No Effect of 1 or 7 Days Green Tea Extract Ingestion on Fat Oxidation during Exercise

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Running title: GTE ingestion and fat oxidation
ABSTRACT

**Purpose:** The aim of this study was to investigate the effects of 1 day and 7 days ingestion of a green tea extract (GTE) on whole body fat oxidation during moderate-intensity exercise.

**Method:** Thirty one males completed two exercise trials (60 min cycle 50% $W_{\text{max}}$). Following the baseline trial (Day 0) subjects were randomly assigned to one of three conditions involving a week supplementation of: 1) 7 days placebo (PLA); 2) 6 days of PLA followed by 1 day of GTE (GTE1); 3) 7 days of GTE ingestion (GTE7). The morning after the supplementation week, subjects consumed an additional supplement and completed a second exercise trial (Day 8). $V_O^2$ and $V_CO_2$ measurements were taken during exercise to calculate whole body fat oxidation rates. Blood samples, for analysis of plasma fatty acids (FAs), glycerol and epigallocatechin gallate (EGCG), were collected at rest and during exercise.

**Results:** On Day 8 the plasma kinetics and maximal plasma concentrations of EGCG were similar in the GTE1 and GTE7 group (206 ± 28 and 216 ± 25 ng∙mL$^{-1}$ respectively). One day of GTE ingestion did not affect markers of lipolysis during the exercise bout. Seven days of GTE ingestion significantly increased plasma glycerol during exercise ($P=0.045$) and plasma FAs during exercise ($P=0.020$) as well as at rest ($P=0.046$). However, fat oxidation did not change in any of the groups.

**Conclusion:** There was no effect of 1 day GTE ingestion on markers of lipolysis or fat oxidation during exercise. Seven days of GTE ingestion increased lipolysis, indicated by increased plasma FA and glycerol concentrations, but did not result in significant changes in fat oxidation.

**Key words:** FAT METABOLISM, EXERCISE TESTING, EGCG, INDIRECT CALORIMETRY
INTRODUCTION

Green tea is produced from the leaves of *Camellia sinensis* (L.) of the Theaceae family. Unlike black and oolong tea, green tea leaves are non-oxidised/non-fermented resulting in high quantities of catechin-polyphenols (catechins) (25). The most abundant catechins are (-)-epicatechin, (-)-epicatechin-3-gallate, (-)-epigallocatechin and (-)-epigallocatechin-3-gallate (EGCG). Regular green tea consumption has been associated with many health benefits. In particular, EGCG has been attributed to protect against cardiovascular disease and cancer [For a full review see: McKay and Blumberg (29)]. There is also a substantial body of literature investigating the effects of green tea ingestion on thermogenesis and fat metabolism (22). While there is ever emerging evidence on the beneficial health effects of green tea and GTE ingestion, overconsumption of green tea and highly concentrated GTE may cause adverse effects (38). However, it is yet to be defined how much green tea in ones diet is classed as detrimental to health. Thus, more in depth studies on the potential harmful effects of green tea consumption are warranted.

Regular GTE ingestion has been found to promote weight loss in some (8, 31, 32, 43), but not all (7, 18) of studies investigating the possible anti-obesity effects of green tea consumption. Wang et al (43) found that consumption of a catechin rich beverage containing caffeine (886 mg catechins and 198 mg caffeine), for 90 days, significantly decreased body weight by 1.7%. Although a small reduction in lean mass partially accounted for the weight loss, GTE intervention led to much larger reductions in body fat, and most notably, reductions in intra-abdominal fat. In addition a reduction in total and subcutaneous fat area was found when a GTE (~625 mg catechins) was ingested daily alongside regular exercise for 12 weeks (28). The body weight and body fat loss, found in this study, may be a result of an upregulation of thermogenesis and substrate oxidation.
It is well established that fatty acids (FAs) are the predominant energy source at rest when fasted (21). It has been suggested that green tea extract (GTE) ingestion may further enhance the relative use of fat as a fuel. Over a 24 hour period, Dulloo et al (13) observed that three feedings of a GTE plus caffeine (375 mg catechins/24 hour and 150 mg caffeine/24 hour) significantly decreased respiratory quotient (RQ) and elevated fat oxidation rates. This contributed to a significant increase in total energy expenditure compared to placebo (2.8%) and caffeine (3.4%). Consumption of a GTE at rest has not always been found to increase fat oxidation. Gregersen et al (16) found no change in substrate metabolism when GTE was ingested, in small but frequent doses, over a 13 hour period. However on balance, studies have found short term (24 hours) GTE ingestion to upregulate fat metabolism at rest (19).

It has been speculated that GTE ingestion enhances lipid catabolism by prolonging and augmenting sympathetic stimulation (12). More specifically, it is thought that EGCG inhibits catechol-O-methyltransferase (COMT), an enzyme that degrades catecholamines such as noradrenaline, resulting in enhanced lipolysis (27). This would result in more available circulating FAs for potential oxidation.

During low to moderate intensity exercise fat oxidation rates are increased 10 fold, compared with resting values (21). Research has found that GTE ingestion may further elevate fat oxidation during exercise, compared to exercise alone, in both animals and humans (6, 30, 34, 39, 42). From our lab (42), a 17% increase in fat oxidation, during a 30 min exercise bout, was found following 1 day ingestion of GTE (890 mg of total catechins; 366 mg EGCG). This study used a supplementation protocol during which healthy lean males consumed GTE capsules (or placebo) in the 24 hours prior to and on the morning of the exercise trial.
The effects of long term GTE supplementation, on fat oxidation during exercise, have also been studied. Shimotoyodome et al (39) observed augmented fat oxidation rates in high-fat fed mice following 15 weeks of GTE (81.3% catechins) ingestion, in combination with exercise training (running 3 days/week for 30min). In humans significantly higher fat oxidation rates (24%) have also been found during exercise, when GTE (570 mg consumed 3 times a week) was ingested over a 2 month period in combination with regular exercise (34), compared to placebo. However, a lower dosage of GTE, consisting of 160 mg of total catechins consumed daily for 3 weeks, did not alter substrate metabolism (14). Together these studies suggest that long term supplementation, of higher doses of GTE, has the potential to upregulate fat metabolism.

Long term GTE supplementation studies in rodents have been associated with increased expression of proteins involved in fat metabolism (fatty acid translocase (FAT)/CD36 and medium-chain acyl-CoA dehydrogenase (MCAD) mRNA (6)), which could be an alternative to the COMT mechanism described above. Because the time course of change in enzyme activity would be days to weeks, this would not explain increases in fat oxidation with short term (24 hour) GTE ingestion. In summary, it appears that both short term and long term GTE ingestion has the potential to increase fat oxidation during exercise, but the mechanisms and time course might be different.

None of the studies investigating the effects of GTE ingestion on metabolism, have included plasma catechin data (5, 13, 14, 16, 34, 42) . It is known that absorption of GTE catechins is generally low, and may be affected by the conditions of consumption (9). Therefore, even when the dose given is known, the rate and degree of catechin uptake is not known. Without these data the supplementation protocol that should be used, to see beneficial metabolic effects is unclear.
Therefore this study aimed to investigate the effects of 1 day and 7 days GTE supplementation on fat oxidation rates during moderate intensity exercise, compared to placebo. Furthermore to elucidate if the duration of GTE ingestion plays a role in upregulating metabolism, during a moderate intensity exercise bout, we compared fat oxidation rates following 1 day and 7 days supplementation compared to baseline.

PARTICIPANTS AND METHODS

Participants

Forty three male participants were recruited for the study. Inclusion criteria included habitual caffeine intake of ≤ 400 mg/day (approximately ≤ 4 cups coffee/ day) to ensure that all participants were low to moderate caffeine consumers and not desensitized to any caffeine effects. This data was obtained from a caffeine consumption questionnaire. Only 5 participants consumed coffee on a daily basis (range 1-4 cups/ day), 16 participants consumed coffee 1-3 times a week and 22 participants were non-coffee drinkers. In addition all volunteers were required to participate in exercise 3-5 times/ week for 30-90 min. Generally, highly trained endurance athletes have high absolute whole body fat oxidation rates (3), as a result of skeletal muscle adaptations from endurance type training. Thus, GTE supplementation may not be potent enough to increase fat oxidation further. Therefore, we recruited subjects who were moderately trained so they could complete the exercise bout at east but not have the skeletal muscle adaptations of an endurance trained athlete.

All participants gave written informed consent to participate in this study and were healthy according to the results of a general health questionnaire. All procedures and protocols were approved by the Life and Sciences Ethical Review Committee at the University of Birmingham.
Preliminary Testing

At least 1 week prior the baseline exercise trial, all participants reported to the Human Performance Laboratory, at the University of Birmingham, for a preliminary fitness test. Participants underwent an incremental exercise test, on an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands), to volitional exhaustion. After a 5 min warm up at 75 W, participants started the test by cycling at 95 W for 3 min. Their effort was increased every 3 min, in incremental steps of 35 W, until they reached voluntary exhaustion. $W_{\text{max}}$ (maximal amount of power) was calculated using the following equation (26).

$$W_{\text{max}} = W_{\text{out}} + [(t \times 180) \times 35]$$

Where $W_{\text{out}}$ is the power output of the last stage completed during the test, and $t$ is the time spent, in seconds, in the final stage. Throughout the test respiratory gas measurements ($VO_2$ and $VCO_2$) were collected continuously using an Online Gas Analyser (Oxycon Pro, Jaeger, Wuerzburg, Germany). $VO_2$ was considered maximal and the test was stopped if 2 out of the 4 following criteria were met. 1) no further increase or a decrease in $VO_2$ even when workload increased 2) a respiratory exchange ratio (RER) of >1.05 3) a heart rate within 10 beats per min of age predicted maximal heart rate 4) a cadence of 50 rpm could not be maintained. Heart rate (HR) was recorded during each stage of the test using a HR monitor (Polar). $W_{\text{max}}$ was used to determine the work load for all subsequent experimental trials (50% $W_{\text{max}}$).
**General Study Design**

In a parallel, double-blind design study, participants completed two exercise tests separated by a week of supplementation. Participants were randomly assigned to one of three supplementation conditions: 1) 7 days placebo (PLA); 2) 6 days of PLA followed by 1 day of GTE (GTE1); 3) 7 days of GTE supplementation (GTE7) (Figure 1). Exercise tests were performed before (baseline, Day 0) and after the supplementation week (Day 8). During each trial participants rested for two hours and then completed a 60 min cycling exercise at 50% of their predetermined $W_{\text{max}}$ (55% $VO_{2\text{max}}$) (Figure 1). This exercise type and duration was used to ensure that participants were in steady state and also working at an exercise intensity which is known to elicit maximal fat oxidation (2).

**Exercise Test**

All participants reported to the Human Performance Laboratory between 0600 and 0800 am after a 10-12 hour overnight fast and having avoided any strenuous exercise and consuming any alcohol and caffeinated beverages in the preceding 24 hours. The exercise bout in the present study was performed following an overnight fast. This is in line with previous research from our lab (42). There is limited research investigating the effects of GTE on fat oxidation during exercise. We wanted to ensure that there were as few confounding factors (such as food intake which would decrease fat oxidation and increase CHO oxidation), as possible. We can therefore be confident that any changes we may see in fat oxidation are a result of the GTE ingestion.

On arrival body weight was recorded (Seca Alpha, Hamburg, Germany) and a flexible 20-gauge Teflon catheter (Venflon; Becton Dickinson, Plymouth, United Kingdom) was
inserted into an antecubital vein. A 3-way stopcock (Connecta; Becton Dickinson, Plymouth, United Kingdom) was attached to the catheter to allow for repeated blood sampling during the experimental period. An initial 15 mL (5 mL collected in Lithium Heparin-containing tubes and 10 mL collected into EDTA-containing tubes) blood sample was collected, participants then rested for 2 hours in a seated position. Before the exercise bout commenced another blood sample (15 mL) was taken (T=120 min). Participants mounted the cycle ergometer after the 2 hour period and began a 60 min cycling exercise at 50% $W_{\text{max}}$ (55% $VO_{2\text{max}}$). Blood samples (15 mL) and 4 min respiratory breath samples (Oxycon Pro, Jaeger, Wuerzburg, Germany) were collected every 10 min during the exercise bout. The catheter was kept patent during both rest and exercise by flushing it with 4-5 mL isotonic saline (0.9% w·v; B Braun, Sheffield, United Kingdom) after every blood sample and every 15 min during the rest period only. HR was recorded continuously with the use of a Polar HR monitor (Polar RS800CX, Polar Electro (UK) Ltd, Warwick, United Kingdom) and Rate of Perceived Exertion (RPE) was recorded every 15 min during the exercise bout.

Following the baseline exercise test (Day 0) participants were supplied with 14 unlabeled cans (330 mL/ can) of either the GTE or PLA beverage. After 7 days of supplementation, participants arrived at the Human Performance Laboratory (Day 8) for a second exercise test. This test was identical to that outlined above however on arrival participants consumed their final test beverage (1 can) before the two hour rest period commenced.

**Supplement**

During the supplementation week, participants consumed 2 cans/ day (15 in total) containing either GTE or placebo. The drinks were consumed one hour before breakfast and
one hour before evening dinner for seven days, and an additional drink (1 can) was consumed on the morning of the second exercise test (Day 8). The supplement was a slightly flavoured peach and apricot beverage (330 mL/can) which was enriched with GTE and caffeine. The placebo beverage was matched for colour and flavour, and contained no catechins but a small amount of caffeine (9.9 mg/can). The amount of GTE in each drink (can) was ~560 mg total catechins (120 mg caffeine), equivalent to ~4 cups of green tea. Of the 560 mg of total catechins ~210 mg was EGCG. To ensure compliance, daily text messages were sent to all participants once a day to remind them to consume the drinks. Participants were instructed to fill in a log sheet detailing the time they consumed each beverage. In addition, participants were required to return all empty cans when they visited the lab for the second exercise trial.

**Diet Control**

Before participating in the first exercise trial participants were shown the controlled diet menu. Foods were replaced if an individual disliked any of the available foods and were replaced with foods that had a similar nutritional content. This was to ensure, as best as we could, that all participants consumed the diet. The same diet was given to all participants to consume in the 24 hour period before both trials. The diet consisted of three meals (breakfast, lunch and dinner) each containing ~50% carbohydrate (CHO), ~35% fat and ~15% protein equating to ~2200 kilocalories (kcal). During this 24 hour control period participants were asked to refrain from any strenuous physical activity and to not consume alcohol or caffeine based beverages.
**Blood Variables**

All tubes were centrifuged at 3500 rpm for 15 min at 4 °C. Aliquots of plasma and serum were immediately frozen in liquid nitrogen and stored at -80 °C for later analysis. Where appropriate, plasma FAs [NEFA-C; Wako Chemicals, Neuss, Germany], and glycerol (Glycerol; Randox, England) were analysed on an ILAB 650 (Instrumentation Laboratory, Cheshire, United Kingdom).

**Plasma EGCG**

To measure the concentrations of deconjugated EGCG, 200 µL EDTA plasma, 20 µL of stabilizer solution (10% ascorbic acid containing 0.1% EDTA), 20 µL of 1.5 mol.L\(^{-1}\) sodium acetate (NaOAc, pH 4.8), and 10 µL of β-glucuronidase (50k U.L\(^{-1}\) in acetate buffer) were mixed and incubated at 37 °C for 45 min. From the supernatant, 5 µL was injected into the high-performance liquid chromatography multiple-reaction monitoring mass spectrometer (HPLC-MRM-MS) system (Agilent 6410 mass spectrometer equipped with an Agilent 1200SL HPLC (Agilent Technologies, Amstelveen, The Netherlands) and an HTC PAL autosampler (CTC Analytics, Zwingen, Switzerland). Samples were analysed batch-wise and controlled by two quality control samples (QCs) per sample batch. EGCG was quantified in plasma by means of 10-point calibration curves. The peak areas of the internal standards as well as the target compounds were determined using Agilent’s MassHunter Quantitative Analysis software (version B.03.02, Agilent Technologies, Santa Clara, CA). A full description of the EGCG analysis can be found in Hodgson et al. (17).
Calculations

From the recorded measurements of \( V\text{CO}_2 \) and \( V\text{O}_2 \) (L\( \cdot \)min\(^{-1} \)), total fat and carbohydrate oxidation was calculated (g\( \cdot \)min\(^{-1} \)) using the following stoichiometric equations (24) assuming that participants were working at a moderate intensity and that protein oxidation was negligible during the exercise bout.

\[
\text{Carbohydrate Oxidation (g\( \cdot \)min\(^{-1} \)) = 4.210 \ V\text{CO}_2 - 2.962 \ V\text{O}_2}
\]

\[
\text{Fat Oxidation (g\( \cdot \)min\(^{-1} \)) = 1.65 \ V\text{O}_2 - 1.701 \ V\text{CO}_2}
\]

Statistical Analysis

The study was designed as a parallel trial with 3 arms: 2 GTE groups (GTE1 or GTE7) and a placebo. Fat Oxidation was measured at baseline and at the end of the intervention period (Day 8). The baseline values obtained were used to adjust for individual levels of the participants. Previous studies with similar numbers of participants, carried out in our lab, have shown a significant difference in fat oxidation of approximately 0.06 g\( \cdot \)min\(^{-1} \) can be obtained.

Data analysis was performed by using SPSS for WINDOWS software (version 17; SPSS Inc, Chicago, IL). Data are expressed as means ± SEMs unless otherwise stated. Differences in the average whole body fat and CHO oxidation and RER, between treatments (PLA, GTE1 and GTE7), were determined using univariate ANCOVA. Differences in average substrate metabolism and plasma blood variables, between Day 0 and Day 8, were determined using paired samples \( t \) test. Differences in plasma FAs and glycerol, during exercise between Day 0 and Day 8, at each time point, were compared using repeated
measures ANOVA (group x time). Plasma EGCG levels were compared between Day 0 and Day 8 of each condition using repeated measures ANOVA. Significance was set at $P < 0.05$.

RESULTS

Subjects

Due to injury and illness four participants did not complete the Day 8 trial therefore they have not been included in the final analysis. Participants that worked at a percentage of $VO_2\text{max}$ that was >10% different between trials were excluded from the final data set (n=5). In addition, following a blind review of the data set, participants with baseline blood metabolite data that were >2SD from the mean were removed (n=3). Therefore data were available and analyzed for 31 subjects (PLA n=10, GTE1 n=11, GTE7 n=10). All subjects were healthy males; there was no significant difference in age, weight, height, BMI and $VO_2\text{max}$ between the groups (Table 1.).

Workload and exercise intensities

On Day 0 the workload of 50% $W_{\text{max}}$ (151 ± 4 W), used during the 60 min exercise bout, elicited a relative $VO_2$ of 56 ± 1, 59 ± 1 and 57 ± 1 %$VO_2\text{max}$ for PLA, GTE1 and GTE7 respectively. $VO_2$ on Day 8 was not significantly different to Day 0 in the PLA and GTE7 groups. In the GTE1 group, $VO_2$ was significantly lower on Day 8 compared to Day 0 (59 ± 1 and 57 ± 1 % $VO_2\text{max}$ Day 0 and Day 8 respectively ($P < 0.05$)).

There was no difference in heart rate between the three groups on Day 0 ($P = .236$) or Day 8 ($P = .727$). In addition there was no difference within groups when Day 0 was
compared to Day 8 (PLA 133 ± 3 and 134 ± 3, GTE1 143 ± 5 and 137 ± 4, GTE7 139 ± 3 and 138 ± 3 bpm on Day 0 and Day 8 respectively). RPE during exercise did not differ between trials in any of the groups (data not shown).

Fat and carbohydrate oxidation

Supplementation of 1 day GTE and 7 days GTE failed to increase average whole body fat oxidation rates, compared to placebo during the 60-min exercise bout (Table 2). In addition, RER did not differ between Day 0 and Day 8 in all three groups (Table 2). Thus, the contribution of fat and CHO to total energy expenditure did not differ between groups (Table 2).

Plasma metabolites

Compared to baseline (Day 0), FAs at rest (t=0) were significantly higher on Day 8 in the GTE7 group (Figure 2). One day ingestion of GTE did not change plasma FAs at rest compared to Day 0 (Figure 2). On Day 8, two hours after an additional GTE beverage (t=120 min), plasma FAs remain significantly higher in the GTE7 group (Day 0: 0.51 ± 0.07 mmol∙L⁻¹ and Day 8: 0.74 ± 0.08 mmol∙L⁻¹). Two hours after GTE ingestion (Day 8) plasma FAs did not change in the GTE1 group compared to Day 0. Plasma FAs at rest (t=0) and following GTE ingestion (t=120min) on Day 8 were significantly higher in the GTE7 group compared to PLA. During exercise plasma FAs was unaffected by PLA or 1 day GTE ingestion (Figure 3A). In the GTE7 group plasma FA during exercise were higher after 7 days of GTE ingestion (Day 8) compared to Day 0 (P=0.020) (Figure 3A).
Compared to baseline (Day 0), there was no difference in plasma glycerol concentrations at rest (t=0) following 1 day and 7 day GTE consumption. Two hours after GTE ingestion (t=120 min), plasma glycerol was unchanged in all three supplementation groups, when Day 0 was compared to Day 8. Compared to PLA, plasma glycerol following GTE ingestion (t= 120 min) on Day 8 was significantly higher in the GTE7 group (35.8 ± 4.0 and 77.5 ± 14.0 µmol∙L\(^{-1}\) respectively). During exercise plasma glycerol concentrations were unchanged after supplementation in PLA and GTE1 but were significantly higher in the GTE7 group when compared to Day 0 (P=0.045) (Figure 3B).

**Plasma EGCG**

As expected, plasma EGCG concentrations were negligible before and after the supplementation period in the placebo group (Figure 4). Following GTE ingestion the EGCG concentrations in the GTE1 and GTE7 groups were significantly higher at rest and during exercise (P<0.001) but the concentrations were very similar in the two GTE groups (Figure 4). Before GTE ingestion (t=0) on Day 8, plasma concentrations of EGCG were significantly higher compared to Day 0 in both GTE1 and GTE7. Concentration at t=0 were around 70 ng∙mL\(^{-1}\) rising to 220-250 ng∙mL\(^{-1}\) 60 min after GTE ingestion and levelling off after this. Even 180 min after ingestion GTE concentrations were still elevated (~240 ng∙mL\(^{-1}\)). On Day 8 there was no significant difference in the area under the curve between GTE1 and GTE7.

**DISCUSSION**

In the present study, after 7 days (but not 1 day) of GTE (plus caffeine) consumption, we observed an increase in plasma FA concentrations at rest and FA and glycerol...
concentrations during exercise. This would indicate that lipolysis was stimulated. However, fat oxidation rates were unchanged following 1 day and 7 days GTE ingestion. This study is, to the best of our knowledge, the first to directly compare the effects of 1 day and 7 days GTE ingestion on fat oxidation during a 60 min exercise bout.

An earlier study from our lab (42) found a 17% increase in fat oxidation during exercise (30 min cycle at 50% \( W_{\text{max}} \)), compared to placebo, following a supplementation period which was identical to the GTE1 group. These studies are similar in design and participant characteristics therefore there is no immediately obvious explanation for the contrasting findings. However, slight differences in GTE composition could have influenced the outcome of our study.

In our previous study (42) the GTE did not contain caffeine. However, in the present study, subjects consumed 240 mg caffeine/ day (in addition to 1200 mg catechins/ day during the supplementation week) and an additional 120 mg of caffeine two hours before the exercise bout. Caffeine has been shown to stimulate glycolysis, evidenced by increased plasma lactate concentrations or reduced lactate clearance (15). Furthermore, there is a clear negative correlation between lactate accumulation and fat oxidation (4). Metabolomic analysis of plasma samples from this study, found an increase in plasma lactate during exercise following 7 days of GTE ingestion (17). It could be suggested that caffeine induced an increase in glycolysis resulting in blunting of fat oxidation. However in the present study, there were no differences in carbohydrate oxidation rates with GTE ingestion. Berube-Parent et al (5) gave subjects a GTE varying in doses of EGCG (270, 600, 900 and 1200 mg) all containing 600 mg of caffeine. No differences in fat oxidation were observed between the GTE groups and placebo. Although plasma FAs and lactate concentrations were not measured, the authors argued that the high dose of caffeine ingested masked the fat metabolism enhancing effects of green tea.
Acute caffeine ingestion has also been associated with increasing FA availability (1, 11, 20), indicative of lipolysis upregulation. Our present study found an increase in plasma FAs at rest and during exercise, following 7 days of caffeinated GTE ingestion. However, this does not explain why circulating FAs and glycerol were not increased following one day of GTE ingestion.

Endurance training affects substrate utilization. In humans, endurance exercise training programs, as short as nine days, have found increased expression of fatty acid translocase (FAT)/CD36 and carnitine palmitoyltransferase I mRNA, both involved in the uptake of FAs in the skeletal muscle for subsequent oxidation (40). This increased expression of fat metabolism enzymes results in fully functional proteins when exercise training programs are extended (23). Rodent studies have reported increases in protein expression, of enzymes involved in fat metabolism, when GTE has been consumed at rest and alongside exercise training. Sae-Tan et al (37) found that rats supplemented with EGCG for 16 weeks, had increased expression of medium-chain acyl-CoA dehydrogenase (MCAD), uncoupling protein 3 and peroxisome proliferator activated receptor (PPAR) α mRNA. All three of these genes are associated with fat metabolism and mitochondrial biogenesis. In particular, PPARα are transcription factors for enzymes involved in β-oxidation. In this study (37) the rats did not exercise however it appears that EGCG supplementation may mimic the adaptations seen following exercise training. Murase et al (6) found that chronic (10 weeks) ingestion of a GTE, in combination with exercise training, increased β-oxidation activity in mice compared to an exercise only group. This enhanced fat metabolism was attributed to increased expression of FAT/CD36 and MCAD mRNA. Therefore, chronic GTE ingestion alongside an exercise training programme may aid training adaptations. However the current evidence for this is found in animal studies, invasive (muscle biopsies) human studies are needed before conclusions can be made on the mechanisms of long term GTE ingestion.
The effect of chronic GTE ingestion, on fat oxidation during exercise, has been studied in humans. Ota et al (34) administered a GTE beverage over a 2 month period in conjunction with exercise training (treadmill exercise; 5km/hour for 30min 3 times a week). In this study participants only consumed the GTE beverage 3 times a week, before or immediately after an exercise training bout. Here the authors observed that after this 2-month period fat oxidation rates were 24% higher in the GTE group, during exercise, compared to those who had received a placebo beverage. The authors gave no explanation for these findings. It is widely suggested that GTE may exert its effects on fat oxidation primarily through the inhibition of COMT, leading to a suppression of adrenaline degradation and in turn prolonging adrenergic drive and promoting lipolysis. It could be speculated that the increase in fat oxidation observed in the study by Ota et al (34) was a result of GTE-induced expression of fat metabolism proteins and enzymes. In the context of the present study, seven days of GTE supplementation may not have been long enough to form functional proteins to upregulate fat oxidation. Thus, future studies should investigate the effects of chronic GTE supplementation on fat oxidation during exercise.

To our knowledge this is the first study, investigating the metabolic effects of GTE, to report plasma EGCG concentrations at rest and during exercise. Circulating EGCG levels, on Day 8, were not different between the two GTE groups. EGCG has a half life of 8 hours (10) therefore we did not expect a difference in plasma concentrations. However, GTE ingestion in the GTE7 group appeared to have an effect on lipolysis and not in the GTE1 condition. Circulating FAs and glycerol levels were elevated at rest and during exercise, both indicative of increased lipolysis. This confirms that long term GTE ingestion may be more effective in enhancing fat metabolism than acute effects, and are not directly related to plasma EGCG concentrations. It is possible that the increases in lipolysis are explained by longer term adaptations, other catechins or caffeine which may be more active than EGCG. However, this
does not explain why we did not observe an increase in fat oxidation following GTE consumption. All studies investigating the effects of GTE ingestion on substrate metabolism have not reported plasma EGCG concentrations. Thus, we do not know if the plasma EGCG concentrations, in the present study, are lower than in those studies that found GTE ingestion to increase fat oxidation rates.

Higher rates of fat oxidation have been found in trained compared with an untrained population (33). It could be argued that we did not find changes in fat oxidation with GTE ingestion because the subjects, in the present study, were relatively well trained. It has indeed been shown that training results in increased fat oxidation although VO2max (an indicator of training status) is a poor predictor (41). It is clear that fat oxidation can be increased quite substantially as a result of nutritional manipulation, even in well trained individuals. For example, glycogen depletion will result in large increases in fat oxidation both in trained and untrained individuals (35). Furthermore, the 17% increase in fat oxidation, with GTE ingestion in the study by Venables et al (42), was found in subjects with similar training status to the subjects in the present study. Thus, it is unlikely that training status, of the subjects, may have masked the effects of GTE ingestion on fat oxidation. In addition, recent findings from Richards et al (36) found that 3 days of EGCG ingestion (total of seven capsules ingested; 135 mg/capsule) increased VO2max by 4.4% compared to placebo. It could be suggested that GTE + caffeine consumption may have increased the VO2max of participants in the GTE7 group. This would have resulted in subjects working at a lower exercise intensity (% VO2max) on the Day 8 trial. However, the study by Richards et al (36) only used EGCG the present study used a GTE (containing all catechins) plus caffeine. The effects of GTE plus caffeine on VO2max are therefore unknown. Furthermore, the study by Richards et al (36) is the only data on the effects of EGCG on VO2max. Thus more studies are needed before clear conclusion in this area can be made.
In the current study we gave all subjects a controlled diet, consisting of 50% CHO, 35% fat and 15% PRO, to consume in the 24 hours before each exercise trial. Unintentionally, this diet only consisted of ~2200 kcal. Although this is lower than the recommended requirements for healthy young males, data on subject’s habitual food intake was not obtained. Therefore we cannot be certain that subject’s were in a state of negative energy balance when they completed each trial. However, we have confidence that all subjects were tested in the same state for both trials thus, eliminating any affects this may have on our results.

In conclusion, the present study showed no effect of 1 day GTE plus caffeine supplementation on lipolysis or whole body fat oxidation during moderate-intensity exercise. The combined effect of GTE plus caffeine for seven days increased lipolysis but did not result in measurable changes in whole body fat oxidation. These results suggest that the often documented COMT mechanisms may be less important than longer term adaptations of fat metabolism caused by GTE plus caffeine supplementation.
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We acknowledge the work by Krishna Mahabir-Jagessar. R.R was involved in the data acquisition, analysis and interpretation of the results, statistical analysis of results and was a significant manuscript writer; A.H was involved in the data acquisition and analysis and interpretation of the study; S.L worked on the interpretation of the results and significant manuscript reviewer/reviser; D.J was involved in the data analysis and manuscript reviewer; N.B worked on the concept and design of the study and was a manuscript reviewer/reviser; D.M reviewed and revised the manuscript and was involved in the concept of the study; A.J was the principal investigator, involved in the concept and design of the study, contributed to the writing and was a significant reviewer of the manuscript.

Disclosure

S.L, D.J and D.M are employees of Unilever. This work was supported by a research grant from Unilever Plc. The results of the present study do not constitute endorsement by the American College of Sports Medicine.
REFERENCES


FIGURE LEGENDS

**Figure 1.** Study design schematic

**Figure 2.** Mean (± SEM) plasma fatty acids (FAs) (mmol·L\(^{-1}\)) at baseline (T=0) on Day 0 (grey bar) and Day 8 (black bar) in the placebo, GTE1 and GTE7 condition. Differences between trials were determined using a paired samples \(t\) test.* \(P<0.05.\)

**Figure 3.A** Mean (±SEM) plasma FAs (mmol·L\(^{-1}\)) during 60-min steady state exercise on Day 0 (O) and Day 8 (●) in the placebo, GTE1 and GTE7 group. **B** Mean (±SEM) plasma glycerol (µmol·L\(^{-1}\)) during 60-min steady state exercise on Day 0 (O) and Day 8 (●) in the placebo, GTE1 and GTE7 group. Differences between trials were determined using a repeated measured ANOVA.* \(P<0.05.\)

**Figure 4.** Mean (±SEM) plasma EGCG (ng·mL\(^{-1}\)) during the whole trial on Day 0 (O) and Day 8 (●) in the placebo, GTE1 and GTE7 group. Differences between trials were determined using a repeated measured ANOVA.* \(P<0.05.\)
Figure 3.

A. PLA

B. PLA

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Figure 4.

PLA

GTE1

GTE7
Table 1. Subjects characteristics. Data is expressed as Means ± standard deviation (SD).

<table>
<thead>
<tr>
<th></th>
<th>PLA  (n=10)</th>
<th>GTE1 (n=11)</th>
<th>GTE7 (n=10)</th>
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<tr>
<td>Age (y)</td>
<td>23 ± 9</td>
<td>21 ± 2</td>
<td>22 ± 5</td>
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<tr>
<td>Weight (kg)</td>
<td>79.0 ± 10.7</td>
<td>77.7 ± 9.6</td>
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<td>Height (m)</td>
<td>1.80 ± 0.04</td>
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<td>BMI (kg·m⁻²)</td>
<td>24.5 ± 2.6</td>
<td>24.2 ± 1.9</td>
<td>24.8 ± 3.1</td>
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<tr>
<td>VO₂ max (mL·kg⁻¹·min⁻¹)</td>
<td>59.3 ± 8.3</td>
<td>54.5 ± 7.1</td>
<td>52.9 ± 5.3</td>
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</table>
Table 2. Mean (±SEM) whole body fat and CHO oxidation (g∙min⁻¹), RER, Percent (%) contribution of fat and CHO to total EE and EE (kJ∙min⁻¹) over the 60-min steady state exercise bout at baseline (Day 0) and post supplementation (Day 8) in the PLA, GTE1 and GTE7 group. No significant differences were found in any of the measurements.

<table>
<thead>
<tr>
<th></th>
<th>PLA</th>
<th>GTE1</th>
<th>GTE7</th>
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<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 8</td>
<td>Diff</td>
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<tr>
<td>Fat Oxidation (g∙min⁻¹)</td>
<td>0.59 (0.05)</td>
<td>0.59 (0.06)</td>
<td>-0.01 (0.051)</td>
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<td>CHO Oxidation (g∙min⁻¹)</td>
<td>1.76 (0.12)</td>
<td>1.85 (0.12)</td>
<td>-0.09 (0.10)</td>
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<td>% Fat Oxidation</td>
<td>44.2 (2.5)</td>
<td>42.5 (3.4)</td>
<td>-1.7 (3.3)</td>
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<tr>
<td>% CHO Oxidation</td>
<td>55.8 (2.5)</td>
<td>57.5 (3.4)</td>
<td>1.7 (3.3)</td>
</tr>
<tr>
<td>RER</td>
<td>0.87 (0.01)</td>
<td>0.87 (0.01)</td>
<td>0.01 (0.01)</td>
</tr>
<tr>
<td>EE (kJ∙min⁻¹)</td>
<td>54.4 (2.8)</td>
<td>55.4 (2.7)</td>
<td>1.1 (0.7)</td>
</tr>
</tbody>
</table>