting smoking, mean (SD) | 25.8(11.4) | 25.3(10.4) | 0.488 |
| Current use of alcohol, n (%) | 414(84.2) | 402(79.6) | 0.063 |
| No. of drinks per week, mean (SD) | 3.4(3.0) | 3.3(2.9) | 0.929 |
| Current use of antibiotics, n (%) | 68 (13.8) | 71 (14.1) | 0.893 |
| Current use of antiviral drugs, n (%) | 15 (3.0) | 17 (3.4) | 0.767 |
| Current use of aspirin, n (%) | 137 (27.8) | 135 (26.8) | 0.722 |
| Current use of non-aspirin NSAIDs, n (%) | 328 (66.5) | 348 (69.1) | 0.395 |
| Current use of acetaminophen, n (%) | 133 (27.0) | 149 (29.6) | 0.365 |
| Current use of statin, n (%) | 105 (21.3) | 112 (22.2) | 0.724 |
| Dietary intake of vitamin C (mg/Kcal), mean (SD) | 78.1 (35.7) | 77.0 (37.4) | 0.661 |
| Less than median (71.57), n (%) | 224(48.7) | 232(51.3) | 0.427 |
| Greater than median (>71.57), n (%) | 236(51.3) | 220(48.7) |  |
| Total cholesterol (mg/dl), mean (SD) | 209.2 (31.7) | 206.8 (30.6) | 0.234 |
| <200, n (%) | 185 (40.3) | 194 (42.9) | 0.671 |
| 200-240, n (%) | 203 (44.2) | 195 (43.2) |  |
| >240, n (%) | 71 (15.5) | 63 (13.9) |  |
| Total triglyceride (mg/dl), mean (SD) | 102.1 (48.9) | 93.6 (43.3) | 0.005 |
| <150, n (%) | 395 (86.1) | 407 (90.0) | 0.064 |
| ≥150, n (%) | 64 (13.9) | 45 (10.0) |  |
| *COMT* Genotypes, n (%) |  |  |  |
| *LL* (low activity) | 150(30.4) | 171(33.9) | 0.388 |
| *HL* (Intermediate activity) | 213(43.1) | 198(39.2) |  |
| *HH* (High activity) | 131(26.5) | 136(26.9) |  |
| a GTE, green tea extract; SD, standard deviation; *COMT, catecheol-O-methyltransferase*; NSAIDs, non-steroidal anti-inflammatory drugs | | | |
| b 2-sided *P* values were derived from *t-*test (for continuous variables) or chi-squared test (for categorical or nominal variables). | | | |
| c The sum of some variables may be less than the total due to missing values. | | | |

Table 2 Average changes of plasma alanine transaminase (ALT) and aspartate transaminase (AST) during the intervention period from baseline in all subjects as well as in subgroups stratified by selected baseline characteristics, the Minnesota Green Tea Trial, 2009-2015

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **ALT (U/L), mean (95% CI)** | | | ***Pint b*** | **AST (U/L), mean (95% CI)** | | | ***Pint b*** |
| **Placebo** | **GTE** | ***Pa*** | **Placebo** | **GTE** | ***Pa*** |
| Mean enzyme level at baseline | 17.9 (17.3, 18.5) | 17.3 (16.7, 17.9) | 0.139 |  | 20.2 (19.8, 20.7) | 19.6 (19.1, 20.0) | 0.023 |  |
| **Mean changes of enzyme from baseline** |  |  |  |  |  |  |  |  |
| All subjects | 0.1 (-1.0, 1.2) | 4.9(3.8, 6.1) | <.0001 |  | 0.2(-0.7, 1.1) | 3.6(2.7, 4.5) | <.0001 |  |
| Subgroups stratified by |  |  |  |  |  |  |  |  |
| BMI (kg/m2) |  |  |  | 0.030 |  |  |  | 0.036 |
| <25 | -0.1 (-1.3, 1.2) | 4.8 (3.5, 6.0) | <.0001 |  | 0.3 (-0.6, 1.3) | 3.6 (2.6, 4.6) | <.0001 |  |
| 25- <30 | 0.4 (-0.9, 1.7) | 4.3 (2.9, 5.7) | <.0001 |  | 0.2 (-0.9, 1.2) | 2.8 (1.8, 3.9) | 0.000 |  |
| ≥30 | -0.3 (-2.3, 1.8) | 7.3 (5.5, 9.1) | <.0001 |  | -0.01 (-1.5, 1.5) | 5.3 (3.9, 6.6) | <.0001 |  |
| *Pc* | 0.699 | 0.005 |  |  | 0.878 | 0.002 |  |  |
| Current Alcohol Drinker |  |  |  | 0.004 |  |  |  | 0.004 |
| No | -1.3 (-3.0, 0.4) | 6.0 (4.5, 7.5) | <.0001 |  | -0.5 (-1.8, 0.8) | 4.5 (3.4, 5.7) | <.0001 |  |
| Yes | 0.3 (-0.8, 1.5) | 4.6 (3.5, 5.8) | <.0001 |  | 0.4 (-0.5, 1.3) | 3.3 (2.4, 4.2) | <.0001 |  |
| *Pc* | 0.038 | 0.047 |  |  | 0.108 | 0.012 |  |  |
| Dietary vitamin C |  |  |  | 0.077 |  |  |  | 0.269 |
| Low (71.57 mg/Kcal) | -0.1 (-1.3, 1.1) | 4.7 (3.5, 5.9) | <.0001 |  | 0.3 (-0.5, 1.2) | 3.4 (2.6, 4.2)) | <.0001 |  |
| High (>71.57 mg/Kcal) | 0.02 (-1.2, 1.2) | 3.4 (2.2, 4.6) | <.0001 |  | 0.02 (-0.8, 0.8) | 2.5 (1.6, 3.3) | <.0001 |  |
| *Pc* | 0.822 | 0.024 |  |  | 0.441 | 0.021 |  |  |
| Total cholesterol level (mg/dl) |  |  |  | 0.338 |  |  |  | 0.576 |
| <200 | -0.8 (-2.1, 0.4) | 4.0 (2.8, 5.3) | <.0001 |  | -0.06 (-0.9, 0.8) | 2.9 (2.1, 3.8) | <.0001 |  |
| 200-240 | 0.3 (-0.9, 1.6) | 4.0 (2.7, 5.2) | <.0001 |  | 0.2 (-0.6, 1.1) | 3.0 (2.2, 3.9) | <.0001 |  |
| >240 | 1.2 (-0.5, 2.8) | 4.8 (3.1, 6.6) | 0.002 |  | 0.7 (-0.4, 1.9) | 2.8 (1.6, 4.0) | 0.014 |  |
| *Pc* | 0.032 | 0.584 |  |  | 0.408 | 0.926 |  |  |
| Total triglyceride level (mg/dl) |  |  |  | 0.815 |  |  |  | 0.829 |
| <150 | -0.3 (-1.4, 0.8) | 3.9 (2.8, 5.0) | <.0001 |  | -0.04 (-0.8, 0.7) | 2.8 (2.1, 3.5) | <.0001 |  |
| ≥150 | 1.5 (-0.3, 3.2) | 5.9 (4.0, 7.9) | 0.001 |  | 1.3 (0.1, 2.5) | 3.9 (2.6, 5.3) | 0.004 |  |
| *Pc* | 0.030 | 0.028 |  |  | 0.022 | 0.086 |  |  |
|  |  | | |  |  | | |  |
| **Table 2 continued** |  | | |  |  | | |  |
|  | **ALT (U/L), mean (95% CI)** | | | ***Pint b*** | **AST (U/L), mean (95% CI)** | | | ***Pint b*** |
|  | **Placebo** | **GTE** | ***Pa*** | **Placebo** | **GTE** | ***Pa*** |
| Current Use of Aspirin |  |  |  | 0.101 |  |  |  | 0.331 |
| No | 0.2 (-0.9, 1.4) | 5.2 (4.1, 6.4) | <.0001 |  | 0.4 (-0.6, 1.3) | 3.9 (2.9, 4.8) | <.0001 |  |
| Yes | -0.2 (-1.6, 1.2) | 4.2 (2.7, 5.6) | <.0001 |  | -0.01 (-1.1, 1.1) | 2.9 (1.8, 4.0) | 0.000 |  |
| *Pc* | 0.508 | 0.095 |  |  | 0.439 | 0.035 |  |  |
| Current Use of Non-aspirin NSAIDs |  |  |  | 0.002 |  |  |  | 0.081 |
| No | -0.9 (-2.3, 0.4) | 5.7 (4.3, 7.1) | <.0001 |  | -0.07 (-1.1, 1.0) | 4.0 (2.9, 5.1) | <.0001 |  |
| Yes | 0.6 (-0.6, 1.8) | 4.6 (3.4, 5.8) | <.0001 |  | 0.4 (-0.5, 1.3) | 3.4 (2.5, 4.3) | <.0001 |  |
| *Pc* | 0.012 | 0.071 |  |  | 0.288 | 0.165 |  |  |
| Current Use of Acetaminophen |  |  |  | 0.087 |  |  |  | 0.390 |
| No | -0.2 (-1.4, 0.9) | 5.0 (3.9, 6.2) | <.0001 |  | 0.1 (-0.8, 1.0) | 3.6 (2.6, 4.5) | <.0001 |  |
| Yes | 1.0 (-0.5, 2.4) | 4.8 (3.4,6.1) | <.001 |  | 0.7 (-0.5, 1.8) | 3.6 (2.5, 4.7) | <.001 |  |
| *Pc* | 0.056 | 0.634 |  |  | 0.228 | 0.984 |  |  |
| Current Use of Statin |  |  |  | 0.572 |  |  |  | 0.707 |
| No | 0.3 (-0.9, 1.4) | 5.0 (3.8, 6.2) | <.0001 |  | 0.3 (-0.6, 1.3) | 3.6 (2.7, 4.6) | <.0001 |  |
| Yes | -0.5 (-2.1, 1.0) | 4.8 (3.2, 6.3) | <.0001 |  | -0.1 (-1.3, 1.1) | 3.5 (2.3, 4.6) | <.0001 |  |
| *Pc* | 0.257 | 0.710 |  |  | 0.399 | 0.735 |  |  |
| Current Use of Antivirus medications |  |  |  | 0.452 |  |  |  | 0.134 |
| No | 0.1 (-1.0, 1.2) | 4.9 (3.8, 6.1) | <.0001 |  | 0.3 (-0.6, 1.2) | 3.5 (2.6, 4.4) | <.0001 |  |
| Yes | -1.1 (-4.3, 2.1) | 5.3 (2.3, 8.4) | 0.004 |  | -0.7 (-3.0, 1.7) | 5.0 (2.7, 7.2) | 0.001 |  |
| *Pc* | 0.441 | 0.782 |  |  | 0.426 | 0.179 |  |  |
| Current Use of Antibiotics |  |  |  | 0.038 |  |  |  | 0.005 |
| No | 0.3(-0.8, 1.5) | 4.8 (3.7, 6.0) | <.0001 |  | 0.4 (-0.5, 1.3) | 3.4 (2.5, 4.3) | <.0001 |  |
| Yes | -1.3 (-3.1, 0.5) | 5.6 (3.9, 7.3) | <.0001 |  | -0.6 (-1.9, 0.8) | 4.7 (3.4, 6.0) | <.0001 |  |
| *Pc* | 0.053 | 0.333 |  |  | 0.114 | 0.017 |  |  |
| GTE, green tea extract; BMI, body mass index; CI, confidence interval; NASID, Nonsteroidal anti-inflammatory drug; All means are from Least Squares Means. | | | | | | | | |
| a 2-sided *P* values were derived from generalized linear mixed models comparing the changes of liver enzyme (ALT or AST) during the intervention period from baseline of women in the GTE group to the corresponding changes in the placebo group after adjustments for age, BMI, smoking status and level of education. | | | | | | | | |
| b 2-sided P values were derived from the same model assessing the interaction between the intervention (GTE or placebo) and the stratifying variables on the liver enzyme (ALT or AST) with the same adjustments. | | | | | | | | |
| c 2-sided P values were derived from the same model comparing the differences in changes of liver enzyme (ALT or AST) from baseline among different levels of stratifying variables within the GTE or placebo group. | | | | | | | | |

Table 3 Odds ratio of developing abnormal liver enzyme (ALT or AST) for women assigned in the GTE versus placebo group during the 12-month treatment period, the Minnesota Green Tea Trial, 2009-2015

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Treatment status by stratifying variable a** | **Alanine transaminase (ALT)** | | |  | **Aspartate transaminase (AST)** | | |
| **Normal** | **Abnormal b** | **OR (95% CI) c** |  | **Normal** | **Abnormal b** | **OR (95% CI) c** |
| All Subjects |  |  |  |  |  |  |  |
| Placebo | 483 | 11 | 1.00 |  | 458 | 36 | 1.00 |
| GTE | 457 | 48 | 4.1 (2.1, 8.2) |  | 412 | 93 | 2.8 (1.8, 4.3) |
| **BMI** |  |  |  |  |  |  |  |
| Normal BMI (< 25 kg/m2) |  |  |  |  |  |  |  |
| Placebo | 267 | 4 | 1.00 |  | 252 | 19 | 1.00 |
| GTE | 251 | 26 | 6.1 (2.1, 18.1) |  | 219 | 58 | 3.3 (1.9, 5.8) |
| Overweight (25- <30 kg/m2) | |  |  |  |  |  |  |
| Placebo | 168 | 7 | 1.00 |  | 161 | 14 | 1.00 |
| GTE | 150 | 14 | 1.8 (0.7, 4.9) |  | 141 | 23 | 1.6 (0.7, 3.3) |
| Obesity (30 kg/m2) |  |  |  |  |  |  |  |
| Placebo | 48 | 0 | N/A |  | 45 | 3 | 1.00 |
| GTE | 56 | 8 | N/A |  | 52 | 12 | 4.9 (1.0, 24.4) |
| **Alcohol Consumption** |  |  |  |  |  |  |  |
| Non-drinkers of alcohol |  |  |  |  |  |  |  |
| Placebo | 77 | 1 | 1.00 |  | 68 | 10 | 1.00 |
| GTE | 89 | 14 | 9.3 (1.2, 75.4) |  | 79 | 24 | 1.8 (0.8, 4.2) |
| Current drinkers of alcohol |  |  |  |  |  |  |  |
| Placebo | 404 | 10 | 1.00 |  | 388 | 26 | 1.00 |
| GTE | 368 | 34 | 3.5 (1.7, 7.3) |  | 333 | 69 | 3.1 (1.9, 5.1) |
| **Use of Antibiotics** |  |  |  |  |  |  |  |
| Non-users of antibiotics |  |  |  |  |  |  |  |
| Placebo | 415 | 10 | 1.00 |  | 396 | 29 | 1.00 |
| GTE | 395 | 38 | 3.7 (1.8, 7.6) |  | 356 | 77 | 3.0 (1.8, 4.8) |
| Current users of antibiotics |  |  |  |  |  |  |  |
| Placebo | 67 | 1 | 1.00 |  | 61 | 7 | 1.00 |
| GTE | 61 | 10 | 9.3 (1.1, 77.7) |  | 55 | 16 | 2.2 (0.8, 5.9) |
| **Use of Anti-viral drugs** |  |  |  |  |  |  |  |
| Non-users of anti-viral drugs | |  |  |  |  |  |  |
| Placebo | 467 | 11 | 1.00 |  | 442 | 36 | 1.00 |
| GTE | 443 | 44 | 3.9 (2.0, 7.7) |  | 399 | 88 | 2.7 (1.7, 4.1) |
|  |  |  |  |  |  |  |  |
| **Table 3 continued** |  | | |  |  | | |
| **Treatment status by stratifying variable a** | **Alanine transaminase (ALT)** | | |  | **Aspartate transaminase (AST)** | | |
| **Normal** | **Abnormal b** | **OR (95% CI) c** |  | **Normal** | **Abnormal b** | **OR (95% CI) c** |
| **Use of Anti-viral drugs** |  |  |  |  |  |  |  |
| Current users of anti-viral drugs | |  |  |  |  |  |  |
| Placebo | 15 | 0 | N/A |  | 15 | 0 | N/A |
| GTE | 13 | 4 | N/A |  | 12 | 5 | N/A |
| **Use of Aspirin** |  |  |  |  |  |  |  |
| Non-users of aspirin |  |  |  |  |  |  |  |
| Placebo | 347 | 9 | 1.00 |  | 327 | 29 | 1.00 |
| GTE | 332 | 37 | 4.2 (2.0, 8.8) |  | 295 | 74 | 2.9 (1.8, 4.8) |
| Current users of aspirin |  |  |  |  |  |  |  |
| Placebo | 135 | 2 | 1.00 |  | 130 | 7 | 1.00 |
| GTE | 124 | 11 | 5.1 (1.0, 27.2) |  | 116 | 19 | 2.2 (0.8, 5.6) |
| **Use of Non-aspirin NSAIDs** | |  |  |  |  |  |  |
| Non-users of Non-aspirin NSAIDs | |  |  |  |  |  |  |
| Placebo | 163 | 2 | 1.00 |  | 157 | 8 | 1.00 |
| GTE | 142 | 14 | 7.4 (1.6, 33.7) |  | 127 | 29 | 5.7 (2.3, 14.3) |
| Current users of Non-aspirin NSAIDs | |  |  |  |  |  |  |
| Placebo | 319 | 9 | 1.00 |  | 300 | 28 | 1.00 |
| GTE | 314 | 34 | 3.4 (1.6, 7.4) |  | 284 | 64 | 2.2 (1.3, 3.6) |
| **Use of Acetaminophen** |  |  |  |  |  |  |  |
| Non-users of Acetaminophen | |  |  |  |  |  |  |
| Placebo | 354 | 6 | 1.00 |  | 336 | 24 | 1.00 |
| GTE | 321 | 34 | 5.4 (2.2, 13.3) |  | 290 | 65 | 2.9 (1.7, 4.8) |
| Current users of Acetaminophen | |  |  |  |  |  |  |
| Placebo | 128 | 5 | 1.00 |  | 121 | 12 | 1.00 |
| GTE | 135 | 14 | 2.7 (0.9, 7.7) |  | 121 | 28 | 2.6 (1.2, 5.6) |
| **Use of Statin** |  |  |  |  |  |  |  |
| Non-users of Statin |  |  |  |  |  |  |  |
| Placebo | 381 | 7 | 1.00 |  | 362 | 26 | 1.00 |
| GTE | 358 | 34 | 4.8 (2.1, 11.0) |  | 322 | 70 | 3.1 (1.9, 5.2) |
| Current users of Statin |  |  |  |  |  |  |  |
| Placebo | 101 | 4 | 1.00 |  | 95 | 10 | 1.00 |
| GTE | 98 | 14 | 3.5 (1.0, 11.9) |  | 89 | 23 | 2.2 (0.9, 5.1) |
|  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |
| **Table 3 continued** |  |  |  |  |  |  |  |
| **Treatment status by stratifying variable a** | **Alanine transaminase (ALT)** | | |  | **Aspartate transaminase (AST)** | | |
| **Normal** | **Abnormal b** | **OR (95% CI) c** |  | **Normal** | **Abnormal b** | **OR (95% CI) c** |
| **Vitamin C intake** |  |  |  |  |  |  |  |
| Low vitamin C intake |  |  |  |  |  |  |  |
| Placebo | 220 | 4 | 1.00 |  | 206 | 18 | 1.00 |
| GTE | 213 | 19 | 4.7 (1.6, 14.2) |  | 191 | 41 | 2.5 (1.4, 4.7) |
| High vitamin C intakes |  |  |  |  |  |  |  |
| Placebo | 230 | 6 | 1.00 |  | 220 | 16 | 1.00 |
| GTE | 206 | 14 | 2.3 (0.8, 6.5) |  | 185 | 35 | 3.0 (1.5, 5.9) |
| **Cholesterol Levels** |  |  |  |  |  |  |  |
| Normal cholesterol level (<200mg/dl) |  |  |  |  |  |  |  |
| Placebo | 182 | 3 | 1.00 |  | 168 | 17 | 1.00 |
| GTE | 177 | 17 | 5.6 (1.6, 20.1) |  | 162 | 32 | 2.0 (1.0, 3.8) |
| Mildly high cholesterol level (200-240mg/dl) |  |  |  |  |  |  |  |
| Placebo | 197 | 6 | 1.00 |  | 191 | 12 | 1.00 |
| GTE | 183 | 12 | 2.0 (0.7, 5.4) |  | 162 | 33 | 3.3 (1.6, 7.0) |
| High cholesterol level (>240mg/dl) |  |  |  |  |  |  |  |
| Placebo | 70 | 1 | 1.00 |  | 66 | 5 | 1.00 |
| GTE | 59 | 4 | 2.4 (0.2,26.5) |  | 52 | 11 | 2.2 (0.7, 7.4) |
| **Triglyceride Levels** |  |  |  |  |  |  |  |
| Normal triglyceride level (<150mg/dl) |  |  |  |  |  |  |  |
| Placebo | 388 | 7 | 1.00 |  | 368 | 27 | 1.00 |
| GTE | 378 | 29 | 3.9 (1.7, 9.3) |  | 341 | 66 | 2.8 (1.7, 4.5) |
| High triglyceride level (150mg/dl) |  |  |  |  |  |  |  |
| Placebo | 61 | 3 | 1.00 |  | 57 | 7 | 1.00 |
| GTE | 41 | 4 | 1.5 (0.3, 8.1) |  | 35 | 10 | 1.6 (0.5, 5.0) |
| a GTE, green tea extract; CI, confidence interval; BMI, body mass index; OR, odds ratio; NSAIDs, non-steroidal anti-inflammatory drugs | | | | | | | |
| b Abnormal ALT > 60 U/L; abnormal AST>35 U/L, at least in one of the monthly liver function test after randomization. | | | | | | | |
| c Odds ratios were derived from unconditional logistic regression models that also included age, smoking status and level of education. | | | | | | | |

106,758 women’s mammograms screened

24,416 invitations mailed to eligible women

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

76 ineligible for the present study

3 missing baseline liver tests

51 none liver tests at any follow-up visit

22 had baseline ALT greater than 60 U/L or baseline AST greater than 35 U/L

999 participants in the present study

505 women in GTE group

494 women in Placebo group

5,473 women further assessed for eligibility via phone screening

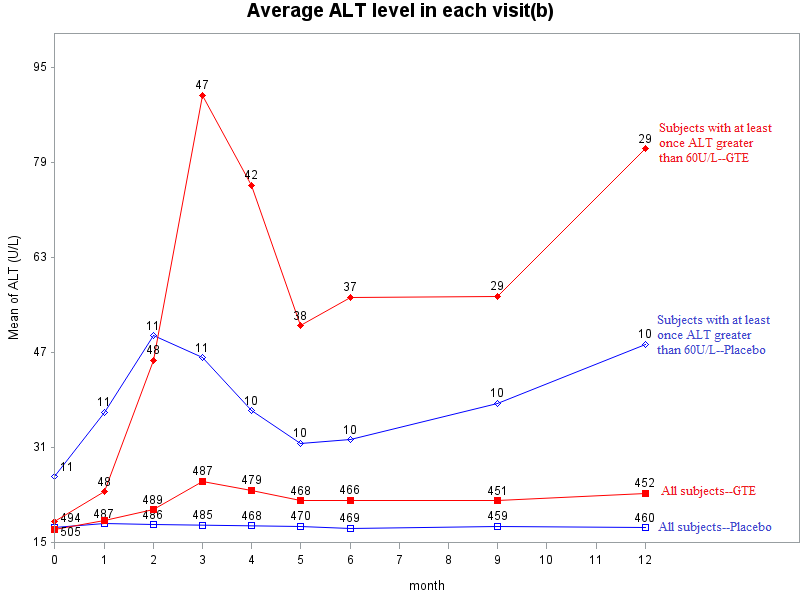
1,081 consented

1,075 randomized

516 non-respondents

516 ineligible

Figure Flow diagram of participant screening, enrollment, randomization, and eligible for the present sub-study, the Minnesota Green Trial, 2009-2015. ALT Alanine transaminase; AST Aspartate transaminase



60U/L

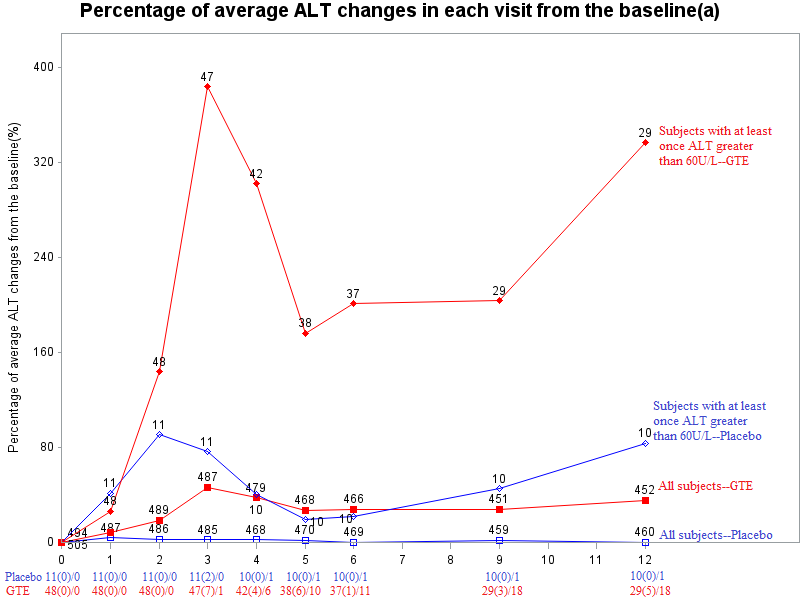
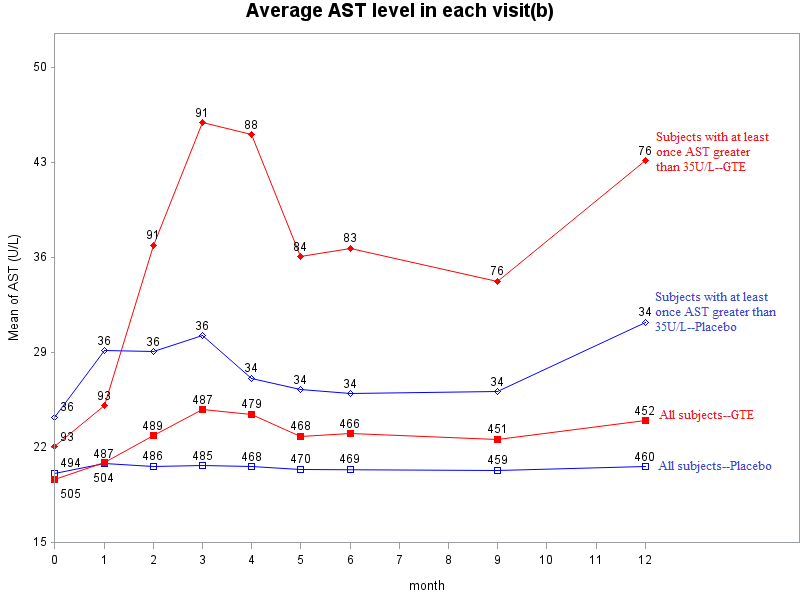
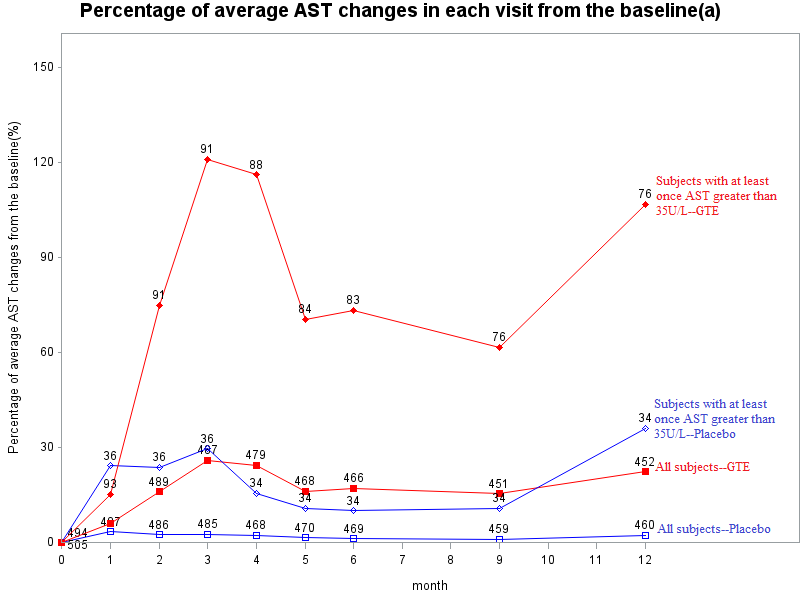


Figure 2 Joint lines of percentage of ALT increases over the baseline (a) and average ALT level (b) at each visit. Each line in the figure shows the outcomes among all subjects or subjects with at least once ALT greater than 60 U/L during 12-month intervention period in GTE or placebo group. For subjects with at least once ALT abnormal elevation, figure 1(a) also shows the number of subjects at each visit, the number of subjects without taking GTE or placebo capsules, and the number of accumulated drop-offs at the same time point. ALT alanine transaminase; GTE green tea extract.

Figure Joint lines of percentage of AST increases over the baseline (a) and average AST level (b) at each visit. Each line in the figure shows the outcomes among all subjects or subjects with at least once AST greater than 35 U/L during 12-month intervention period in GTE or placebo group. AST aspartate transaminase; GTE green tea extract.



35U/L



# appendix: SUPPLEMENTAL TABLES

Supplemental Table 1 Baseline mean concentrations of alanine transaminase (ALT) and aspartate transaminase (AST) in all subjects as well as in subgroups stratified by selected characteristics of study participants by treatment status (GTE or placebo) , the Minnesota Green Trial, 2009-2015

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | **ALT (U/L), mean (95% CI)** | | |  | **AST (U/L), mean (95% CI)** | | |
| **Placebo** | **GTE** | ***P\**** | **Placebo** | **GTE** | ***P\**** |
| Mean enzyme levels at baseline | 17.9 (17.3, 18.5) | 17.3 (16.7, 17.9) | 0.139 |  | 20.2 (19.8, 20.7) | 19.6 (19.1, 20.0) | 0.023 |
| Mean enzyme levels within stratified categories | |  |  |  |  |  |  |
| BMI (kg/m2) |  |  |  |  |  |  |  |
| <25 | 17.3 (16.5, 18.1) | 17.0 (16.2, 17.8) | 0.621 |  | 20.7 (20.2, 21.3) | 20.0 (19.4, 20.5) | 0.043 |
| 25- <30 | 18.2 (17.3, 19.2) | 17.9 (16.9, 18.9) | 0.580 |  | 19.7 (19.0, 20.4) | 19.4 (18.7, 20.1) | 0.540 |
| 30 | 20.4 (18.6, 22.2) | 17.5 (15.9, 19.1) | 0.016 |  | 19.6 (18.3, 20.9) | 18.5 (17.4, 19.6) | 0.214 |
| *P\** | 0.006 | 0.399 |  |  | 0.030 | 0.056 |  |
| Current Alcohol Drinker |  |  |  |  |  |  |  |
| No | 19.2 (17.8, 20.6) | 17.2 (16.0, 18.4) | 0.031 |  | 21.9 (20.9, 22.9) | 19.6 (18.8, 20.4) | <0.01 |
| Yes | 17.7 (17.1, 18.4) | 17.4 (16.7, 18.1) | 0.441 |  | 19.9 (19.4, 20.4) | 19.6 (19.1, 20.1) | 0.283 |
| *P\** | 0.0568 | 0.768 |  |  | <0.001 | 0.948 |  |
| Dietary vitamin C intake (mg/Kcal) |  |  |  |  |  |  |  |
| Less than median intake level (71.57) | 18.0 (17.1, 18.9) | 16.9 (16.1, 17.8) | 0.068 |  | 20.3 (19.6, 20.9) | 19.3 (18.7, 20.0) | 0.026 |
| Greater than median intake level (>71.57) | 17.8 (16.9, 18.7) | 18.0 (17.1, 18.9) | 0.790 |  | 20.3 (19.6, 20.9) | 19.8 (19.1, 20.4) | 0.272 |
| *P\** | 0.772 | 0.077 |  |  | 0.987 | 0.289 |  |
| Baseline blood cholesterol level (mg/dl) | |  |  |  |  |  |  |
| <200 | 18.6 (17.7, 19.6) | 17.7 (16.8, 18.7) | 0.158 |  | 20.5 (19.8, 21.2) | 19.8 (19.1, 20.5) | 0.142 |
| 200-240 | 17.3 (16.4, 18.2) | 17.7 (16.7, 18.6) | 0.537 |  | 20.1 (19.5, 20.8) | 19.5 (18.8, 20.2) | 0.145 |
| >240 | 17.7 (16.2, 19.1) | 15.6 (14.1, 17.2) | 0.062 |  | 20.0 (19.0, 21.1) | 19.0 (17.9, 20.1) | 0.181 |
| *P\** | 0.106 | 0.051 |  |  | 0.666 | 0.431 |  |
| Baseline blood triglycerides level (mg/dl) | |  |  |  |  |  |  |
| <150 | 17.6 (16.9, 18.3) | 17.2 (16.5, 17.9) | 0.429 |  | 20.3 (19.8, 20.8) | 19.4 (18.9, 19.9) | 0.007 |
| 150 | 19.5 (18.0, 21.1) | 18.6 (16.8, 20.4) | 0.441 |  | 20.0 (18.8, 21.1) | 20.3 (19.0, 21.6) | 0.678 |
| *P\** | 0.023 | 0.164 |  |  | 0.581 | 0.215 |  |
|  |  |  |  |  |  |  |  |
| **Supplemental Table 1 continued** | | |  |  |  | |  |
|  | **ALT (U/L), mean (95% CI)** | | |  | **AST (U/L), mean (95% CI)** | | |
|  | **Placebo** | **GTE** | ***P\**** |  | **Placebo** | **GTE** | ***P\**** |
| Current Use of Aspirin |  |  |  |  |  |  |  |
| No | 17.8 (17.0, 18.5) | 17.1 (16.4, 17.9) | 0.184 |  | 20.2 (19.7, 20.7) | 19.6 (19.1, 20.1) | 0.051 |
| Yes | 18.2 (17.2, 19.3) | 17.9 (16.8, 18.9) | 0.627 |  | 20.3 (19.5, 21.1) | 19.7 (18.9, 20.5) | 0.250 |
| *P\** | 0.445 | 0.627 |  |  | 0.859 | 0.809 |  |
| Current Use of Non-aspirin NSAIDs | |  |  |  |  |  |  |
| No | 18.8 (17.8, 19.8) | 16.5 (15.5, 17.5) | 0.001 |  | 20.4 (19.7, 21.1) | 19.7 (19.0, 20.5) | 0.165 |
| Yes | 17.4 (16.7, 18.2) | 17.7 (17.0, 18.5) | 0.519 |  | 20.2 (19.6, 20.7) | 19.5 (19.0, 20.1) | 0.075 |
| *P\** | 0.023 | 0.519 |  |  | 0.566 | 0.684 |  |
| Current Use of Acetaminophen |  |  |  |  |  |  |  |
| No | 18.2 (17.5, 18.9) | 17.3 (16.5, 18.0) | 0.047 |  | 20.3 (19.8, 20.8) | 19.6 (19.0, 20.1) | 0.023 |
| Yes | 17.1 (16.0, 18.2) | 17.5 (16.5, 18.5) | 0.546 |  | 20.0 (19.0, 20.1) | 19.7 (19.0, 20.4) | 0.539 |
| *P\** | 0.077 | 0.675 |  |  | 0.508 | 0.754 |  |
| Current Use of Statin |  |  |  |  |  |  |  |
| No | 17.4 (16.7, 18.0) | 16.8 (16.1, 17.5) | 0.167 |  | 20.0 (19.5, 20.5) | 19.2 (18.6, 19.7) | 0.011 |
| Yes | 19.6 (18.3, 20.8) | 19.1 (18.0, 20.3) | 0.595 |  | 21.1 (20.2, 22.0) | 21.0 (20.1, 21.8) | 0.835 |
| *P\** | 0.001 | 0.000 |  |  | 0.021 | <0.01 |  |
| Current Use of Antivirus |  |  |  |  |  |  |  |
| No | 18.0 (17.3, 18.6) | 17.4 (16.8, 18.0) | 0.174 |  | 20.2 (19.8, 20.7) | 19.7 (19.2, 20.1) | 0.051 |
| Yes | 16.3 (13.2, 19.3) | 15.7 (12.8, 18.6) | 0.786 |  | 20.7 (18.5, 22.9) | 17.8 (15.8, 19.9) | 0.062 |
| *P\** | 0.286 | 0.246 |  |  | 0.675 | 0.087 |  |
| Current Use of Antibiotics |  |  |  |  |  |  |  |
| No | 17.8 (17.1, 18.4) | 17.2 (16.5, 17.8) | 0.156 |  | 20.1 (19.7, 20.6) | 19.6 (19.1, 20.1) | 0.081 |
| Yes | 18.6 (17.1, 20.2) | 18.2 (16.8, 19.7) | 0.705 |  | 20.9 (19.8, 22.0) | 19.5 (18.5, 20.6) | 0.071 |
| *P\** | 0.313 | 0.178 |  |  | 0.210 | 0.876 |  |
| GTE, green tea extract; CI, confidence interval; BMI, body mass index; NSAIDs, non-steroidal anti-inflammatory drugs | | | | | | | |
| \*2-sided *P* values were derived from generalized linear models comparing the baseline liver enzymes (ALT or AST) between treatment groups, or between different categories of stratifying variable within placebo/GTE group after adjustments for age, smoking status and level of education. | | | | | | | |

Supplemental Table 2 The changes of alanine transaminase (ALT) and aspartate transaminase (AST) during the GTE treatment by *COMT* genotype, the Minnesota Green Tea Trial, 2009-2015

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Mean changes from baseline (95% CI) | **ALT (U/L), mean (95% CI)** | | | ***Pint\**** | **AST (U/L), mean (95% CI)** | | | | | ***Pint \**** | |
| **Placebo** | **GTE** | ***P*\*** | **Placebo** | **GTE** | ***P*\*** | |  | |
| All subjects | 0.1 (-1.0, 1.2) | 4.9(3.8, 6.1) | <.0001 |  | 0.2(-0.7, 1.1) | 3.6(2.7, 4.5) | | <.0001 | |  | |
| Stratifying Variables |  |  |  |  |  |  | |  | |  | |
| *COMT* Genotypes |  |  |  | 0.914 |  |  | |  | | 0.683 | |
| *LL* (low activity) | 0.2 (-1.2, 1.6) | 5.2 (3.8, 6.5) | <.0001 |  | 0.6 (-0.5, 1.7) | 3.6 (2.5, 4.6) | | <.0001 | |  | |
| *HL* (Intermediate activity) | -0.3 (-1.6, 1.0) | 4.3 (3.1, 5.7) | <.0001 |  | -0.02 (-1.0, 1.0) | 3.5 (2.4, 4.5) | | <.0001 | |  | |
| *HH* (High activity) | 0.5 (-0.9, 1.9) | 5.4 (4.1, 7.0) | <.0001 |  | 0.2 (-0.9, 1.3) | 3.7 (2.6, 4.8) | | <.0001 | |  | |
| *P*\* | 0.478 | 0.202 |  |  | 0.419 | 0.897 | |  | |  | |
| GTE, green tea extract; *COMT catecheol-O-methyltransferase*; CI, confidence interval; BMI, body mass index; All means are from Least Squares Means. | | | | | | | | | | | |
| \*All 2-sided *P* values were derived from generalized linear mixed models comparing the changes of liver enzyme (ALT or AST) during the intervention period from baseline between treatment groups, or among different categories of *COMT* genotype, as well as interactions after adjustments for age, BMI, smoking status and level of education. | | | | | | | | | | | |

Supplemental Table 3 Demographic details and alanine transaminase (ALT) examinations of subjects who had at least once ALT greater than 60 U/L and stopped taking GTE or placebo during the 12-month study period, the Minnesota Green Trial, 2009-2015

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| ID | Age at baseline | Treatment Assignment | *COMT* genotype | Baseline | | Date of Randomization | First Abnormal Elevation | | Highest ALT Test | | Last ALT Test | | Date of Stopping taking GTE | Status | Note |
| Date | ALT (U/L) | Date | ALT (U/L) | Date | Value (U/L) | Date | Value (U/L) |
| 20 | 54.3 | GTE | *LL* | 11/16/2009 | 14 | 12/2/2009 | 6/14/2010 | 68 | 6/14/2010 | 68 | 8/23/2010 | 33 | 6/14/2010 | Withdrawal | She was off the study for 2 months before her ALT got back |
| 63 | 51 | GTE | *HH* | 3/4/2010 | 17 | 4/16/2010 | 8/3/2010 | 65 | 8/3/2010 | 65 | 11/2/2010 | 41 | 8/3/2010 | Withdrawal | Her ALT was high again when retaking the GTE. |
| 72 | 54.3 | GTE | *LL* | 3/24/2010 | 17 | 4/23/2010 | 7/2/2010 | 118 | 7/2/2010 | 118 | 8/12/2010 | 22 | 7/2/2010 | Withdrawal | She was terminated from the study immediately when her ALT was 118U/L. |
| 92 | 55.3 | Placebo | *HL* | 4/7/2010 | 21 | 5/24/2010 | 9/28/2010 | 67 | 9/28/2010 | 67 | 10/25/2010 | 53 | 9/28/2010 | Withdrawal | She was terminated from the study immediately when her AST was 90U/L. |
| 116 | 62.1 | GTE | *HH* | 4/22/2010 | 17 | 7/1/2010 | 9/16/2010 | 117 | 9/16/2010 | 117 | 11/24/2010 | 58 | 9/16/2010 | Withdrawal | Her ALT was high again when retaking the GTE. |
| 132 | 61.6 | GTE | *HL* | 5/26/2010 | 17 | 7/18/2010 | 11/29/2010 | 268 | 11/29/2010 | 268 | 12/20/2010 | 82 | 11/29/2010 | Withdrawal | She was terminated from the study immediately when her ALT was 268U/L. |
| 145 | 53.4 | GTE | *HL* | 5/27/2010 | 28 | 7/28/2010 | 9/3/2010 | 80 | 9/3/2010 | 80 | 3/28/2011 | 29 | 9/3/2010 | Withdrawal | Her ALT was high again when retaking the GTE. She asked to withdraw from the study. |
| 197 | 51.7 | GTE | *HH* | 7/16/2010 | 16 | 9/28/2010 | 2/4/2011 | 383 | 2/23/2011 | 399 | 3/8/2011 | 31 | 2/4/2011 | Withdrawal | She was terminated from the study immediately when her ALT was 383U/L. |
| 218 | 54.5 | GTE | *LL* | 9/1/2010 | 12 | 10/14/2010 | 1/18/2011 | 483 | 1/18/2011 | 483 | 4/5/2011 | 38 | 1/18/2011 | Withdrawal | She was terminated from the study immediately when her ALT was 483U/L. |
| **Supplemental Table 3 continued** | | | | | |  |  | |  | |  | |  |  |  |
| ID | Age at baseline | Treatment Assignment | *COMT* genotype | Baseline | | Date of Randomization | First Abnormal Elevation | | Highest ALT Test | | Last ALT Test | | Date of Stopping taking GTE | Status | Notes |
| Date | ALT (U/L) | Date | ALT (U/L) | Date | Value (U/L) | Date | Value (U/L) |
| 240 | 64.1 | GTE | *HL* | 10/14/2010 | 14 | 11/9/2010 | 2/17/2011 | 128 | 2/17/2011 | 128 | 4/15/2011 | 47 | 2/17/2011 | Withdrawal | She was terminated from the study immediately when her ALT was 128U/L. |
| 461 | 65.4 | GTE | *HL* | 12/20/2011 | 18 | 1/19/2012 | 5/10/2012 | 102 | 8/6/2012 | 143 | 8/22/2012 | 83 | 5/10/2012 | Withdrawal | Her ALT was high again when retaking the GTE. |
| 475 | 62.8 | GTE | *HL* | 12/23/2011 | 30 | 1/26/2012 | 3/15/2012 | 81 | 3/15/2012 | 81 | 8/13/2012 | 27 | 3/15/2012 | Withdrawal | She asked to withdraw from the study. |
| 477 | 52.8 | GTE | *LL* | 12/9/2011 | 16 | 2/1/2012 | 8/9/2012 | 70 | 11/2/2012 | 2055 | 11/6/2012 | 1762 | 11/2/2012 | Withdrawal | She was terminated from the study immediately when her ALT was 2055U/L. |
| 558 | 57.1 | GTE | *HL* | 2/8/2012 | 13 | 3/6/2012 | 6/19/2012 | 497 | 6/19/2012 | 497 | 8/8/2012 | 59 | 6/19/2012 | Completed - ITT | She was terminated from the study immediately when her ALT was 497U/L. |
| 608 | 56.4 | GTE | *HL* | 2/20/2012 | 27 | 4/2/2012 | 7/11/2012 | 192 | 7/11/2012 | 192 | 8/7/2012 | 79 | 7/11/2012 | Withdrawal | She was terminated from the study immediately when her ALT was 192U/L. |
| 833 | 63.8 | GTE | *HH* | 6/12/2012 | 20 | 7/20/2012 | 11/1/2012 | 114 | 1/2/2013 | 303 | 2/25/2013 | 59 | 11/1/2012 | Completed - ITT | Her ALT was high again when retaking the GTE. She was terminated from the study immediately when her ALT was 303U/L. |
| 864 | 65.9 | GTE | *LL* | 6/22/2012 | 14 | 8/9/2012 | 11/20/2012 | 896 | 11/20/2012 | 896 | 1/10/2013 | 80 | 11/20/2012 | Completed - ITT | She was terminated from the study immediately when her ALT was 896U/L. |
| 992 | 68.5 | GTE | *LL* | 10/9/2012 | 17 | 12/13/2012 | 2/21/2013 | 93 | 3/21/2013 | 120 | 5/24/52013 | 54 | 2/21/2013 | Withdrawal | Her ALT was high again when retaking the GTE. |
| **Supplemental Table 3 continued** | | | | | | | | | | |  |  |  |  |  |
| ID | Age at baseline | Treatment Assignment | *COMT* genotype | Baseline | | Date of Randomization | First Abnormal Elevation | | Highest ALT Test | | Last ALT Test | | Date of Stopping taking GTE | Status | Notes |
| Date | ALT (U/L) | Date | ALT (U/L) | Date | Value (U/L) | Date | Value (U/L) |
| 996 | 67.6 | GTE | *HH* | 11/15/2012 | 26 | 12/27/2012 | 3/7/2013 | 243 | 3/7/2013 | 243 | 3/21/2013 | 41 | 3/7/2013 | Withdrawal | She was terminated from the study immediately when her ALT was 243U/L. |
| GTE, green tea extract; *COMT catecheol-O-methyltransferase*; *LL* low activity; *HL* intermediate activity; *HH* high activity; AST aspartate transaminase; ITT intention-to-treat | | | | | | | | | | | | | | | |