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

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Abstract

RANDELL, R. K., A. B. HODGSON, S. B. LOTITO, D. M. JACOBS, N. BOON, D. J. MELA, and A. E. JEUKENDRUP. No Effect of 1 or 7 d of Green Tea Extract Ingestion on Fat Oxidation during Exercise. Med. Sci. Sports Exerc., Vol. 45, No. 5, pp. 883–891, 2013. Purpose: The aim of this study was to investigate the effects of 1 and 7 d of green tea extract (GTE) ingestion on whole body fat oxidation during moderate-intensity exercise. Methods: Thirty-one men completed two exercise trials (60-min cycle, 50% VO2peak). After the baseline trial (day 0), subjects were randomly assigned to one of three conditions involving a week supplementation of the following: 1) 7 d of placebo, 2) 6 d of placebo followed by 1 d of GTE (GTE1), and 3) 7 d of GTE ingestion (GTE7). The morning after the supplementation week, subjects consumed an additional supplement and completed a second exercise trial (day 8). VO2, VCO2, and plasma hormone measurements were taken during exercise to calculate whole body fat oxidation rates. Blood samples, for analysis of plasma fatty acids (FA), glycerol, and epigallocatechin gallate, were collected at rest and during exercise. Results: On day 8, the plasma kinetics and maximal plasma concentrations of epigallocatechin gallate were similar in the GTE1 and GTE7 group (206 ± 28 and 216 ± 25 ng/mL, 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 FA during exercise (P = 0.020) as well as at rest (P = 0.046). However, fat oxidation did not change in any of the groups. Conclusions: There was no effect of 1 d of 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

Green tea is produced from the leaves of Camellia sinensis (L.) of the Theaceae family. Unlike black and oolong tea, green tea leaves are nonoxidized/nonfermented, resulting in high quantities of catechin-polyphenols (catechins) (24). 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 [28]). There is also a substantial body of literature investigating the effects of green tea ingestion on thermogenesis and fat metabolism (21). Although there is ever emerging evidence on the beneficial health effects of green tea and green tea extract (GTE) ingestion, the overconsumption of green tea and highly concentrated GTE may cause adverse effects (37). However, it is yet to be defined how much green tea in one’s 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 (8,30,31,42) but not all (7,17) of studies investigating the possible antiobesity effects of green tea consumption. Wang et al. (42) found that the consumption of a catechin-rich beverage containing caffeine (886 mg catechins and 198 mg caffeine), for 90 d, 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 wk (27). The body weight and body fat loss, found in this study, may be a result of an up-regulation of thermogenesis and substrate oxidation.

It is well established that fatty acids (FA) are the predominant energy source at rest when fasted (20). It has been suggested that GTE ingestion may further enhance the relative use of fat as a fuel. For a 24-h period, Dulloo et al. (13) observed that three feedings of a GTE plus caffeine (375 mg catechins/24 h and 150 mg caffeine/24 h) significantly
decreased respiratory quotient and elevated fat oxidation rates. This contributed to a significant increase in total energy expenditure compared with placebo (2.8%) and caffeine (3.4%). The 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, for a 13-h period. However, on balance, studies have found short-term (24 h) GTE ingestion to up-regulate fat metabolism at rest (18).

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 (26). This would result in more available circulating FA for potential oxidation.

During low- to moderate-intensity exercise, fat oxidation rates are increased 10-fold compared with resting values (20). Research has found that GTE ingestion may further elevate fat oxidation during exercise compared with exercise alone in both animals and humans (6,29,33,38,41). From our laboratory (41), a 17% increase in fat oxidation, during a 30-min exercise bout, was found after 1 d of GTE ingestion (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 h before 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. (38) observed augmented fat oxidation rates in high-fat fed mice after 15 wk of GTE (81.3% catechins) ingestion, in combination with exercise training (running 3 d wk\(^{-1}\) for 30 min). In humans, significantly higher fat oxidation rates (24%) have also been found during exercise, when GTE (570 mg consumed three times a week) was ingested for a 2-month period in combination with regular exercise (34) compared with placebo. However, a lower dosage of GTE, consisting of 160 mg of total catechins consumed daily for 3 wk, did not alter substrate metabolism (14). Together, these studies suggest that long-term supplementation of higher doses of GTE has the potential to up-regulate fat metabolism.

Long-term GTE supplementation studies in rodents have been associated with increased expression of proteins involved in fat metabolism (FA translocase [FAT]/CD36 and medium-chain acyl-CoA dehydrogenase [MCAD] messenger RNA [mRNA] [6]), which could be an alternative to the COMT mechanism described earlier. 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 h) 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 (5,13,14,16,33,41). 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. Furthermore, to elucidate if the duration of GTE ingestion plays a role in up-regulating metabolism, during a moderate-intensity exercise bout, we compared fat oxidation rates after 1- and 7-d supplementation compared with baseline.

**PARTICIPANTS AND METHODS**

**Participants.** Forty-three male participants were recruited for the study. Inclusion criteria included habitual caffeine intake of ≤400 mg d\(^{-1}\) (approximately 4 cups of coffee per day or less) to ensure that all participants were low to moderate caffeine consumers and not desensitized to any caffeine effects. These data were obtained from a caffeine consumption questionnaire. Only 5 participants consumed coffee on a daily basis (one to four cups per day), 16 participants consumed coffee one to three times per week, and 22 participants were non-coffee drinkers. In addition, all volunteers were required to participate in exercise three to five times per 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 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 wk before 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, the 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. \(W_{max}\) (maximal amount of power) was calculated using the following equation (26):

\[
W_{max} = W_{out} + \left[ \frac{(t - 180) \times 35}{t} \right]
\]

where \(W_{out}\) 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 (\(\text{VO}_2\) and \(\text{VCO}_2\)) were collected continuously using an Online Gas...
Analyser (Oxycon Pro, Jaeger, Wuerzburg, Germany). VO₂ was considered maximal, and the test was stopped if two of the four following criteria were met: 1) no further increase or a decrease in VO₂ even when workload increased, 2) a respiratory exchange ratio (RER) of >1.05, 3) an HR within 10 beats per min of age-predicted maximal HR, and 4) a cadence of 50 rpm could not be maintained. HR was recorded during each stage of the test using an HR monitor (Polar). Ẇₘₐₓ was used to determine the workload for all subsequent experimental trials (50% Wₘₐₓ).

**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 d of placebo (PLA), 2) 6 d of PLA followed by 1 d of GTE (GTE1), and 3) 7 d of GTE supplementation (GTE7) (Fig. 1). Exercise tests were performed before (baseline, day 0) and after the supplementation week (day 8). During each trial participants rested for 2 h and then completed a 60-min cycling exercise at 50% of their predetermined Wₘₐₓ (55% VO₂max) (Fig. 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 6:00 and 8:00 a.m. after a 10- to 12-h overnight fast and having avoided any strenuous exercise and consuming any alcohol and caffeinated beverages in the preceding 24 h. The exercise bout in the present study was performed after an overnight fast. This is in line with previous research from our laboratory (41). 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, UK) was inserted into an antecubital vein. A three-way stopcock (Connecta; Becton Dickinson) 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 ethylenediaminetetraacetic acid [EDTA]–containing tubes) blood sample was collected, and then participants rested for 2 h 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-h period and began a 60-min cycling exercise at 50% Wₘₐₓ (55% VO₂max). Blood samples (15 mL) and 4-min respiratory breath samples (Oxycon Pro) 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, UK) after every blood sample and every 15 min during the rest period only. HR was recorded continuously using a Polar HR monitor (Polar RS800CX, Polar Electro Ltd., Warwick, UK), and RPE was recorded every 15 min during the exercise bout. After the baseline exercise test (day 0), participants were supplied with 14 unlabeled cans (330 mL per can) of either the GTE or PLA beverage. After 7 d of supplementation, participants arrived at the Human Performance Laboratory (day 8).
for a second exercise test. This test was identical with that outlined previously; however, on arrival, participants consumed their final test beverage (one can) before the 2-h rest period commenced.

**Supplement.** During the supplementation week, participants consumed two cans per day (15 in total) containing either GTE or placebo. The drinks were consumed 1 h before breakfast and 1 h before evening dinner for 7 d, and an additional drink (one can) was consumed on the morning of the second exercise test (day 8). The supplement was a slightly flavored peach and apricot beverage (330 mL per can), which was enriched with GTE and caffeine. The placebo beverage was matched for color and flavor and contained no catechins but a small amount of caffeine (9.9 mg per can). The amount of GTE in each drink (can) was ~560 mg total catechins (120 mg caffeine), equivalent to approximately four 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 laboratory 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-h period before both trials. The diet consisted of three meals (breakfast, lunch, and dinner) each containing ~50% CHO, ~35% fat, and ~15% protein equating to ~2200 kcal. During this 24-h 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 FA (NEFA-C; Wako Chemicals, Neuss, Germany) and glycerol (Glycerol; Randox, England) were analyzed on an ILAB 650 (Instrumentation Laboratory, Cheshire, UK).

**Plasma EGCG.** To measure the concentrations of deconjugated EGCG, 200 μL of 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 analyzed batchwise and controlled by two quality control samples per sample batch. EGCG was quantified in plasma using 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).

**Calculations.** From the recorded measurements of $\dot{VCO}_2$ and $\dot{VO}_2$ (L·min⁻¹), total fat and total CHO oxidation were calculated (g·min⁻¹) using the following stoichiometric equations (23), assuming that participants were working at a moderate intensity and that protein oxidation was negligible during the exercise bout:

$$\text{carbohydrate oxidation (g·min}^{-1}) = 4.210\dot{VCO}_2 - 2.962\dot{VO}_2 \quad \text{[2]}$$

$$\text{fat oxidation (g·min}^{-1}) = 1.65\dot{VCO}_2 - 1.701\dot{VCO}_2 \quad \text{[3]}$$

**Statistical analysis.** The study was designed as a parallel trial with three arms: two 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 laboratory, have shown that a significant difference in fat oxidation of approximately 0.06 g·min⁻¹ can be obtained.

Data analysis was performed by using the Statistical Package for the Social Sciences for Windows (Version 17; SPSS Inc., Chicago, IL). Data are expressed as means ± SEM 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 FA and glycerol, during exercise between day 0 and day 8, at each time point, were compared using repeated-measures ANOVA (group × 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.** Because of 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 VO2max, 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 >2 SD from the mean were removed ($n = 3$). Therefore, data were available and analyzed for 31 subjects (PLA, $n = 10$; GTE1, $n = 11$; and GTE7 $n = 10$). All subjects were healthy males; there was no significant difference in age, weight, height, BMI, and VO2max between the groups (Table 1).
Workload and exercise intensities. On day 0, the workload of 50% \( W_{\text{max}} \) (151 \( \pm \) 4 W), used during the 60-min exercise bout, elicited a relative \( V_O_2 \) of 56% \( \pm \) 1%, 59% \( \pm \) 1%, and 57% \( \pm \) 1% \( V_O_{2\text{max}} \) for PLA, GTE1, and GTE7, respectively. \( V_O_2 \) on day 8 was not significantly different from day 0 in the PLA and GTE7 groups. In the GTE1 group, \( V_O_2 \) was significantly lower on day 8 compared with day 0 (59% \( \pm \) 1% and 57% \( \pm \) 1% \( V_O_{2\text{max}} \) day 0 and day 8, respectively, \( P < 0.05 \)).

There was no difference in HR between the three groups on day 0 (\( P = 0.236 \)) or day 8 (\( P = 0.727 \)). In addition, there was no difference within groups when day 0 was compared with day 8 (PLA, 133 \( \pm \) 3 and 134 \( \pm \) 3 bpm; GTE1, 143 \( \pm \) 5 and 137 \( \pm \) 4 bpm; GTE7, 139 \( \pm \) 3 and 138 \( \pm \) 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 CHO oxidation. One 7 d of GTE supplementation failed to increase average whole body fat oxidation rates compared with 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 with baseline (day 0), FA at rest (\( t = 0 \)) were significantly higher on day 8 in the GTE7 group (Fig. 2). One day ingestion of GTE did not change plasma FA at rest compared with day 0 (Fig. 2). On day 8, 2 h after an additional GTE beverage (\( t = 120 \) min), plasma FA remain significantly higher in the GTE7 group (day 0: 0.51 \( \pm \) 0.07 mmol L\(^{-1}\) and day 8: 0.74 \( \pm \) 0.08 mmol L\(^{-1}\)). Two hours after GTE ingestion (day 8), plasma FA did not change in the GTE1 group compared with day 0. Plasma FA at rest (\( t = 0 \)) and after GTE ingestion (\( t = 120 \) min) on day 8 were significantly higher in the GTE7 group compared with PLA. During exercise, plasma FA was unaffected by PLA or 1 d of GTE ingestion (Fig. 3A). In the GTE7 group, plasma FA during exercise were higher after 7 d of GTE ingestion (day 8) compared with day 0 (\( P = 0.020 \)) (Fig. 3A).

Compared with baseline (day 0), there was no difference in plasma glycerol concentrations at rest (\( t = 0 \)) after 1 and 7 d of GTE consumption. Two hours after GTE ingestion (\( t = 120 \) min), plasma glycerol was unchanged in all three supplementation groups, when day 0 was compared with day 8. Compared with PLA, plasma glycerol after GTE ingestion (\( t = 120 \) min) on day 8 was significantly higher in the GTE7 group (35.8 \( \pm \) 4.0 and 77.5 \( \pm \) 14.0 mmol 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 with day 0 (\( P = 0.045 \)) (Fig. 3B).

Plasma EGCG. As expected, plasma EGCG concentrations were negligible before and after the supplementation period in the placebo group (Fig. 4). After 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 (Fig. 4). Before GTE ingestion (\( t = 0 \)) on day 8, plasma concentrations of EGCG were significantly higher compared with day 0 in both GTE1 and GTE7. Concentration at \( t = 0 \) were

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**Table 1. Subject characteristics.**

<table>
<thead>
<tr>
<th></th>
<th>PLA (( n = 10 ))</th>
<th>GTE1 (( n = 11 ))</th>
<th>GTE7 (( n = 10 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>23 ( \pm ) 9</td>
<td>21 ( \pm ) 2</td>
<td>22 ( \pm ) 5</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>79.0 ( \pm ) 10.7</td>
<td>77.7 ( \pm ) 9.6</td>
<td>79.2 ( \pm ) 13.2</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.80 ( \pm ) 0.04</td>
<td>1.79 ( \pm ) 0.07</td>
<td>1.78 ( \pm ) 0.05</td>
</tr>
<tr>
<td>BMI (kg m(^{-2}))</td>
<td>24.5 ( \pm ) 2.6</td>
<td>24.2 ( \pm ) 1.9</td>
<td>24.8 ( \pm ) 3.1</td>
</tr>
</tbody>
</table>

*Data are expressed as mean \( \pm \) SD.*

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**Table 2. Mean \( \pm \) SEM whole body fat and CHO oxidation (g min\(^{-1}\)), RER, percentage of contribution of fat and CHO to total EE and EE (kJ min\(^{-1}\)) for the 60-min steady-state exercise bout at baseline (day 0) and postsupplementation (day 8) in the PLA, GTE1, and GTE7 group.**

<table>
<thead>
<tr>
<th></th>
<th>PLA</th>
<th>GTE1</th>
<th>GTE7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat oxidation (g min(^{-1}))</td>
<td>0.59 (0.05)</td>
<td>0.59 (0.06)</td>
<td>0.59 (0.06)</td>
</tr>
<tr>
<td>CHO oxidation (g min(^{-1}))</td>
<td>1.76 (0.12)</td>
<td>1.85 (0.12)</td>
<td>1.85 (0.12)</td>
</tr>
<tr>
<td>% CHO oxidation</td>
<td>55.8 (2.5)</td>
<td>57.5 (3.4)</td>
<td>57.5 (3.4)</td>
</tr>
<tr>
<td>RER</td>
<td>0.87 (0.01)</td>
<td>0.87 (0.01)</td>
<td>0.87 (0.01)</td>
</tr>
<tr>
<td>EE (kJ min(^{-1}))</td>
<td>54.4 (2.8)</td>
<td>55.4 (2.7)</td>
<td>55.4 (2.7)</td>
</tr>
</tbody>
</table>

No significant differences were found in any of the measurements.

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**Figure 2.—Mean \( \pm \) SEM plasma FA (mmol L\(^{-1}\)) at baseline (\( t = 0 \)) on day 0 (black bar) and day 8 (gray bar) in the placebo, GTE1, and GTE7 condition. Differences between trials were determined using a paired samples \( t \)-test. \( * P < 0.05 \).**
around 70 ng mL\(^{-1}\) rising to 220–250 ng mL\(^{-1}\) 60 min after GTE ingestion and leveling 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 d (but not 1 d) 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 after 1 and 7 d of GTE ingestion. This study is, to the best of our knowledge, the first to directly compare the effects of 1 and 7 d of GTE ingestion on fat oxidation during a 60-min exercise bout.

An earlier study from our laboratory (41) found a 17% increase in fat oxidation during exercise (30-min cycle at 50% \(W_{\text{max}}\)), compared with placebo, after a supplementation period which was identical with 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 (41), the GTE did not contain caffeine. However, in the present study, subjects consumed 240 mg of caffeine per day (in addition to 1200 mg catechins per day during the supplementation week) and an additional 120 mg of caffeine 2 h 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 after 7 d of GTE ingestion. 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 CHO 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 the placebo. Although plasma FA 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 (1,11,19), indicative of lipolysis up-regulation. Our present study found an increase in plasma FA at rest and during exercise, after 7 d of caffeinated GTE ingestion. However, this does not explain why circulating FA and glycerol were not increased after one day of GTE ingestion.

Endurance training affects substrate use. In humans, endurance exercise training programs, as short as 9 d, have found increased expression of FAT/CD36 and carnitine palmitoyl-transferase I mRNA, both involved in the uptake of FA in the skeletal muscle for subsequent oxidation (39). This increased expression of fat metabolism enzymes results in fully functional proteins when exercise training programs are extended (22). 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. (36) found that rats supplemented with EGCG for 16 wk had increased expression of MCAD, uncoupling protein 3 and peroxisome proliferator-activated receptor α mRNA. All three of these genes are associated with fat metabolism and mitochondrial biogenesis. In particular, peroxisome proliferator-activated receptor α are transcription factors for enzymes involved in β-oxidation. In this study (36), the rats did not exercise; however, it appears that EGCG supplementation may mimic the adaptations seen after exercise training. Murase et al. (29) found that chronic (10 wk) ingestion of a GTE, in combination with exercise training, increased β-oxidation activity in mice compared with 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 program 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. (33) administered a GTE beverage for a 2-month period in conjunction with exercise training (treadmill exercise; 5 km h⁻¹ for 30 min, three times a week). In this study, participants only consumed the GTE beverage three 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 with 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. (33) was a result of GTE-induced expression of fat metabolism proteins and enzymes. In the context of the present study, 7 d of GTE supplementation may not have been long enough to form functional proteins to up-regulate 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 h (10); therefore, we did not expect a difference in plasma concentrations. However, GTE ingestion in the GTE7 group appeared to affect lipolysis and not in the GTE1 condition. Circulating FA 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 after 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 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 (32). 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 (40). 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 (34). Furthermore, the 17% increase in fat oxidation, with GTE ingestion in the study by Venables et al. (41), 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. (35) found that 3 d of EGCG ingestion (total of seven capsules ingested; 135 mg per capsule) increased VO2max by 4.4% compared with 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, whereas 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. (35) is the only data on the effects of EGCG on VO2max. Thus, more studies are needed before a 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 h 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 were not obtained. Therefore, we cannot be certain that the subjects 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 d of GTE plus caffeine supplementation on lipolysis or whole body fat oxidation during moderate-intensity exercise. The combined effect of GTE plus caffeine for 7 d 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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The results of the present study do not constitute endorsement by the American College of Sports Medicine.

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