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The Effect of Green Tea Extract on Endurance Performance in Young Adults

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CHAPTER I
INTRODUCTION

Over the past several years, many published studies have provided evidence that consumption of green tea (GT), green tea extract (GTE), or epigallocatechin gallate (EGCG, the main component of GT) can result in weight loss, increased fat oxidation, and elevated energy expenditure (EE) in mice and humans both at rest and while exercising (1-8). Meta-analyses and review articles have also confirmed these results (9-12). Furthermore, consumption of GTE has been shown to result in increased exercise endurance in mice (4, 13, 14) and improved maximal oxygen uptake (VO_{2max}) in humans (15). These findings suggest that consumption of GT or GTE might improve exercise endurance in humans, possibly by increasing fat oxidation (4). In contrast, a recent study by Dean et al. (16) found no significant increase in exercise endurance in cyclists consuming EGCG. However, they only examined the effect of short-term (6 days), moderate dose (270 mg) supplementation of caffeine-free EGCG. The objective of this study was to determine if the daily consumption of decaffeinated GTE, for 4-5 weeks, could improve exercise endurance in humans, as measured by 2.5 mile running time trials conducted on an indoor track.

CHAPTER II
REVIEW OF LITERATURE

Background

GT, one of the most consumed beverages in the world (17, 18), has received a great deal of attention in the last few decades as a potential enhancer of health. Research has suggested that consumption of GT could promote weight loss (2, 3, 8, 19-22); protect against cardiovascular disease (2, 10, 23-25) and cancer (26-31); scavenge free radicals more effectively than vitamins C and E (32, 33); reduce insulin resistance in diabetics (5, 7); combat the HIV virus (34, 35); protect against neurodegenerative diseases such as Alzheimer Disease and Parkinson Disease (36); and reduce all of the risk factors related to metabolic syndrome (a condition that refers to a group of cardiometabolic risk factors that usually includes dyslipidemia, central obesity, hyperglycemia, and hypertension) (21, 37). These health benefits associated with GT consumption are attributed to a class of polyphenols known as catechins (10, 18, 33, 38). Catechins present in GT include EGCG, epicatechin gallate, gallic catechin, and epigallocatechin. EGCG has received the most attention, as it is considered to be the most potent and biologically active catechin in GT (3, 38-40).

While some of the catechins found in GT are also found in small amounts in chocolate, some fruits and vegetables, some nuts, and carob flour, GT contains the highest amounts of them per typical serving size (41). Black tea also contains some of these same catechins, but has significantly less than GT because the fermentation process used to make black tea changes the chemical structure of the polyphenols found in the tea leaf (42).

The exact amount of catechins in GT varies greatly depending on where and how it is grown and harvested, how long and in which manner it is stored, and how much of it and by which methods it is prepared (10, 43). Estimates range from ~100-250 mg of total catechins (43) and ~26-226 mg EGCG per cup of tea. It is also estimated that the median amount of EGCG per cup of GT is ~ 150 mg (15). Those estimates are fairly consistent with the amounts of GT catechins measured by the USDA. Their “Flavonoid Database” lists an estimated average amount of ~125 mg of catechins and ~77 mg of EGCG per 1 g of GT leaves infused in water (41). The 1.5 -2.0 g of dry tea contained in many tea bags and recommended for use by distributors of loose leaf tea (44) would result in, based on USDA data, 187-250 mg of total catechins and 116-155 mg of EGCG in a cup of tea. Also, new extraction methods have recorded levels of catechins in GT higher than previously measured (45), suggesting there could be more total catechins in GT than previously thought. Although the exact amount of total catechins and EGCG in a “typical” cup of tea is difficult to estimate, based on the above research, it would be reasonable to expect an average cup of tea to contain approximately 90-170 mg EGCG and 150-250 mg total catechins.

The effect of GT on weight loss, fat oxidation, and EE in mice and humans

In 1999, Dulloo et al. (3) conducted one of the earliest studies that examined the potential of GT consumption to increase EE and fat oxidation. In this double-blind, crossover study design, participants spent three separate 24-hr trials in a respiratory chamber. During each trial, the participants consumed 2 capsules with each of their 3

meals (6 capsules per trial). The 6 capsules contained, in total, one of the following: (1) GTE, containing 375 mg catechins (270 mg EGCG) and 150 mg caffeine; (2) 150 mg caffeine only; or (3) placebo (inert cellulose). The order of the trials for each subject was randomly assigned. During the 24-hour period spent in the respiratory chamber, participants' diet, activity pattern (sedentary), meal pattern, and sleeping habits were the same. Results showed that, relative to caffeine and placebo, consumption of GTE significantly increased EE ($P < 0.01$), and lowered the respiratory quotient (RQ) ($P < 0.001$), indicating increased fat oxidation. Treatment with caffeine equal to that contained in the GTE had no significant effect on either measurement.

This study had some strengths. It was well controlled as participants were confined to a laboratory setting with activity levels and diet strictly matched. Also, researchers not only compared GT to placebo, but also compared it directly with an equal amount of caffeine. Because of this design, the results of this study strongly suggest that the increased EE and fat oxidation elicited in the subjects consuming GTE were due, in large part, to the effects of the catechins in the GTE, and not primarily due to its caffeine content or variations in the participants' diets or activity levels.

Several studies conducted on mice published over the past decade found results similar to that of Dulloo et al. (3). These studies showed that consumption of GTE resulted in significant reductions in body weight, abdominal fat mass, cholesterol, and serum triacylglycerols in mice fed both normal and high fat (HF) diets (46-48). A 2008 study (49) looked at the effect of EGCG on mice fed a HF diet. Compared with control mice fed a HF diet, mice that consumed EGCG while on a HF diet (HFE) had

significantly less body weight gain (33-41% less), lower percent body fat, and lower visceral fat weight (37% less) after long term (16 week) EGCG supplementation ($P < 0.05$ for all measures). Mice fed the HFE diet also had significantly ($P < 0.05$) lower blood glucose (25% decrease), plasma insulin (61% reduction), plasma cholesterol, liver weight, liver triacylglycerols, and plasma alanine aminotransferase concentrations (a marker of liver damage and predictor of nonalcoholic fatty liver disease) compared with mice fed the control HF diet (49, 50).

This study had many strengths. It examined the effects of EGCG alone, thus removing the potential for caffeine to affect the results. It controlled for and examined a number of markers that reflect fat metabolism. Furthermore, compared with some previous animal studies, the amount of EGCG given to the mice was lower (3.2 g/kg food intake) and thus more relevant to humans (49). Relative to their food intake, mice consumed the amount of EGCG equivalent to a human on a 2,000 kcal diet consuming 8-10 cups of green tea per day, which is considered a safe amount (51-53). The authors suggested that the reductions in body weight, fat gain, and insulin resistance found in mice supplemented with EGCG were likely due to decreased fat absorption, anti-inflammatory effects, decreased fatty acid synthesis, and increased fatty acid oxidation (49). The results of these mice studies reinforced the earlier findings of Dulloo et al. (3) that consumption of GT can induce thermogenesis and increase fat oxidation.

In 2009, Thielecke and Boschmann (37) reviewed the effects of short- and long-term consumption of GT on metabolic syndrome in humans. The authors reviewed 12 human studies on the effects of GT consumption on weight loss and 9 studies on GT

consumption and its effects on EE and fat oxidation. The majority of these studies showed significant decreases in weight and fat mass, and increases in EE and fat oxidation for GT treatment vs. placebo ($P < 0.05$). Studies showed body weight reductions ranging from 0.7 kg to 3.5 kg, and body fat reductions ranging from 0.7 kg to 1.8 kg in GT groups. GT had a significant effect on body weight and body fat reduction in studies where subjects consumed a moderate energy-restricted diet as well as in those studies where subjects consumed a normal diet.

A 2009 meta-analysis (12) examined the effects of GT on weight loss (WL) and weight maintenance (WM). These researchers controlled for high habitual caffeine intake and ethnicity. GT studies on Asian populations and those with low habitual caffeine intake have found a greater effect of GT on WL than studies on non-Hispanic white populations and those on habitually high caffeine users. Eleven WL or WM studies met their inclusion criteria of being randomized, with blinded participants, using a GT or GTE intervention compared with placebo, and a length of 12 weeks. Their analysis revealed that GT plus caffeine significantly reduced or maintained BW when compared with placebo ($P < 0.001$). While neither the amount of habitual caffeine intake nor ethnicity individually affected WL or WM significantly ($P > 0.05$), the interaction of ethnicity and caffeine intake was a significant moderator ($P = 0.04$)

In a recent systematic review and meta-analysis, Phung et al. (11) analyzed randomized control trials looking at the relationship between GT or GTE consumption and anthropometric measures such as body mass index (BMI), body weight, waist circumference (WC), and waist-to-hip ratio (WHR). Fifteen studies met inclusion criteria

and were divided into 3 separate groups: six studies that compared GT or GTE plus caffeine (GT+C) with a caffeine-matched control, five studies that compared GT+C with a caffeine-free control, and two that compared decaffeinated GTE (GT-C) with a caffeine-free control. Using a random-effects model, a weighted mean difference of change from baseline (with 95% Confidence Intervals [CIs]) was calculated for each group of studies.

Results of the meta-analysis revealed the following: (1) GT+C showed statistically significant reductions in BMI, body weight, and WC compared with the caffeine only group; (2) GT+C significantly decreased body weight when compared with a caffeine-free control; (3) GT-C did not show any statistically significant changes in anthropomorphic measures compared with the caffeine-free placebo. The significant results found in the pooled data of first two groups of studies support previous research that consumption of GT can promote weight and fat loss (however, the authors stated that the clinical significance is “modest”). The authors concluded that there might be a synergistic effect between catechins and caffeine in increasing energy expenditure and fat oxidation. While the authors did not find any effect of decaffeinated GT alone on anthropomorphic measures, they only examined two studies that tested this, with one finding some significant effects on BMI and weight loss and the other not finding such effects. Their conclusions were in contrast to previous studies on mice discussed above that found a pronounced effect of decaffeinated GT or EGCG on body weight.

Lastly, Thielecke et al. (6) published a study last month (April 2010, after the publication of the above meta-analyses) that found that short-term (3 days), consumption

of decaffeinated, “low” dose (300 mg) EGCG by obese men significantly increased ($P < 0.05$) postprandial fat oxidation compared with placebo. This effect was equal to the effect of consumption of 200 mg caffeine. While EGCG did not significantly increase fasting fat oxidation over placebo, 200 mg caffeine did (by 26.3%, $P < 0.05$), and EGCG plus caffeine did so to an even greater extent (by 35.4%, $P < 0.01$ vs. placebo and EGCG alone). Both fasting and post-prandial RQ values were significantly lower for EGCG + caffeine when compared with placebo ($P < 0.001$).

Mechanisms of action of GT consumption on weight loss

Several mechanisms have been proposed to explain why consumption of GT is linked to reductions in body fat, weight, and central adiposity. Firstly, EGCG receptors are found in most cells, which potentially explains the far-reaching effects of GT (39). GT has been proposed to reduce body fat by increasing fat oxidation, decreasing lipogenesis, and decreasing fat absorption (1, 5, 37, 39). It is proposed that GT increases fat oxidation by prolonging the action of norepinephrine, a catecholamine released by the sympathetic nervous system, through inhibition of catechol-O-methyl-transferase (COMT), an enzyme that degrades norepinephrine (3, 40). Elevated levels of norepinephrine increase hepatic fat oxidation and overall mobilization of lipids for oxidation from adipocytes throughout the body (1, 3, 39, 40).

GT is further theorized to promote fat oxidation and weight loss through activation of thermogenesis in brown adipose tissue (3, 40). GT catechins have been shown to work synergistically with caffeine (a natural component of green tea) to

increase the respiration rates of brown adipose tissue in mice more than an equal amount of caffeine alone did. Combinations of ephedra, caffeine, and GT have also been shown to produce weight loss in humans (3, 40). However, such weight loss did not appear to be occurring in humans via activation of brown adipose tissue because, until recently, it was thought that adult humans do not have any significant amounts of this thermogenic tissue.

However, three recent studies (54-56) provided strong evidence that adult humans do contain significant stores of brown adipose tissue. It is now known that increased action of norepinephrine or being subjected to cold can activate brown adipose tissue in humans. Because GT has been shown to increase or prolong the action of norepinephrine (3, 13, 40), it is plausible that GT activates brown adipose tissue in humans as it does in mice. This could explain some of the positive results that have shown GT to be an aid in increasing energy expenditure, thermogenesis, and weight loss.

Several reviews (9-11) have suggested other possible mechanisms through which GT consumption might aid in fat reduction, including decreasing energy intake and absorption (via reductions in appetite, food consumption, blood glucose, lipid emulsification, lipid digestion and absorption, lipid transport, and insulin concentrations). It is theorized that those actions result in positive effects on adipocytes such as increased apoptosis and lipolytic activity, and decreased growth, differentiation, lipogenic activity, and glucose intake. This can lead to increased energy expenditure via increased thermogenesis, fat oxidation, muscle cell glucose uptake (due to increased GLUT 4

activity), fecal lipid excretion, tumor necrosis factor α , and decreased hepatic liver production (39).

The effects of GT consumption on exercise endurance in mice

The early research demonstrating that the consumption of GT can increase fat oxidation in humans and rodents led some researchers to hypothesize that GT could potentially be a useful aid for increasing exercise endurance. This hypothesis was based on the fact that the ability to oxidize more fat during exercise is a likely advantage for endurance athletes (4, 13, 57). This is because glycogen stores are limited, and therefore the body will increasingly rely on fat for fuel as muscles become depleted of glycogen (58). In fact, one of the adaptations that occurs as a result of exercise is an increased ability to oxidize fat (57), which delays glycogen depletion. Furthermore, research has shown that muscles do not have to be completely empty of glycogen in order for fatigue to occur (58, 59). It has been shown that reduced glycogen content in muscles will immediately result in athletes choosing a slower pace and easier effort when beginning a 1 hour time trial (58). This may be due to the fact that any significant decrease in glycogen stores could signal the brain, possibly via feedback sent to the CNS from chemoreceptors in muscle, that exhaustion is near and therefore cause the brain to signal muscles to reduce their effort (58-60).

Therefore, an ability to keep glycogen stores relatively high during exercise might delay the stress signals sent to the brain that result in decreased muscular effort (58) and increasing one's use of fat for fuel could help one achieve that end. Also, increased use of

fatty acids for energy in place of glucose tends to be associated with lower lactate levels, which is associated with lower fatigue levels (4, 13). Renowned Italian distance running coach Renato Canova, who works closely with several of the best Kenyan distance runners, has written extensively on the importance of fat oxidation for endurance running (61).

All of the aforementioned reasons explain why the ability to increase use of fat for energy, even during shorter endurance exercise events, which do not highly deplete glycogen stores, could be advantageous for delaying fatigue. Because GT has been shown to increase fat oxidation in mice and humans, the hypothesis that it might be able to increase exercise endurance was a natural next step to investigate.

Murase et al. (4, 13) conducted two studies on mice that became the first to directly measure the effect of GTE on exercise endurance. In each study the researchers gave three groups of mice the same endurance training and the same diet for 8-10 weeks. One group received 0.2% GTE (wt/wt) in their diet, another group received 0.5% GTE, while the exercise-control (Ex-cont) group received no GTE. These studies compared the effects of GTE and placebo on endurance capacity (running and swimming to exhaustion), energy metabolism, and fat oxidation. Mice given endurance training plus 0.5% GTE improved endurance capacity by 24-30% more than Ex-cont mice. These findings were statistically significant ($P < 0.05$, $P < 0.001$). Mice given 0.2% GTE also increased their endurance capacity compared to Ex-cont mice in both studies, but the differences were not statistically significant.

These two studies also revealed that compared to Ex-cont mice, the mice fed 0.5% GTE had significantly ($P < 0.05$ and $P < 0.01$) lower respiratory exchange ratios (RER's) both at rest and during exercise, higher muscle β -oxidation, and higher concentrations of plasma non-esterified fatty acids (NEFA). Immediately after exercise, mice fed GTE also had significantly lower levels of plasma lactate and lower reductions in muscle glycogen content, the latter by an impressive 84%. Furthermore, GTE mice had lower levels of muscle malonyl-CoA content. All of these measurements indicated an increased utilization of fat both at rest and during exercise.

Murase et al. (13) found that EGCG consumption also significantly improved endurance in mice. This suggests that the endurance-enhancing effect of GT consumption is strongly mediated by EGCG. However, because the effects were not as pronounced as those found with GTE, EGCG is likely not the only component of GT that influences endurance and fat metabolism.

In both of Murase et al.'s studies (4, 13), the GTE consumed by the mice contained a negligible amount of caffeine. In the second study (4) they state that their GTE "did not contain caffeine". However, earlier in the article they state that it contained 0.1% caffeine, the same amount as their first study. Furthermore, in their first study (13), consumption of decaffeinated EGCG elicited much of the same effects as did consumption of GTE. Therefore, the authors concluded that it was the catechins present in GT, not the caffeine, that produced the significant effects on measured endurance and fat metabolism.

Other studies looking at the effects of GT on physical capacity and endurance in mice observed similar physiological improvements with GT consumption (38, 47, 48). The findings from these studies suggest that GT consumption increases whole body lipid utilization and decreases carbohydrate use during exercise. As discussed above and demonstrated in the time to exhaustion results, such actions appear to be advantageous for delaying fatigue during endurance exercise (4, 13).

The effects of GT consumption on fat oxidation in exercising humans

With GT/GTE consumption having been shown to increase fat oxidation in humans and mice at rest and to improve endurance and fat oxidation in exercising mice, the next step was to determine if GT could enhance fat oxidation in humans during exercise. Two recent studies demonstrated just this (1, 5). Venables et al. (5) found that the acute (24 hr.) consumption of decaffeinated GTE significantly ($P < 0.01$) increased fat oxidation in human subjects during a single moderate exercise bout (workload = 60% VO_{2max} ; average HR 135 bpm). Subjects oxidized 17% more fat during exercise after consuming GTE (3 capsules in 24 hours containing a total of ~ 900 mg polyphenols and 370 mg EGCG) than they did after consuming placebo (5).

A randomized, double-blind design by Maki et al. (1) also demonstrated that GT consumption might increase fat oxidation in exercising humans. Obese participants who completed a 12-week protocol of exercise and daily consumption of a GTE beverage (containing 625 mg GT catechins, including 215 mg EGCG) had significantly higher

decreases in total abdominal fat area ($P = 0.013$) and subcutaneous abdominal fat area ($P = 0.019$) compared with obese subjects who only exercised during this period (1).

Both the Venables et al. (5) and Maki et al. (1) studies showed that GT consumption appears to enhance the fat oxidation effects of exercise in humans. Venables et al.(5) showed this effect using a very short term (24 hrs), moderate dose supplementation of decaffeinated GTE (= ~ 3-4 cups GT per day), while Maki et al. did so using a longer term (12 weeks), low dose supplementation of a GTE beverage (= ~ 2 cups GT/d) containing a small amount of caffeine. Taken together, these two studies strongly suggest that acute or regular consumption of low to moderate doses of GT catechins, containing no or little caffeine, can result in increased fat oxidation in exercising humans.

Mechanisms of action of GT consumption on increased fat oxidation during exercise

Although the exact mechanisms through which GT consumption increases lipid usage during exercise is not fully understood, they appear to be similar to those mechanisms by which GT promotes weight loss. First, GT decreases the malonyl-CoA content of muscles, which results in increased plasma fatty acid (FA) concentrations in the blood (4). If such an increase occurs during exercise, the body may adapt to the increased FA exposure by utilizing greater amounts of lipids (4, 47). Second, GT inhibits COMT, an enzyme that degrades norepinephrine, a catecholamine produced by the sympathetic nervous system, which becomes very active during stressful situations such

as exercise. Norepinephrine is released to augment physiologic functions necessary to survive, or more efficiently handle, such stress (3, 4, 62).

Norepinephrine interacts with a series of adrenergic receptors (α - 1 and 2, and β - 1, 2, and 3) in order to induce its effects. Stimulation of these receptors triggers synthesis of cyclic adenosine monophosphate (cAMP), a secondary messenger, which in turn activates protein kinase A (PKA) resulting in increased action by hormone sensitive lipase (62). This metabolic cascade amplifies various physiologic functions including heart rate, peripheral blood flow, glycogenolysis, glucose production, and lipolysis in adipocytes. All of these actions are potentially beneficial during exercise. By stimulating lipolysis in fat cells, the pool of available FA's that can be used for fuel is increased. Furthermore, catecholamines such as norepinephrine directly increase fatty acid availability in skeletal muscle by increasing the breakdown of triacylglycerols via hydrolysis, an action controlled by PKA stimulation of hormone sensitive lipase. Because hormone sensitive lipase is already activated in muscle during exercise, GT consumption might heighten muscle triacylglycerol breakdown during exercise via prolonging the action of norepinephrine (62).

The effects of GT consumption on exercise endurance in humans

With evidence that consumption of GT enhances fat oxidation in humans both at rest and during exercise, and exercise endurance in mice, researchers next tested whether consumption of GT could improve exercise endurance in humans. In a randomized

double-blind study, Richards et al. (15) demonstrated that very short term consumption of EGCG (405 mg/d for 2 d, and 135 mg taken 2 hrs before exercise test) significantly ($P=0.04$) increased maximal oxygen uptake (VO_{2max}) in healthy adults engaged in stationary cycling exercise. VO_{2max} (maximal cardiac output x maximal arterial-venous oxygen difference) is considered an important measurement or predictor of an individual's potential aerobic or endurance exercise ability (15). Because this study did not demonstrate an increase in the participants' maximal cardiac output, the authors concluded that EGCG was likely exerting its effects on VO_{2max} by increasing the maximal arterial-venous oxygen difference.

The authors suggested that this could occur through the antioxidant properties of EGCG, an augmentation of the metabolic response to β -adrenergic receptor stimulation (which occurs during exercise) or the uncoupling of electron transport from ATP synthesis. The authors speculated the last possibility because research has shown that EGCG consumption can increase uncoupling protein-2 gene expression in 3T3-L1 fat cells. Thus, it is thought that the increased energy expenditure and lipid oxidation found in humans consuming EGCG might be due to the uncoupling of electron transport from ATP synthesis (15).

The Richards et al. (15) study only looked at the effect of GTE consumption on a physiological marker (VO_{2max}) for endurance potential and did not truly test its effect on endurance performance in humans. However, just recently a study was published (16) that examined the relationship between GTE consumption and endurance performance.

The study used a 3-way crossover, randomized, placebo-controlled, double-blind, diet-controlled research design. All participants, who were trained cyclists, received, in random order, 3 different treatments (placebo 270 mg, EGCG 270 mg, and placebo 270 mg + caffeine 3 mg/kg) over a 6-d period and 1 hr before exercise testing. Exercise testing involved each participant completing 3 exercise trials consisting of 60 min of cycling at 60% $\text{VO}_{2\text{max}}$ immediately followed by a self-paced 40-km cycling time trial. This study did not find any effect of EGCG consumption on exercise endurance, or on fat oxidation.

While this was a well-controlled and well-designed study, it had some limitations. While EGCG appears to be the most active nutritional constituent of GT, it is not the only catechin present in GT that exerts physiological effects (13). Also, long-term consumption of GT is thought to have the most profound effect on an individual's physiology. Therefore, six days of supplementation might not be a long enough duration to determine the potential of GT consumption to improve exercise endurance. In fact, in the studies that demonstrated improved endurance in mice consuming GTE, significant improvements were not measured until eight weeks of supplementation had been completed (13).

Furthermore, the effects of GTE on the mice were dose-dependent, with the mice consuming the smaller amounts of GTE (= to a human consuming ~ 4 cups of GT/d) showing non-significant improvements in endurance (13). The mice that demonstrated significantly increased endurance compared to placebo mice consumed an amount of GTE that would be equivalent to a human consuming approximately 10 cups of GT per

day. The subjects in this human study only consumed the amount of EGCG found in ~ 2 cups of GT a day. In short, the researchers might have found different results if they had used a whole GTE supplement rather than EGCG, and/or used a higher dose of catechins, and/or a longer supplementation period.

In conclusion, several studies clearly demonstrate that consumption of GT can improve exercise endurance in mice (4, 13, 14) and increase whole body lipid utilization in mice and humans during exercise and at rest (1-5, 7, 8, 19-21, 37, 47). One study showed an improved VO_{2max} in humans who consumed EGCG (15). Together, these findings suggest that consumption of GT or GTE could result in improved endurance ability in humans. However, this hypothesis has only been tested once before (16). While that study failed to find a significant effect of EGCG on endurance, it had several limitations. Therefore, the objective of this study was to determine if the daily consumption of a higher dose decaffeinated GTE, for 4-5 weeks, could improve exercise endurance in humans, as measured by 2.5 mile running time trials conducted on an indoor track.

CHAPTER III**METHODS**

Participants

In order to be eligible for the study, participants had to be aged 18-40 yrs, not have a history of liver problems or any other chronic illness such as ulcers, diabetes, gastro-intestinal problems, not be currently ill, and not be pregnant. Also, participants must have been performing aerobic exercise at least twice a week for 20-30 minutes at a fairly vigorous pace, had consumed green tea or green tea extract without any adverse effects, and not be a cigarette smoker, excessive drinker (more than 5 alcoholic drinks per day), or illegal drug user. Therefore, all participants were healthy, physically active undergraduate or graduate students, with one faculty member participating (see Table 1). All participants had previously signed medical waivers to engage in demanding physical activity at the Georgia State University (GSU) Recreation Center. The Institutional Review Board at GSU approved the experimental protocol (see Appendix A).

Table 1: Participant characteristics

| <u>Exclusion Criteria</u> | <u>Inclusion Criteria</u> |
|------------------------------------|--|
| History of chronic illness | Age 18-40 (mean age: 23) |
| Currently ill | Currently perform vigorous aerobic exercise 2-3 x /wk |
| Pregnant | Signed medical waiver to exercise at GSU Recreation Center |
| Smoker | Prior consumption of GT with no side effects |
| Heavy drinker or illegal drug user | |

Subjects were recruited using fliers posted at the GSU Recreation Center (see Appendix B); in-person recruiting at the Recreation Center indoor track; in-person recruiting at selected Health Science classes; and e-mails sent to all Division of Nutrition students (see Appendix C). The objective, methodology, and risks of the study were explained to each subject and written informed consent was obtained (see Appendix D). Sixteen subjects agreed to participate.

Design

This study was designed as a randomized, placebo-controlled, double-blinded study. The participants were randomly and alternately divided into the A or B group in the order that they volunteered for the study. Participants were not informed whether they were receiving intervention (GTE) or placebo. The researcher was also blinded to which group was receiving which treatment.

Run Trials

Participants completed two running trials (RTs) separated by 4-5 weeks. Each run trial consisted of running 20 laps on the GSU Recreation Center indoor track (length: ~ 1/8 of a mile) for a total of ~ 2.5 miles. Participants were instructed to give their best effort in completing the distance as fast as they could. Every 2-3 laps they were given an update on how many laps they had completed. Lap counts were monitored and recorded by using a list numbered 1-20 for each participant and crossing off the appropriate

number as each lap was completed. Times were recorded via an Ironman Timex® stopwatch, and were backed up by the Recreation Center track clock.

RTs took place over several days, and 1-5 participants ran at the same time for each trial, with start times staggered by 30 sec to 2 min. During each RT, a few other runners not connected to the study were jogging on the track. While there was some concern that these other runners could interfere with the progress of the study participants, they did not do so. In fact, these other joggers gave the runners who completed their trials alone some sense of “company” or “competition” similar to that which the runners who ran at the same time as other participants experienced. An equal number of participants (n=2) from each treatment group ran their RT’s alone, while the others ran at the same time as other participants.

Intervention (GTE) and placebo treatments

The GTE capsules were obtained from Food Science of Vermont (Essex Junction, VT). Each capsule contained a total of 450 mg of GT polyphenols, which included 350 mg of EGCG , and also included a small amount of rice flour and vegetable cellulose (see Appendix E). Each capsule contained the amount of GT catechins and EGCG equivalent to about 2.5-3 cups of GT. Participants were instructed to consume 2 capsules/d, for a total of 900 mg polyphenols and 700 mg EGCG, roughly equivalent to about 5-6 cups of GT/d.

Placebo capsules were made using empty vegetable cellulose capsules, equal in the size to the GT capsules (size “0”), that were obtained from Wonder Laboratories (White House, TN). The capsules were filled with organic Buckwheat and Teff flour purchased from the local farmer’s market. Capsules were filled in sanitary conditions using a capsule filling machine purchased from Wonder Laboratories (White House, TN).

While the color of the GT and placebo capsules was not identical, neither one appeared green. Furthermore, participants were asked not to compare their capsules to those in the other group. Informal pretrial testing with individuals not participating in the study indicated that which capsules contained GTE or placebo could not be discerned. However, when asked to guess which treatment group they were in, the majority of participants were able to guess correctly (70% correct). None of them stated that they based their guess on the appearance of the capsules, but that they either guessed randomly or based on how they felt during the study.

Placebo and GT capsules were placed into identical, unmarked white bottles set aside in their respective groups. A researcher not connected with this study randomly determined which treatments would be labeled group A and group B, and then labeled the bottles appropriately. The researcher recorded this information separately from the rest of the study data, such that the researcher conducting the study and the participants were blinded as to the identity of groups A and B. Bottles were distributed to participants upon completion of their first RT, with participant 1 in group A, participant 2 in group B, and alternating from there. Participants were instructed to take 2 capsules/d (one in the morning, one at night) with food. They were given enough capsules to allow them to

consume that amount for 4-5 wk's. The initial study protocol planned for the supplementation period to last five weeks. However, due to time constraints, participants who volunteered late only took the supplement for 4 wks.

Diet and exercise records and controls

During the study period, participants were instructed not to change their diet from what they normally ate and to complete a 3-d food record on the USDA's website, MyPyramidTracker. Participants were also instructed not to consume any supplements other than multi-vitamins or fish oil; limit coffee consumption to no more than one cup a day (or ~ 150- 200 mg of caffeine from other sources); and to consume no tea beverages at all during the study period. These instructions were given to ensure that participants did not consume any supplements, beverages, or food that contained tea catechins or similar polyphenols. Participants were asked to limit caffeine consumption during the study because there is some evidence that, when consuming GT, high caffeine users demonstrate less of an increased fat oxidation effect than do lower caffeine users (12, 22). Lastly, participants were instructed to keep an exercise diary on their physical activity.

Participants were instructed to record the amount and type of exercise they engaged in (if any) on the day before RT1 (baseline run trial) and to repeat that type and amount of activity on the day before RT2 (post-intervention run trial). They were also instructed to consume the same diet on the day of their two RT's, and not to consume

coffee or any caffeine-containing beverages or foods within four hours of their RTs.

Records were checked at the end of the study to determine compliance.

Statistical analysis

Statistical analyses were conducted using independent (unpaired) *t*-tests (2-tailed) to compare between group (GTE and placebo) totals for calorie intake during the study, and endurance performance for RT1, RT2, and RT1-RT2 (endurance improvement).

Paired *t*-tests were conducted to compare within group change for RT1-RT2. The level of statistical significance was set at $P < 0.05$. Statistical analyses were completed using the Statistical Package for Social Sciences (SPSS version 17.0).

CHAPTER IV

RESULTS

Of the 16 participants that began the study, 14 completed it (GTE, $n=7$; placebo, $n=7$), which is an 88% completion rate. The two participants that dropped out did so for unknown reasons. These two participants that dropped out were two of only three participants who could not complete the 2.5 mile RT's without walking part of the way.

Whether or not the data of the 3rd participant who walked (participant #5, placebo group) should be included in the data analysis is unclear. Although participants were told that walking was allowed during the RT's, this participant was the only one to complete the study that did so, and she walked the majority of the distance in both RT's. Furthermore, she was one of only two participants to get slower from RT1 to RT2, and the only one to do so to a large degree: she ran RT2 7.35% slower than her RT1, while the other participant who did not improve in RT2 on her time from RT1 was only slower by 0.78%. However, her percentage change from RT1 to RT2 was within three standard deviations (SD's) of the mean change for all participants, thus putting her just within the acceptable statistical range. The mean percentage change for all participants was $2.26\% \pm$ SD 3.56% , with a range of -8.42% , 12.94% . Therefore, the decision was made to run two sets of analyses, one including participant #5, and one not. Analysis 1 contained data from all 14 participants, and Analysis 2 contained 13 participants, as it did not include the data from participant #5.

Run Performance

Data Analysis 1:

Between Group Comparison

The mean times for the baseline run trial (RT1) were 1169 sec for the placebo group and 1256 sec for the GTE group. There was no significant difference between the groups at baseline ($P = 0.49$). The mean times for the post-intervention run trial (RT2) were 1151 sec for placebo, and 1228 sec for GTE, and again there were no significant difference between groups. The mean improvement in performance, from RT1 to RT2 for the placebo group was 18 sec, or 2.09%. The mean improvement in performance for the GTE group was 28 sec, or 2.43%. For the major endpoint of the study, there was no significant difference between the endurance performance improvements of the 2 groups ($P = 0.74$) (see Table 2).

TABLE 2: Between and Within Group Comparisons of Group Mean Run Times

| | DATA ANALYSIS 1 | | | DATA ANALYSIS 2 | | |
|-------------------------|-----------------|--------------|---------------------------|-----------------|--------------|---------------------------|
| | PLA (n=7) | GTE (n=7) | P Value between groups | PLA (n=6) | GTE (n=7) | P Value between groups |
| RT1, sec | 1169 | 1256 | 0.49 | 1101 | 1256 | 0.18 |
| RT2, sec | 1151 | 1228 | 0.58 | 1060 | 1228 | 0.16 |
| RT1-RT2, sec | 18 | 28 | 0.74 | 41 | 28 | 0.38 |
| P Value within group | 0.48 | 0.02* | | 0.02* | 0.02* | |

GTE= green tea extract group, PLA = placebo group; RT1= run trial 1 (baseline run trial), RT2 = run trial 2 (post-intervention run trial); RT1-RT2 = Improvement in performance from baseline trial to post-intervention trial.

* $P < 0.05$, statistically significant

Within Group Comparison

The mean 18 sec improvement from RT1 to RT2 by the placebo group was not statistically significant ($P = 0.48$). However, the mean improvement of 28 sec from RT1 to RT2 by the GTE group was significant ($P = 0.02$) (see Table 2).

Data Analysis 2:

Between Group Comparison

The mean times for the baseline run trial (RT1) were 1101 sec for placebo ($n = 6$), and 1256 sec for the GTE ($n = 7$). There was no significant difference between the groups at baseline ($P = 0.18$). The mean times for the post-intervention run trial (RT2) were 1060 sec for placebo, and 1228 sec for GTE, and again there were no significant difference between groups ($P = 0.16$). The mean improvement in performance, from RT1 to RT2, for the placebo group was 41 sec, or 3.66%. The mean improvement in performance for the GTE group was 28 sec, or 2.43%. There was no significant difference between the improvements of the two groups ($P = 0.38$) (see Table 2).

Within Group Comparison

The mean 41 sec improvement from RT1 to RT2 by the placebo group was statistically significant ($P = 0.02$). The mean improvement of 28 sec from RT1 to RT2 by the GTE group was also significant ($P = 0.02$) (see Table 2).

Diet

Diet information was incomplete. Ten of the fourteen participants (GTE, n=5; Placebo, n=5) recorded 3-d diet records on MyPyramidTracker. Based on the participant data recorded, the GT group consumed an average of 1942 calories per day during the study period, and the placebo group consumed an average of 1833. There was no significance difference between the total calorie consumption of groups during the study (P = 0.32).

CHAPTER V
DISCUSSION AND CONCLUSIONS

Consumption of GT, GTE, or EGCG has been shown to increase fat oxidation in mice and humans at rest and while exercising (1-3, 5, 13, 21, 22, 48), increase exercise endurance in mice (4, 13), and increase VO_{2max} in humans (15). The only published study to directly measure the effect of consumption of GTE on endurance performance (measured with cycling time trials) in humans did not find significant results (16). Our study used a higher dose and longer period of GTE supplementation than did that study. Furthermore, we measured endurance performance via running (2.5 miles) rather than cycling time trials. Our study also did not find a significant difference in endurance performance between groups consuming placebo and GTE. However, under our one data analysis we did find that the GTE group significantly improved their performance in the second RT compared to baseline, while the placebo group did not. Yet under the other data analysis, with one potential outlier excluded, both placebo and GTE groups significantly improved from baseline to RT2. Therefore, the results of our study taken as a whole do not demonstrate efficacy of GTE consumption to increase exercise endurance.

This study had some important strengths and findings. This was only the second study to directly test the hypothesis that GT consumption can improve exercise endurance in humans. The design of this study more closely resembled, in duration and in GTE amount and composition, the mouse studies conducted by Murase et al. (4, 13), than did the other study (16) to test the effect of GTE on endurance performance. We found that moderately high GTE consumption (~900 mg polyphenols, 700 mg EGCG), for a moderately long period of time (4-5 weeks) results in virtually no reported side-effects by human participants. The RTs were conducted on an indoor track, rather than a

treadmill, which is an exercise scenario more relevant to real-world activities and competitions. Lastly, this study measured actual endurance performance rather than a physiological marker of potential endurance (such as VO_{2max} , or lactate threshold, etc).

However, despite its strengths, this study had limitations that likely reduced our ability to find significant results. For instance, the sample size was smaller than anticipated. It was estimated that 35-40 of participants would be required to reach the desired statistical power, higher than the number of participants completing the study. Also, some aspects of the study could not be well controlled. While the intention of the study was to examine free-living humans without strict controls, time constraints and some lack of participant adherence to the study protocol resulted in more differences between individuals and possibly the two groups than anticipated.

For example, participants ran on different days and under slightly different circumstances. Some ran at the same time as other participants, some separately, and some ran one trial with others, and the other trial separately. We planned to have participants run in a few large groups. In that case, even if they were not all running together, they would have had similar competitive, group experiences. However, this was not possible due to scheduling conflicts. Thus, the participants who ran together likely had a more competitive atmosphere than the ones who ran individually. While these discrepancies were approximately equal between treatment groups, in such a small sample size, they might have affected the outcome.

Likewise, some participants did not do exactly the same type and amount of exercise before each of the two trials, as had been requested of them. For example, one

participant raced a half-marathon (13.1 miles) the day before the baseline trial, but only ran an 8 mile practice run the day before the second trial, with the result being that she was likely more rested for the second trial. Participation in the study required that participants had been performing “aerobic exercise at least twice a week for 20-30 minutes at a fairly vigorous pace”, and they were expected to maintain that amount of exercise during the study. However, based on exercise logs provided by the participants, some did not meet that standard during the study period. In the studies that reported increased endurance in mice that consumed GTE, the mice exercised intensely two to three times a week, and in the case of one of the studies, that included once a week to exhaustion (4, 13).

Catecholamines, such as norepinephrine, are released during exercise from sympathetic nerves, which stimulates fat breakdown and usage (4, 62). GT catechins have been shown to inhibit COMT, the enzyme that breaks down catecholamines (4). Therefore, GT consumption might have a more marked effect on increasing fat oxidation in individuals who regularly engaged in activities that stimulate the sympathetic nervous system and result in catecholamine release, such as exercise. In other words, GT catechins may work synergistically with exercise to produce weight loss and increased fat oxidation, and possibly increase endurance (4, 16). In the absence of exercise or other catecholamine-stimulating activities, consumption of GT catechins might not have such a pronounced effect. In this study, some participants did not regularly exercise to the degree requested, and this might have limited the effects of GTE on endurance performance.

Another possible limitation of this study was amount of GTE consumed by participants. Participants were asked to consume a daily amount of GTE that contained 900mg GT polyphenols, and included 700 mg EGCG, equal to ~ 5-6 cups of GT per day. While this is not considered a low amount in relation to other studies in humans, it is less than what was required to produce significantly increased endurance in mice. Those studies by Murase et al. (4, 13) only showed significant effects of GTE on endurance in mice consuming an amount of GTE equivalent to a human consuming ~ 10 cups of GT per day. The mice consuming a lower amount (~ 3-4 cups of GT a day) also improved their endurance compared with control mice, but this difference was not statistically significant. Furthermore, while overall compliance with capsule intake in our study was fairly high (90%), some participants did miss doses, and this could have further diluted the effect of GT consumption on endurance.

Another possible limitation was study length. The study period was only 4-5 weeks. Based on results of the studies conducted by Murase et al. (4, 13) on mice, and in some weight loss studies conducted on humans, GTE supplementation appears to have a more significant effect on endurance and fat oxidation when consumed for a duration of 8-12 weeks, which is two to three times the length of this study (1, 4, 11, 13, 16) .

Further studies are needed which address the limitations of this study. Future studies would benefit from a longer study duration (8-12 weeks), a slightly higher amount of GTE supplementation (equivalent to ~ 6-8 cups GT per day), and a larger sample size (~ n= 40). Other improvements could include having study participants complete 2-3 run trials at baseline and post-intervention to better control for individual variances,

increasing the distance of the run trials to 5-6 miles to more accurately reflect endurance performance, and having all participants take part simultaneously in the same run trials to better control for the effects of competition on run performance. Increased adherence to the study protocol, through emphasis on the importance of following the study protocol or use of incentives, would also be a key improvement.

One method to accomplish these changes could be to invite members of a running team or exercise/nutrition clients of the researcher to participate. This would ensure that participants would have regular contact with the researcher, and their workouts would either be directed by or at least more easily monitored by him. This would allow for more control of differences in exercise regimens during the study period that could effect results. Lastly, the one could attempt to strictly control food intake during the study by contacting a prepared foods supplier to see if they would be interested in taking part in the study. Under that scenario, participants could consume the prepared foods for two meals each day, and follow dietary guidelines for the 3rd meal, thus enabling strong diet control for the study.

CONCLUSIONS

While this study and the only published study (16) to measure the effects of GTE consumption on exercise endurance in humans did not demonstrate significant results, both studies had many limitations, and there is still a considerable amount of rationale that suggests that GT consumption might have this effect (4, 13, 15, 16). This rationale is based on research that has shown that GT consumption increases both endurance and

physiological markers for endurance in mice, and physiological markers for endurance in humans. In fact, it is reported that a study that has just been conducted at the University of Glasgow on the effect of GT consumption on endurance performance is producing positive results (63); however, it is not published at this time and it remains to be seen if results will truly be statistically significant. Therefore, while the promise that GT has shown for many years as a potential ergogenic aid still exists, it remains to be realized at this time.

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APPENDICES

APPENDIX A
IRB APPROVAL OF PROTOCOL E-MAILS

From: svogtner1@gsu.edu [svogtner1@gsu.edu]
Sent: Friday, December 18, 2009 12:48 PM
To: Eric Green; vganji@gsu.edu
Subject: Protocol Approved

The Protocol you submitted has been approved by the IRB.

Protocol Title: The Effect of Green Tea Extract on Exercise Endurance in Humans
PI: Ganji, Vijay
Protocol Number: H10144

To view this Protocol, please go to this page
<https://irbwise.gsu.edu///sub/submissionview.form?submissionId=14410>

From: svogtner1@gsu.edu [svogtner1@gsu.edu]
Sent: Friday, December 18, 2009 12:45 PM
To: Eric Green; vganji@gsu.edu
Subject: Approved Consent Forms Stamped

This is an automated message from IRBWISE. Consent Forms for Protocol, Protocol H10144, have been approved and stamped.

PLEASE PRINT AND USE ONLY THE STAMPED VERSION FOR ENROLLING PARTICIPANTS. YOU CAN ALSO ACCESS YOUR APPROVED DOCUMENTS ON IRBWISE.

Download Stamped Consent Forms at
<https://irbwise.gsu.edu///associateddocuments.srv?submissionId=14410>

INSTITUTIONAL REVIEW BOARD

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In Person: Alumni Hall
30 Courtland St, Suite 217

December 18, 2009

Principal Investigator: Ganji, Vijay

Student PI: Eric Green

Protocol Department: Nutrition

Protocol Title: The Effect of Green Tea Extract on Exercise Endurance in Humans

Submission Type: Protocol H10144

Review Type: Expedited Review

Approval Date: December 18, 2009

Expiration Date: December 17, 2010

The Georgia State University Institutional Review Board (IRB) reviewed and approved the above referenced study and enclosed Informed Consent Document(s) in accordance with the Department of Health and Human Services. The approval period is listed above.

Federal regulations require researchers to follow specific procedures in a timely manner. For the protection of all concerned, the IRB calls your attention to the following obligations that you have as Principal Investigator of this study.

1. When the study is completed, a Study Closure Report must be submitted to the IRB.
2. For any research that is conducted beyond the one-year approval period, you must submit a Renewal Application 30 days prior to the approval period expiration. As a courtesy, an email reminder is sent to the Principal Investigator approximately two months prior to the expiration of the study. However, failure to receive an email reminder does not negate your responsibility to submit a Renewal Application. In addition, failure to return the Renewal Application by its due date must result in an automatic termination of this study. Reinstatement can only be granted following resubmission of the study to the IRB.
3. Any adverse event or problem occurring as a result of participation in this study must be reported immediately to the IRB using the Adverse Event Form.
4. Principal investigators are responsible for ensuring that informed consent is obtained and that no human subject will be involved in the research prior to obtaining informed consent. Ensure that each person giving consent is provided with a copy of the Informed Consent Form (ICF). The ICF used must be the one reviewed and approved by the IRB; the approval dates of the IRB review are stamped on each page of the ICF. Copy and use the stamped ICF for the coming year. Maintain a single copy of the approved ICF in your files for this study. However, a waiver to obtain informed consent may be granted by the IRB as outlined in 45CFR46.116(d).

All of the above referenced forms are available online at <https://irbwise.gsu.edu>. Please do not hesitate to contact Susan Vogtner in the Office of Research Integrity (404-413-3500) if you have any questions or concerns.

Sincerely,

Susan Laury, IRB Chair

VOLUNTEERS NEEDED
FOR GREEN TEA/Exercise RESEARCH
STUDY!

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

Be a part of a Georgia State University Division of Nutrition research study

Research has shown that consumption of green tea can enhance fat burning in mice and humans, and increase exercise endurance in mice. Therefore, it is hypothesized that green tea could enhance exercise endurance in humans, and it is this hypothesis that will be tested.

STUDY DETAILS:

- Study will last for 5 weeks
- Participants will consume capsules of either green tea extract or placebo (made of vegetable cellulose filled with a small amount of rice flour) each day
- Participants will take part in 2 run/walk trials (one at the beginning of the study, one at the conclusion). Each trial will consist of “running” 3.1 miles (5k) as quickly as one can (walking is allowed if one is unable to run the whole way).

You are Eligible to Enroll if You:

- **Are aged 18-40**
- **Currently perform aerobic exercise at least twice a week for 20-30 minutes**
- **Have consumed green tea or green tea extract before without any adverse effects**
- **Do not have a history of liver problems, ulcers, diabetes, GI problems (Crohn, Celiac, IBT, etc), and are not pregnant, do not smoke, and do not regularly consume more than 5 alcohol drinks per day**

If interested, or you have any questions, please contact Eric Green at egreen19@student.gsu.edu, or Dr Vijay Ganji at vganji@gsu.edu . Thank you for your consideration.

**APPENDIX C
RECRUITMENT E-MAIL TO GSU DIVISION OF NUTRITION STUDENTS**

Hi all,

My name is Eric Green, and under the advisement of Dr. Ganji, I am completing my master's thesis project. My project is conducting a research study on "The Effect of Green Tea on Exercise Endurance in Humans." In order to carry this out, I need volunteers from GSU to be participants in the study. It is a quite simple design, and would not involve a lot of your time (and should be fun too).

The details of the study are on the two documents I have attached. Please read the documents attached and e-mail me back if you are interested or have any questions regarding the study. If you are interested, I will really appreciate your participation! (and if you know anyone else at GSU that might be interested, please let them know about the study)

Lastly, if anyone is interested in assisting me in timing some of the study volunteers at the recreation center indoor track, that would also be very helpful. You can also contact me if you'd like to do that.

The study will begin in a few weeks

Thank you for your time and consideration.

Eric

APPENDIX D
IRB APPROVED INFORMED CONSENT FORM

Georgia State University

Department of Nutrition

Informed Consent

Title: Effect of Green Tea Extract on Endurance Ability in Humans

Principal Investigator: Dr Vijay Ganji, Ph.D., R.D.

Student, P.I. Eric Green, Masters in Nutrition Candidate

I. Purpose:

You are being invited to take part in a research study. The purpose of the study is to investigate the effect of green tea extract on exercise endurance ability (as measured by 5 kilometer/3.1 mile run/walk time). In order to participate, you must meet the following criteria:

- You are aged 18-40
- You currently perform aerobic exercise (any one or combination of the following: brisk walking, jogging/running, swimming, bicycling, rollerblading, stairmaster, elliptical machine, aerobics class, etc) at least twice a week for 20-30 minutes, at a fairly vigorous pace.
- You have consumed green tea or green tea extract before without any adverse effects
- You do not have a history of liver problems, or any other chronic illness (ulcers, diabetes, GI problems [Crohn, Celiac, IBT, etc], etc)
- You are not currently pregnant
- You are not currently ill
- You do not currently smoke cigarettes, drink to excess (more than 5 alcoholic drinks per day), or use illegal drugs

The study will last 5 weeks. Approximately 40 participants will be recruited to take part in

the study. Participation will include the following activities:

Two 5-kilometer (5k, 3.1 miles) run/walk timed trials: approximately 30 minutes
Consent Form Approved by Georgia State University IRB February 01, 2010 - December 17, 2010
each.

- Daily recording (for 5 weeks) of exercise performed the day: 5 minutes/day
- Record 3 days of food intake (each day's record should take about 20-30 minutes. Only 3 days are needed, you are not doing this every day)
- Twice-daily consumption of green tea extract or placebo pill: 1 minute/day.

II . Procedures :

The study will take place during winter/spring 2009 (exact dates to be determined soon). Participation will involve the following:

- At the beginning and end of the study, you will be asked to participate in a 5k run/walk trial (you do not need to run the whole way if you are unable to. You must simply complete the 3.1 miles as quickly as you can).
- Eric Green will be recording the times for the trials, and will likely be assisted by other graduate students from the GSU division of nutrition. The trials will be

conducted on the GSU indoor track.

Each day during the study, you will be asked to take a capsule (either green tea extract or inert placebo) with breakfast and dinner, and write down how much you exercised that day. Taking the capsule should take no more than 30 seconds, and the recording of your exercise for the day should take you no more than 10 minutes total to complete each day.

All participants are eligible to receive free nutrition counseling during this study if they desire it. Simply contact Eric Green for details of this benefit (egreen19@student.gsu.edu).

III. Risks:

In this study, you should not experience any more risks than you would in a normal day of life.

However, in extremely rare cases, a few individuals have experienced liver problems that were believed to be due to consumption of green tea or green tea extract. This could have been due to unknown contaminants in the tea or extract, individuals having an extremely rare genetic mutation that made them not able to tolerate green tea, or other unknown possibilities. Although any adverse reaction to the amount of green tea extract being used in this study is extremely unlikely, we are being as safe as possible by only accepting participants who have met certain criteria (listed above). To ensure participant safety, the following safety measures will be used:

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been due to unknown contaminants in the tea or extract, individuals having an extremely rare genetic mutation that made them not able to tolerate green tea, or other unknown possibilities. Although any adverse reaction to the amount of green tea extract being used in this study is extremely unlikely, we are being as safe as possible by only accepting participants who have met certain criteria (listed above). To ensure participant safety, the following safety measures will be used:

- * The green tea extract used in the study will be one that has been independently verified to contain no contaminants or impurities, and to contain the amount of green tea claimed on the bottle.

- * The amount of daily green tea extract consumed by the subjects will be equal to approximately 5 cups of green tea. This is a moderate amount that has been consumed safely by millions of people all over the world. It is also equal to 1/4 the amount found to cause no adverse effects in humans in recent trials, and is 400 times less than the no observable adverse effects limit (NOAEL) found in mice (i.e., 400 x the amount of green tea extract that you will be consuming, per pound of body weight, was consumed by mice with no adverse health effects found in the mice)

- * The participants will be instructed to consume the supplement twice a day with food (since food consumption slows absorption).

- * Only those individuals who have consumed green tea before, and who have not experienced any discomfort when consuming it, will be included in the study.

- * Those individuals with a history of liver problems will be excluded from the study.

- * All subjects will be instructed to immediately stop taking the capsules if they experience any minor discomforts (e.g., dizziness, or any Gastro-Intestinal(GI) disturbances. GI disturbances commonly include stomach pain, heartburn, diarrhea, constipation, nausea and vomiting).

- * All subjects will be instructed to immediately stop taking the capsules and contact their doctor if they experience any of the following symptoms which could indicate a liver disorder: abdominal pain or jaundice (symptoms of jaundice include a yellowing of skin

or the whites of the eyes, red, orange, or dark urine, and severe lethargy).

Again, if you participate in this study, you should not experience any more risks than

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you would in a normal day of life. However, the above safety measures are being put in place in order to ensure participant safety to the highest degree possible.

IV. Benefits:

Participation in this study may benefit you personally. For instance, by participating in the

study you may be motivated to be more active since you know that you will be taking part

in two 5k timed trials. Furthermore, even short term green tea consumption has been shown in clinical trials to reduce body fat and improve markers of cardiovascular health (e.g., decrease blood pressure, LDL cholesterol, oxidative stress, and a marker of chronic inflammation). However, there is no guarantee that you will experience these benefits during this study. Also, you may be benefiting society by helping to determine if green tea

can improve exercise endurance. Such knowledge could help people become more active and lead healthier lives.

V. Voluntary Participation and Withdrawal:

Participation in research is voluntary. You do not have to be in this study. If you decide to be in the study and change your mind, you have the right to drop out at any time. Whatever you decide, you will not lose any benefits to which you are otherwise entitled.

VI. Confidentiality:

We will keep your records private to the extent allowed by law. The only private information we will be receiving from you is your name and e-mail address. Dr Vijay Ganji, Ph.D., R.D., from the Division of Nutrition, will have access to the information you provide. Information may also be shared with those who make sure the study is done correctly (GSU Institutional Review Board, the Office for Human Research Protection (OHRP) and/or the Food and Drug Administration (FDA)). We will use your initials rather than your name on study records. The information you provide will be stored on password and firewall-protected computers. Your name and other identifying information *Consent Form Approved by Georgia State University IRB February 01, 2010 - December 17, 2010* will not appear when we present this study or publish its results. The findings will be summarized and reported in group form. You will not be identified personally.

VII. Contact Persons:

Contact Dr Vijay Ganji (at (404) 413-1236 or vganji@gsu.edu), and/or Eric Green (at (404)

408-9779 or egreen19@student.gsu.edu) if you have any questions about this study. If you

have questions or concerns about your rights as a participant in this research study, you may

contact Susan Vogtner in the Office of Research Integrity at 404-413-3513 or svogtner1@gsu.edu.

VIII. Copy of Consent Form to Subject:

We will give you a copy of this consent form to keep.

If you are willing to volunteer for this research, please sign below.

Participant Date

Principal Investigator or Researcher Obtaining Consent Date

APPENDIX E
GREEN TEA EXTRACT

Where Science and Nature Come Together. ®

FOODSCIENCE®
of Vermont

Green Tea-70

- A dietary supplement to support proper immune system function.*
- Green Tea is a water soluble antioxidant that is 100 times more effective than Vitamin C and 25 times better than Vitamin E.*
- Green Tea comes from the tea plant, *Camellia sinensis*, which is an excellent source of potent polyphenols (bioflavonoids with powerful antioxidant properties).* The four primary polyphenols in Green Tea are epicatechin, epicatechin gallate, epigallocatechin, and epigallocatechin-3-gallate (EGCG). Research has identified EGCG as the most active agent in Green Tea.*
- Green Tea-70 is a unique formula because it contains the highest percentage of EGCG currently on the market.* Each 500 mg capsule of Green Tea-70 is standardized to contain 70% or 350 mg of EGCG per 500 mg capsule. Most competitors only provide 35 to 40% EGCG.
- EGCG is an important nutrient that supports:
 - Immune system function*
 - Cardiovascular health*
 - Skin health*
 - Joint comfort*
 - Intestinal health*
 - Liver function*
 - Weight loss*
 - Dental care*
 - Respiratory health*
- EGCG supports liver health as well as detoxification and elimination functions.* Green Tea may increase the activity of antioxidants and detoxifying enzymes within the small intestine, liver, and lungs.*

- EGCG helps to support cholesterol levels within normal ranges and balances the ratios of HDL and LDL cholesterol.*
- Research demonstrates that EGCG supports the activity of B-Cells to produce higher antibody response and immune activity of the T-Cells and macrophages.*
- EGCG is shown to support skin health by recycling aged cells and supporting new cell growth.* It offers antioxidant support against the free radicals that attack collagen (the skin's structural protein) and decreases the activity of the enzyme that breaks down collagen to help keep skin firm and healthy.*
- EGCG may support metabolism due to its thermogenic effect and helps to maintain normal blood insulin levels.* EGCG supports the burning of fats and helps control appetite, which helps to support weight loss and weight management.*

Supplement Facts

Each capsule contains:

| | |
|--|--------|
| Green Tea (<i>Camellia sinensis</i>) Extract | 500 mg |
| yielding: Total Polyphenols | 450 mg |
| Epigallo-catechin-3-gallate (a polyphenol) | 350 mg |

Other ingredients: rice flour, vegetable cellulose.

- **Warning:** If you are pregnant or nursing, consult your health care practitioner before taking this or any nutritional product.
- **Suggested Use:** As a dietary supplement, take 1 capsule once or twice daily.

Sold Exclusively Through Retailers.

0300726.060 (60 Capsules)

*This statement has not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure or prevent any disease.

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1-800-874-9444 • www.foodscienceofvermont.com

**APPENDIX F
PARTICIPANT RAW TIME DATA**

| PARTICIPANT | <u>GROUP</u> | <u>RT1</u> | <u>RT2</u> | <u>RT1-RT2</u> | <u>% IMPROVEMENT</u> |
|--------------------|---------------------|-------------------|-------------------|-----------------------|-----------------------------|
| 1 | GTE | 1122 | 1099 | 23 | 2.05% |
| 2 | PLA | 1156 | 1140 | 16 | 1.38% |
| 3 | PLA | 1579 | 1695 | -116 | -7.35% |
| 4 | GTE | 1557 | 1518 | 39 | 2.50% |
| 5 | PLA | 1344 | 1257 | 87 | 6.47% |
| 6 | GTE | 1290 | 1300 | -10 | -0.78% |
| 7 | PLA | 963 | 930 | 33 | 3.43% |
| 8 | GTE | 1238 | 1212 | 26 | 2.10% |
| 9 | GTE | 974 | 914 | 60 | 6.16% |
| 10 | PLA | 1010 | 1004 | 6 | 0.59% |
| 11 | PLA | 1139 | 1096 | 43 | 3.78% |
| 12 | GTE | 1053 | 1008 | 45 | 4.27% |
| 13 | PLA | 995 | 932 | 63 | 6.33% |
| 14 | GTE | 1557 | 1546 | 11 | 0.71% |

APPENDIX G DATA ANALYSIS

DATA ANALYSIS 1

Independent T-Test

Group Statistics

| | Group | N | Mean | Std. Deviation | Std. Error Mean |
|------------|-------|---|-----------|----------------|-----------------|
| RT1 | PLA | 7 | 1169.4286 | 222.78005 | 84.20295 |
| | GTE | 7 | 1255.8571 | 231.43424 | 87.47392 |
| RT2 | PLA | 7 | 1150.5714 | 267.30997 | 101.03367 |
| | GTE | 7 | 1228.1429 | 243.00235 | 91.84626 |
| Difference | PLA | 7 | -18.8571 | 65.49155 | 24.75348 |
| | GTE | 7 | -27.7143 | 23.07751 | 8.72248 |

Independent Samples Test

| | | t-test for Equality of Means | | |
|------------|-----------------------------|------------------------------|-----------------|-----------------------|
| | | Sig. (2-tailed) | Mean Difference | Std. Error Difference |
| RT1 | Equal variances assumed | .490 | -86.42857 | 121.41591 |
| | Equal variances not assumed | .490 | -86.42857 | 121.41591 |
| RT2 | Equal variances assumed | .580 | -77.57143 | 136.54134 |
| | Equal variances not assumed | .581 | -77.57143 | 136.54134 |
| Difference | Equal variances assumed | .742 | 8.85714 | 26.24531 |
| | Equal variances not assumed | .745 | 8.85714 | 26.24531 |

Paired T-Test for PLACEBO GROUP

Paired Samples Statistics

| | Mean | N | Std. Deviation | Std. Error Mean |
|--|------|---|----------------|-----------------|
| | | | | |

| | | | | | |
|--------|-----|-----------|---|-----------|-----------|
| Pair 1 | RT1 | 1169.4286 | 7 | 222.78005 | 84.20295 |
| | RT2 | 1150.5714 | 7 | 267.30997 | 101.03367 |

Paired Samples Correlations

| | | N | Correlation | Sig. |
|--------|-----------|---|-------------|------|
| Pair 1 | RT1 & RT2 | 7 | .981 | .000 |

Paired Samples Test

| | | Paired Differences | | |
|--------|-----------|--------------------|----------------|-----------------|
| | | Mean | Std. Deviation | Std. Error Mean |
| Pair 1 | RT1 - RT2 | 18.85714 | 65.49155 | 24.75348 |

Paired Samples Test

| | | Paired Differences | | | | |
|--------|-----------|---|----------|------|----|-----------------|
| | | 95% Confidence Interval of the Difference | | | | |
| | | Lower | Upper | t | df | Sig. (2-tailed) |
| Pair 1 | RT1 - RT2 | -41.71244 | 79.42672 | .762 | 6 | .475 |

Paired T-Test for GREEN TEA EXTRACT GROUP

Paired Samples Statistics

| | | Mean | N | Std. Deviation | Std. Error Mean |
|--------|-----|-----------|---|----------------|-----------------|
| Pair 1 | RT1 | 1255.8571 | 7 | 231.43424 | 87.47392 |
| | RT2 | 1228.1429 | 7 | 243.00235 | 91.84626 |

Paired Samples Correlations

| | | N | Correlation | Sig. |
|--------|-----------|---|-------------|------|
| Pair 1 | RT1 & RT2 | 7 | .996 | .000 |

Paired Samples Test

| | | Paired Differences | | |
|--------|-----------|--------------------|----------------|-----------------|
| | | Mean | Std. Deviation | Std. Error Mean |
| Pair 1 | RT1 - RT2 | 27.71429 | 23.07751 | 8.72248 |

Paired Samples Test

| | | Paired Differences | | | | |
|--------|-----------|---|----------|-------|----|-----------------|
| | | 95% Confidence Interval of the Difference | | | | |
| | | Lower | Upper | t | df | Sig. (2-tailed) |
| Pair 1 | RT1 - RT2 | 6.37115 | 49.05742 | 3.177 | 6 | .019 |

DATA ANALYSIS 2**Independent T-Test****Group Statistics**

| Group | | N | Mean | Std. Deviation | Std. Error Mean |
|-------|-----|---|-----------|----------------|-----------------|
| RT1 | PLA | 6 | 1101.1667 | 142.88375 | 58.33205 |
| | GTE | 7 | 1255.8571 | 231.43424 | 87.47392 |
| RT2 | PLA | 6 | 1059.8333 | 128.78263 | 52.57529 |

| | | | | | |
|------------|-----|---|-----------|-----------|----------|
| | GTE | 7 | 1228.1429 | 243.00235 | 91.84626 |
| Difference | PLA | 6 | -41.3333 | 30.05772 | 12.27101 |
| | GTE | 7 | -27.7143 | 23.07751 | 8.72248 |

Independent Samples Test

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | |
|------------|-----------------------------|---|------|------------------------------|--------|
| | | F | Sig. | t | df |
| RT1 | Equal variances assumed | 1.489 | .248 | -1.417 | 11 |
| | Equal variances not assumed | | | -1.471 | 10.121 |
| RT2 | Equal variances assumed | 2.650 | .132 | -1.517 | 11 |
| | Equal variances not assumed | | | -1.590 | 9.369 |
| Difference | Equal variances assumed | .462 | .511 | -.924 | 11 |
| | Equal variances not assumed | | | -.905 | 9.342 |

Independent Samples Test

| | | t-test for Equality of Means | | |
|------------|-----------------------------|------------------------------|-----------------|-----------------------|
| | | Sig. (2-tailed) | Mean Difference | Std. Error Difference |
| RT1 | Equal variances assumed | .184 | -154.69048 | 109.15700 |
| | Equal variances not assumed | .172 | -154.69048 | 105.13950 |
| RT2 | Equal variances assumed | .157 | -168.30952 | 110.91838 |
| | Equal variances not assumed | .145 | -168.30952 | 105.82956 |
| Difference | Equal variances assumed | .375 | -13.61905 | 14.73179 |
| | Equal variances not assumed | .388 | -13.61905 | 15.05521 |

Independent Samples Test

| | | t-test for Equality of Means | |
|------------|-----------------------------|---|----------|
| | | 95% Confidence Interval of the Difference | |
| | | Lower | Upper |
| RT1 | Equal variances assumed | -394.94342 | 85.56246 |
| | Equal variances not assumed | -388.57663 | 79.19568 |
| RT2 | Equal variances assumed | -412.43924 | 75.82019 |
| | Equal variances not assumed | -406.28248 | 69.66343 |
| Difference | Equal variances assumed | -46.04351 | 18.80541 |
| | Equal variances not assumed | -47.48734 | 20.24925 |

Paired T-Test for PLACEBO GROUP

Paired Samples Statistics

| | | Mean | N | Std. Deviation | Std. Error Mean |
|--------|-----|-----------|---|----------------|-----------------|
| Pair 1 | RT1 | 1101.1667 | 6 | 142.88375 | 58.33205 |
| | RT2 | 1059.8333 | 6 | 128.78263 | 52.57529 |

Paired Samples Correlations

| | | N | Correlation | Sig. |
|--------|-----------|---|-------------|------|
| Pair 1 | RT1 & RT2 | 6 | .981 | .001 |

Paired Samples Test

| | | Paired Differences | | |
|--|--|--------------------|----------------|-----------------|
| | | Mean | Std. Deviation | Std. Error Mean |
| | | | | |

Paired Samples Test

| | | Paired Differences | | |
|--------|-----------|--------------------|----------------|-----------------|
| | | Mean | Std. Deviation | Std. Error Mean |
| Pair 1 | RT1 - RT2 | 41.33333 | 30.05772 | 12.27101 |

Paired Samples Test

| | | Paired Differences | | | | |
|--------|-----------|---|----------|-------|----|-----------------|
| | | 95% Confidence Interval of the Difference | | t | df | Sig. (2-tailed) |
| | | Lower | Upper | | | |
| Pair 1 | RT1 - RT2 | 9.78969 | 72.87698 | 3.368 | 5 | .020 |

USE ALL. COMPUTE filter_\$(Group = 2). VARIABLE LABEL filter_\$ 'Group = 2 (FILTER)'. VALUE LABELS filter_\$ 0 'Not Selected' 1 'Selected'. FORMAT filter_\$ (f1.0). FILTER BY filter_\$. EXECUTE. T-TEST PAIRS=RT1 WITH RT2 (PAIRED) /CRITERIA=CI(.9500) /MISSING=ANALYSIS.

Paired T-Test for GTE GROUP

Notes

| | | |
|-------|---------------------------|----------------------|
| | Output Created | 26-Apr-2010 11:10:16 |
| | Comments | |
| Input | Active Dataset | DataSet0 |
| | Filter | Group = 2 (FILTER) |
| | Weight | <none> |
| | Split File | <none> |
| | N of Rows in Working Data | 7 |
| | File | |

| | | |
|------------------------|-----------------------|--|
| Missing Value Handling | Definition of Missing | User defined missing values are treated as missing. |
| | Cases Used | Statistics for each analysis are based on the cases with no missing or out-of-range data for any variable in the analysis. |
| | Syntax | T-TEST PAIRS=RT1 WITH RT2 (PAIRED) /CRITERIA=CI(.9500) /MISSING=ANALYSIS. |
| Resources | Processor Time | 0:00:00.016 |
| | Elapsed Time | 0:00:00.015 |

[DataSet0]

Paired Samples Statistics

| | Mean | N | Std. Deviation | Std. Error Mean |
|------------|-----------|---|----------------|-----------------|
| Pair 1 RT1 | 1255.8571 | 7 | 231.43424 | 87.47392 |
| RT2 | 1228.1429 | 7 | 243.00235 | 91.84626 |

Paired Samples Correlations

| | N | Correlation | Sig. |
|------------------|---|-------------|------|
| Pair 1 RT1 & RT2 | 7 | .996 | .000 |

Paired Samples Test

| | |
|--|--------------------|
| | Paired Differences |
|--|--------------------|

| | | Mean | Std. Deviation | Std. Error Mean |
|--------|-----------|----------|----------------|-----------------|
| Pair 1 | RT1 - RT2 | 27.71429 | 23.07751 | 8.72248 |

Paired Samples Test

| | | Paired Differences | | | | |
|--------|-----------|---|----------|-------|----|-----------------|
| | | 95% Confidence Interval of the Difference | | | | |
| | | Lower | Upper | t | df | Sig. (2-tailed) |
| Pair 1 | RT1 - RT2 | 6.37115 | 49.05742 | 3.177 | 6 | .019 |