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Running Title: Duration of dGTE ingestion and fat oxidation

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ABSTRACT

Purpose: The aim of this study was to investigate if duration of decaffeinated green tea extract (dGTE) ingestion plays a role in augmenting fat oxidation rates during moderate-intensity exercise. Method: In a cross-over, placebo controlled design, 19 healthy males [\(\bar{x}\pm SD\) age: \(21\pm2\) y; weight \(75.0\pm7.0\) kg; body mass index (BMI) \(23.2\pm2.2\) kg\(\cdot\)m\(^{-2}\); maximal oxygen consumption \(\dot{V}O_{2}\text{max}\) \(55.4\pm4.6\) mL\(\cdot\)kg\(^{-1}\)\(\cdot\)min\(^{-1}\)] ingested dGTE and placebo (PLA) for 28 days, separated by a 28 day wash-out period. On the first day (dGTE 1 or PLA 1) and following 7 days (dGTE 7 or PLA 7) and 28 days (dGTE 28 or PLA 28) participants completed a 30-min cycle exercise bout (50\% \(W_{\text{max}}\)), 2 hours after ingestion. Indirect calorimetry was used to calculate rates of whole body fat and carbohydrate oxidation during exercise. Blood samples were collected at rest and during exercise for analysis of plasma fatty acids (FA), glycerol and epigallocatechin gallate (EGCG). Results: Ingestion of dGTE did not significantly change whole body fat oxidation rates during exercise on day 1, 7 or 28 compared to PLA. There were also no changes in plasma concentrations of FA and glycerol at rest and during exercise as a result of dGTE ingestion at any time point compared to PLA. Plasma EGCG concentrations, immediately prior to the exercise bout, in the three dGTE trials were elevated compared with PLA but not different between 1, 7 and 28 days. Conclusion: In contrast to previous reports we found that duration of dGTE ingestion had no effect on whole body fat oxidation rates or fat metabolism-related blood metabolites during exercise in physically active healthy males.

Key words: DECAFFEINATED GTE, SUBSTRATE METABOLISM, EGCG, INDIRECT CALORIMETRY
INTRODUCTION

Carbohydrate and fat are the most important substrates for energy metabolism during exercise. However, the relative contribution of these two substrates differs depending on the exercise duration and intensity. At low to moderate exercise intensities (up to \( \sim 60\% \ VO_2\text{max} \)) fat oxidation increases in absolute terms (g\(\cdot\)min\(^{-1}\)) (1, 23, 31). With further increases in exercise intensity absolute rates of fat oxidation decrease and substrate use shifts to predominately carbohydrate (1, 23, 31). An ability to oxidize fat at high rates during exercise is considered an advantage for endurance trained athletes. Muscle glycogen stores are relatively small thus, in theory, any intervention that increases the capacity of skeletal muscle to oxidize fat could result in muscle glycogen sparing and in turn enhance endurance capacity (10, 11).

A number of studies have investigated the potential health benefits of green tea/ green tea extract (GTE) ingestion (for a full review of the health effects of green tea ingestion see Suzuki et al. (28)) and there is a growing interest in the potential of GTE to increase fat oxidation. Tea is produced from the leaves of *Camellia sinensis* L. of the *Theaceae* family (26). Green tea is unique in that it contains relatively large quantities of catechin-polyphenols (catechins) (15) due to the post-harvest handling process. These catechins are proposed as the active ingredients in GTE with the most abundant being (-)-epigallocatechin-3-gallate (EGCG). Green tea also contains caffeine. The concentration of catechins and caffeine in green tea is highly variable and can differ depending on the type of tea/ extract, infusion time and ratio of tea leaves to water. Studies have used a variety of GTE/ green tea differing in composition, duration of ingestion and sample populations in order to investigate fat oxidation rates at rest (3, 6, 24, 29) and during exercise (7, 21, 33). For a more detailed review the reader is referred to (9).
To gain an insight into the mechanisms, studies have investigated the effects of acute GTE ingestion on substrate metabolism (3, 6, 8, 29, 33). Under exercise conditions Venables et al. (33) found that ingestion of decaffeinated GTE (total: 890 ± 13 mg catechin including 366 ± 5 mg EGCG), in the 24 hour before an exercise bout, increased fat oxidation during a 30 min steady state cycle (60% $V\dot{O}_2\text{max}$) by 17% (+ 0.06 g∙min$^{-1}$) in a group of physically active healthy males. In a follow up study (22), physically active males completed an exercise bout (60 min steady state cycle at 55% $V\dot{O}_2\text{max}$) before and after one or 7 days ingestion of a caffeinated GTE beverage (~1200 mg catechins/ day plus 240 mg caffeine/ day). In this study fat oxidation rates were unchanged following one day of GTE ingestion (22). Ingestion of GTE for seven days significantly increased plasma FAs concentrations but did not result in measurable changes in whole body fat oxidation (22). It was suggested that the consumption of caffeine may have increased glycolytic flux, and in turn inhibited fat oxidation. To date these are the only studies which have investigated the substrate enhancing effects of GTE during exercise following acute ingestion.

In rodents it has been found that chronic (8 - 15 weeks) ingestion of GTE can significantly increase in fat oxidation rates during exercise compared to an exercise only control (18, 19, 27). It is believed that chronic ingestion may cause augmentations in fat oxidation through an upregulation of mitochondrial proteins. This has been demonstrated in animal studies performed under resting (17, 25) and exercise conditions (18) in which increased expression of oxidative enzymes was observed following 8-10 weeks of EGCG ingestion. For example, Murase et al. (18) found that following a 10 week exercise training period mice that consumed high doses of EGCG (0.2 and 0.5% of diet) had significantly greater expression of fatty acid translocase (FAT)/ CD36 and medium-chain acyl-CoA dehydrogenase (MCAD) mRNA compared to
exercise only mice. These observed skeletal muscle adaptations were accompanied by increased β-oxidation rates during an exercise bout (18).

In a population of healthy non-physically active humans, two studies have observed a significant increase in fat oxidation rates during moderate intensity exercise following an 8 – 10 week period of GTE ingestion (12, 21). Although changes in skeletal muscle oxidative enzyme expression were not measured these findings suggest that chronic ingestion of GTE may result in an upregulation of fat oxidation during exercise in healthy untrained adults.

While many studies have investigated the independent effects of acute and chronic GTE ingestion, there is no study which has directly compared the length of ingestion (both acute and chronic) on substrate metabolism during exercise. The available chronic studies in humans, although suggesting beneficial effects, have used untrained individuals. Therefore, it is unknown if longer term ingestion of GTE would have the same effect in a physically active population. Thus the aim of this study was to investigate the acute and long term effects of decaffeinated GTE (dGTE) ingestion on whole body fat oxidation rates during moderate intensity exercise (50% \(W_{\text{max}}\)). Substrate metabolism was measured following a single dose (dGTE 1), and after 7 (dGTE 7) and 28 (dGTE 28) days of ingestion, in physically active healthy males. We hypothesized that ingestion of dGTE will alter fat oxidation during a 30 min steady state exercise bout compared to placebo at all time points. Furthermore, we hypothesized that dGTE 28 will result in greater alterations of fat oxidation compared to dGTE 1 and dGTE 7.
PARTICIPANTS AND METHODS

Participants

For the purpose of this study, participants were recruited from the student population of the University of Birmingham. Inclusion criteria included habitual participation in exercise 3-5 times/week for 30-90 min and caffeine intake of ≤ 400 mg/day (approximately < 4 cups of coffee/tea or caffeinated soda) estimated using a caffeine consumption questionnaire. 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.

The response variable of main interest was whole body fat oxidation during exercise. However, earlier studies on green tea and fat oxidation during exercise contrasted in terms of their design (parallel vs. cross-over, acute vs. chronic, administered doses, populations, etc) therefore no reliable power calculation could be carried out. When a sample size for a pilot investigation cannot be calculated, it is recommended that a minimum of 12 participants per group be considered (14). Statistically significant effects of green tea on energy metabolism were previously reported using a sample size of 10-12 (6, 33). Therefore 20 participants were recruited to account for potential drop-out.

Twenty individuals were recruited, one volunteer dropped out of the study after the first exercise test, due to illness not related to the intervention. Therefore, 19 healthy lean males [mean ± SD age: 21 ± 2 y; weight 75.0 ± 7.0 kg; body mass index (BMI) 23.2 ± 2.2 kg/m²; maximal oxygen consumption ($\dot{VO}_2_{max}$) 55.4 ± 4.6 mL·kg⁻¹·min⁻¹] completed the study.
Preliminary Testing

At least 1 week prior to the first exercise trial, all participants reported to the Human Performance Laboratory, at the University of Birmingham, for preliminary tests. Participants completed an incremental cycle test to exhaustion ($VO_2^{max}$ test), to establish maximal oxygen uptake ($VO_2^{max}$) and maximal power output ($W^{max}$). A 20 min steady state cycle (steady state) at 50% $W^{max}$ (55% $VO_2^{max}$) was also performed, to ensure that the exercise intensity was set correctly for all subsequent exercise trials. The two tests are described in more detail below.

$VO_2^{max}$ test: All participants were given a 5 min warm up at 75 $W$ on an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands). The test started at 95 $W$, every 3 min the effort was increased in incremental steps of 35 $W$, until voluntary exhaustion was reached. During each stage of the test respiratory gas measurements ($VO_2$ and $VCO_2$) were collected using an online gas analyzer (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) if $VO_2$ did not increase 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 RS800CX, Polar Electro (UK) Ltd, Warwick, United Kingdom) and $W^{max}$ was calculated using the following equation (16):

$$W^{max} = W_{out} + [(t/180)\cdot35]$$

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. $W^{max}$ was used to determine the work load for all subsequent experimental trials (50% $W^{max}$).
Steady state: After 15 minutes of rest participants completed a steady state cycle. This involved participants cycling for 20 min at a pre-determined exercise intensity of 50% \( W_{\text{max}} \) (55% \( VO_2_{\text{max}} \); calculated from the \( VO_2_{\text{max}} \) test). To ensure the correct intensity was set \( W \) a 4 min measurement of \( VO_2 \) was obtained, using an online gas analyzer (Oxycon Pro, Jaeger, Wuerzburg, Germany), every 5 min. If the recorded \( VO_2 \) values did not equate to 55% \( VO_2_{\text{max}} \) (± >5%) the resistance on the cycle ergometer was adjusted accordingly.

General Study Design

In a double blind, cross-over, counterbalanced design, participants completed two 28 day periods of dGTE and placebo ingestion, separated by a 28 day wash-out period. On the first day (Day 1) of supplementation (dGTE 1 or PLA 1), 2 hours after ingestion, participants completed a 30 min cycle exercise bout at 50% \( W_{\text{max}} \) (an exercise protocol employed by Venables et al.(33) ). Participants continued to ingest the assigned supplement, on a daily basis, for a total of 28 days. Following the same protocol as day 1, additional exercise trials were completed on the morning following 7 days (dGTE 7 or PLA 7) and 28 days (dGTE 28 or PLA 28) of ingestion. All exercise trials were identical in design (Figure 1).

Exercise Trial

All participants reported to the Human Performance Laboratory between 0600 and 0800 am, after a 10-12 hour overnight fast. 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 whole trial.

An initial 15 mL (5 mL collected in sodium fluoride-containing tubes and 10 mL collected into EDTA-containing tubes) blood sample was collected (t=0). Participants then consumed two capsules with 200 mL of water and rested for 2 hours in a seated position. The catheter was kept patent, during rest and exercise, by flushing with 4-5 mL isotonic saline (0.9 % w·v; B Braun, Sheffield, United Kingdom) after every blood sample and every 30 min during the rest period. Before the exercise bout commenced a second blood sample (15 mL) was taken (t=120).

Participants mounted the cycle ergometer after the 2 hour period and began a 30 min cycling exercise at 50% $W_{\text{max}}$ (55% $V\dot{O}_2\text{max}$). Blood samples (15 mL) and 4 min respiratory breath samples (Oxycon Pro, Jaeger, Wuerzburg, Germany) were collected every 10 min during the exercise bout (Figure 1). HR was recorded continuously with the use of a Polar HR monitor (Polar RS800CX, Polar Electro (UK) Ltd, Warwick, United Kingdom) and Rating of Perceived Exertion (RPE) was recorded every 10 min during the exercise bout.

**Nutritional Intervention**

Ingestion of dGTE or PLA began on the morning of the first exercise trial (dGTE 1 or PLA 1) for each supplementation period, 2 hours before the exercise bout commenced. Participants continued to ingest the assigned supplement for a total of 28 days. On a daily basis participants ingested 4 capsules/ day containing dGTE or placebo. Two capsules were consumed an hour before lunch and two additional capsules were consumed an hour before dinner. The time of
supplement ingestion differed on the exercise trial days when two capsules were consumed in the 
morning (in a fasted state) and the following two capsules were consumed one hour prior to 
dinner.

Participants received the capsules in white (opaque) containers that were sealed. The containers 
were labeled with a number (corresponding to the assigned nutritional intervention, unknown to 
the experimenters and participants) and instructions on when to consume the capsules. The 
containers were filled with enough capsules to last one week (28 in total). Therefore, participants 
visited the laboratory weekly to collect a new container of capsules and to return the empty one. 
This was used as a measure to monitor ingestion adherence.

The composition of the dGTE and PLA capsules can be found in Table 1. A negligible amount of 
caffeine was present in the dGTE (0.82%). The placebo capsules contained cellulose (~270 
mg/capsule). All capsules were identical in color (blue and white) and size (Size 0). Participants 
were contacted daily (~11 am) via text message to remind them to consume two capsules an hour 
before lunch and an additional two capsules an hour before dinner.

**Diet Control**

All participants were given the same controlled diet to consume in the 24 hour period before all 
trials. The diet consisted of three meals (breakfast, lunch and dinner) each containing ~50% 
carbohydrate (CHO), ~35% fat and ~15% protein. Our pre-test day meal equated to a total of 
2700 kilocalories (kcal). The participants differed slightly in body weight (range 63 – 87 kg) and 
activity levels (30-90 min, 3-5 times a week). Although no exercise was performed in the 24 
hours before the exercise tests we wanted to ensure that all participants were roughly in energy
balance in this standardized 24 hour period. Furthermore, during this 24 hour control period participants were asked to refrain from any 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 analyzed on an ILAB 650 (Instrumentation Laboratory, Cheshire, United Kingdom).

**Plasma EGCG**

Samples were analyzed for plasma EGCG concentrations at t=0 and t=120 only. To measure the concentrations of deconjugated EGCG, EDTA plasma (200 µL), stabilizer solution (20 µL, 10 % ascorbic acid containing 0.1 % EDTA), sodium acetate (20 µL of 1.5 mol·L⁻¹ NaOAc, pH 4.8), and β-glucuronidase (10 µL, 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 batch-wise and controlled by two quality control
samples (QCs) per sample batch. The reference material was pure EGCG (Sigma-Aldrich Company Ltd, Dorset, UK). 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).

**Calculations**

From the recorded breath-by-breath measurements of $VCO_2$ and $VO_2$ (mL·min$^{-1}$), total fat and carbohydrate oxidation was calculated (g·min$^{-1}$) using the following stoichiometric equations (13) assuming that participants were working at a moderate intensity and that protein oxidation was negligible during the exercise bout.

\[
\text{Carbohydrate oxidation} = 4.210 \cdot VCO_2 - 2.962 \cdot VO_2 \\
\text{Fat Oxidation} = 1.65 \cdot VO_2 - 1.701 \cdot VCO_2
\]

**Statistical Analysis**

The statistical analysis was performed using SAS software v9.2 (SAS Institute, Cary North Carolina) and $p < 0.05$ was used as the criterion for statistical significance. Data are expressed as mean ± SEMs unless otherwise stated.
The analysis of substrate metabolism data was conducted via a linear mixed model. Treatment, treatment order, and supplementation duration (dGTE 1, dGTE 7, and dGTE 28) were employed as fixed effects in the model, with participant treated as a random effect. A repeated measures covariance structure was employed to accommodate the potential for greater correlation between participants within a treatment arm. The Kenward-Roger method of estimating denominator degrees of freedom in tests of fixed effects was employed. The Tukey-Kramer method of adjustment for multiple comparisons was employed to assess the treatment by supplementation interaction.

For resting blood metabolites, analysis of covariance was used for all data at t=0, when participants had not consumed any supplement (dGTE or placebo). Therefore the blood metabolite data from t=0, from each separate supplementation period, was replaced by the average.

For analysis of FA and glycerol, change from pre-exercise level (t=120) were analyzed via a linear mixed model. Treatment, trial, time and their interactions were treated as fixed effects, as was treatment order. Standardized baseline (t=120) scores were included as a covariate in interaction with trial (thus allowing for differing baselines in each trial). Participant was treated as a random effect. A repeated measures variance structure allowed for possible correlation between observations on a particular visit.

The statistical analysis for plasma EGCG concentration was performed using SPSS for WINDOWS software (version 19; SPSS Inc, Chicago, IL). Data are expressed as means ± SEMs unless otherwise stated. Differences in average plasma EGCG concentration, between dGTE 1 and PLA 1, dGTE 7 and PLA 7, dGTE 28 and PLA 28, at t=0 and t=120, were determined using
paired samples t test. Differences in plasma EGCG concentration at t=0 and t=120 between the three dGTE trials were determined using a one-way ANOVA. Significance was set at $p <0.05$.

RESULTS

Workload and exercise intensity

Participants cycled at an average workload of 139 ± 3 W. This workload equated to an average relative exercise intensity of 55.8 ± 0.3% $\dot{V}O_2$max. Measurements of mean (average of the three breath by breath recordings obtained every 10 minute during the exercise) absolute $\dot{V}O_2$ were similar between exercise bouts (Table 2). Thus, participants worked at the same exercise intensity during all trials (Table 2).

Substrate Metabolism

Rates of whole body fat oxidation, measured every 10 min during exercise in the dGTE 1, dGTE 7 and dGTE 28, compared to placebo, are shown in Figure 2. Average whole body fat oxidation rates during the 30 min exercise bout was not statistically different following dGTE 1 (Effect size (ES)= -0.3), dGTE 7 (ES= 0.10) and dGTE 28 (ES= 0.08) compared to placebo. Furthermore, there was no statistical difference in CHO oxidation rates following ingestion of dGTE at any of the measured time points. Therefore total energy expenditure (EE) and the relative contribution of fat and CHO oxidation to total EE was not statistically different in all three dGTE exercise trials compared to placebo (Table 2).
Plasma FA and glycerol at rest

At t=0, plasma FA ($p = 0.13$, $ES = -0.15$) and glycerol ($p = 0.25$, $ES = 0.26$) concentrations were not different in the dGTE 1 trial compared to PLA 1. In addition, following 7 days and 28 days ingestion of dGTE there was no statistically significant difference in plasma FA ($ES = 0.20$ and $ES = 0.50$ for 7 days and 28 days respectively) and glycerol ($ES = 0.28$ and $ES = 0.44$ for 7 days and 28 days respectively) at t=0.

Plasma FA and glycerol during exercise

Plasma FAs ($ES = -0.41$) and glycerol ($ES = -0.48$) during exercise in the dGTE 1 trial were not statistically different from PLA 1 (Figure 3). Furthermore, there was no statistical difference in plasma FAs ($ES = 0.10$ and $ES = 0.16$ for 7 days and 28 days respectively) and glycerol ($ES = -0.21$ and $ES = 0.05$ for 7 days and 28 days respectively) during exercise in the dGTE 7 and dGTE 28 compared to PLA 7 and PLA 28 respectively (Figure 3).

Plasma EGCG

For the analysis of EGCG, outliers were determined as any data point that was ±2SD away from the mean. Furthermore, it has been found that under basal fasted conditions blood concentrations of EGCG range between 0-20 ng·mL$^{-1}$ in healthy males (22). In the PLA 28 trial two participants had plasma EGCG concentrations which were significantly greater than 2SDs and > 20 ng·mL$^{-1}$. Therefore both these data points were removed from the final analysis of all blood and breath by breath variables.
Plasma EGCG concentration have been found to range from \( \sim 120 - 560 \text{ ng}\cdot\text{mL}^{-1} \) (average \( \sim 260 \text{ ng}\cdot\text{mL}^{-1} \)) two hours following consumption of GTE (\( \sim 600 \text{ mg total catechins} \)) (22). In addition, Chow et al. (5) found on average plasma EGCG to be \( \sim 800 \text{ ng}\cdot\text{mL}^{-1} \), following ingestion of 400 mg EGCG. In the present study one participant’s plasma EGCG concentrations, at t=120 in the dGTE 1 trial, was negligible and did not increase above baseline. Furthermore, in the dGTE 28 trial one participant’s plasma EGCG concentrations was >2SD away from the mean at t=0 and did not increase at t=120. Therefore these data points were removed from the final analysis. The removal of these data points did not affect the main outcome of this data.

In the dGTE 1 trial at t=0 there was no difference in plasma EGCG concentrations compared to PLA 1 (\( p > 0.05 \)) (Figure 4 A). After 7 and 28 days ingestion of dGTE (dGTE 7 and dGTE 28) at t=0 circulating levels of plasma EGCG were significantly higher compared to PLA 7 and PLA 28 (\( p < 0.001 \) and \( p < 0.05 \) respectively) (Figure 4 A). On the morning of the exercise trials at t=120 plasma EGCG concentrations, in all dGTE trials, were significantly increased compared to placebo (\( p < 0.05 \) in all trials compared to placebo) (Figure 4 B).

At t=0 there was a significant difference in plasma EGCG in the dGTE 1 trial compared to dGTE 7 (\( p = .003 \)) and dGTE 28 (\( p = .002 \)) (Figure 4B). This was expected as no dGTE had been consumed in the dGTE 1 trial at this time point. However, at t=120 there was no difference in any of the three dGTE trials (Figure 4B).

**DISCUSSION**

In the present study ingestion of dGTE did not elicit changes in fat oxidation rates, irrespective of the length of the supplementation period. To our knowledge this is the first human study to
investigate, in a placebo controlled cross over design, the effects of dGTE ingestion on fat oxidation during exercise over a period of 28 days.

The findings presented here are in contrast to previous work investigating the effects of dGTE ingestion on fat oxidation, by Venables et al. (33). Venables et al. (33) found acute (24 hour) ingestion of encapsulated dGTE increased fat oxidation rates by 17% during exercise compared to placebo. During this study (33) healthy physically active males ingested dGTE in the 24 hours prior to, and an additional dose ~1 hour before the exercise bout. In the present study our acute ingestion trial involved participants consuming a single bolus of dGTE prior to the exercise trial (dGTE 1). This difference in methodology could explain the inconsistencies in findings following acute GTE ingestion. Our EGCG data show elevated plasma levels in the fasted basal state following 7 days of dGTE ingestion. Furthermore, a recent study from our lab found elevated fasted plasma EGCG concentrations after consuming GTE in the previous 24 hours (one dose consumed an hour before breakfast and an addition dose one hour before dinner) (22). EGCG has a half life of 8-10 hours (4), although no change in fat metabolism was observed in our previous study (22) these data suggest that prior GTE feeding elevates fasted basal plasma EGCG levels. Thus, the cumulative effect of acute GTE ingestion (in the days before) and the additional dose on the morning of the trial may result in acute augmentations of fat oxidation.

Furthermore in the present study, 7 and 28 days of dGTE feeding did not result in measureable changes in fat oxidation compared to placebo. In a recent study by Randell et al. (22) ingestion of a caffeinated GTE beverage for 7 days did not alter substrate metabolism during a 60 min exercise bout, despite increases in plasmas FA concentrations. Additionally, Eichenberger et al. (7), found no influence of three weeks ingestion of a caffeinated GTE (160 mg catechins/ day) on any indices of fat metabolism during a two hour exercise bout. Exercise may be a prerequisite
for the green tea to exert its long term effects. Only studies which have combined chronic GTE ingestion (8-10 weeks) and an exercise training program, in untrained adults, have found an upregulation of fat oxidation during exercise (12, 21). Unfortunately, no study to date has measured skeletal muscle oxidative enzymes, to confirm the increases seen in animals (18). Future studies may wish to explore this theory. However, these studies suggest that GTE may be more potent when consumed on a daily basis alongside an endurance training program in untrained individuals.

Previously, ingestion of a caffeine-free GTE has been found to augment fat oxidation during exercise (33). More recently ingestion of a caffeinated GTE was not found to significantly alter exercise metabolism (7, 22). Randell et al. (22) suggested that the caffeine present may have inhibited the upregulation of fat metabolism. Therefore in the present study we administered a dGTE. However, independent of length of ingestion dGTE did not alter fat oxidation during exercise. Taken together these data imply that it is still unknown what the optimal composition of GTE is in order to increase fat metabolism during exercise.

Other factors may contribute to the effectiveness of GTE feeding on fat oxidation during exercise. One of these is the training status of the test participants. Endurance exercise training is known to cause skeletal muscle adaptations in favor of fat metabolism (30). Higher rates of absolute fat oxidation have been found in trained compared with untrained/ sedentary populations (2, 20). The participants in the present study were physically active (exercising 3-5 times a week) with an average \( \dot{V}O_2 \) max of 55.2 mL·kg\(^{-1}\)·min\(^{-1}\) (ranging from \( \dot{V}O_2 \) max: 49 to 64 mL·kg\(^{-1}\)·min\(^{-1}\)). Only one study has found GTE to increase fat oxidation in physically active males (33). Other studies (including the present study), using a similar cohort of individuals, have not replicated these findings (7, 22).
It is currently unknown if training status or aerobic capacity (indicated by $V\dot{O}_2$max) affects the apparent metabolic impact of GTE. To explore this hypothesis, we performed a simple post-hoc analysis and divided participants into groups with either a $V\dot{O}_2$max value above or below the mean of this population. The average $V\dot{O}_2$max in the low group was 51 mL· kg$^{-1}$·min$^{-1}$ and in the high group 59 mL· kg$^{-1}$·min$^{-1}$ ($p<0.05$). The percent change in mean whole body fat oxidation rates observed in the dGTE 1, dGTE 7 and dGTE 28 trials were greater in the low $V\dot{O}_2$max group (-2.9 ± 11.9, 12.0 ± 10.5, 16.0 ± 16.8% for the three time points respectively) than that observed in the high $V\dot{O}_2$max group (-6.6 ± 3.8, -2.6 ± 6.3, 2.2 ± 5.7% for the three time points respectively). Although there was no statistical difference between the two groups at any time points, it became apparent that there were more ‘responders’ in the low $V\dot{O}_2$max group, compared to the high $V\dot{O}_2$max group. Thus, $V\dot{O}_2$max may be a predictor for the metabolic effects of GTE ingestion. It should also be acknowledged that $V\dot{O}_2$max was only measured once (during the preliminary testing visit) in the present study. Over the time course of the study (12 weeks) $V\dot{O}_2$max may have decreased or increased in some participants. This could have contributed to some individuals eliciting a more blunted response to dGTE ingestion (and vice versa if $V\dot{O}_2$max decreased). Taken together future studies may wish to specifically test the potency of GTE effects on participants differing in aerobic capacity ($V\dot{O}_2$max).

A steady state exercise bout, at a fixed exercise intensity, has been employed in all studies investigating the effects of GTE on substrate metabolism. However, this exercise protocol does not account for the large inter-individual variation in fat oxidation rates and the exercise intensity at which maximal fat oxidation rates occur (FATMAX). This could account for the inconsistencies in findings between studies. Achten et al. (1) found, in a group of 18 moderately
trained cyclists, that FATMAX ranged from 42 – 84% $\dot{V}O_2$max (mean 64 ± 4% $\dot{V}O_2$max). Furthermore, a large scale study using 300 males and females found that FATMAX ranged from 25 to 77% $\dot{V}O_2$max (mean 48 ± 1% $\dot{V}O_2$max) (32). It is currently unknown at what exercise intensity (below or above FATMAX), GTE is most effective. Furthermore, it is likely that there are individual differences in the exercise intensity at which the effects of GTE feeding are optimal.

In conclusion 1 day, 7 days or 28 days of dGTE ingestion did not alter fat oxidation rates during exercise in physically active healthy males, compared to placebo. Plasma concentrations of FAs and glycerol were also unchanged with dGTE ingestion. Thus, this study suggests that dGTE feeding alone, irrespective of duration, has no measurable change in fat metabolism and any change that may have occurred are small and inconsistent. However, we suggest that future studies should investigate the effects of aerobic capacity ($\dot{V}O_2$max) on GTE feeding and the potential individual differences in the exercise intensity at which GTE elicits maximal effects on augmenting fat oxidation.

Acknowledgements

We acknowledge the work by Dr Sarah Aldred and Dr Theo Mulder. 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; M.R worked on the statistical analysis of the data and reviewed the manuscript; 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, M.R 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


CAPTIONS

Figure 1: Exercise trial protocol. Following ingestion of either dGTE or PLA (first downwards pointed arrow) participants rested for two hours (white box). The second downwards pointed arrow indicates the start of the exercise bout which involved a 30 min steady state cycle at ~50% Wmax (grey box). Upward pointed arrows represent blood samples and the start of each breath sample measurement are represented by an X (collection time lasted 4 min).

Figure 2. Mean (± SEM) fat oxidation rates (g·min⁻¹), measured every 10 min during exercise on Day 1, Day 7 and Day 28; placebo (open circles) and dGTE (filled circles).

Figure 3. Mean (± SEM) plasma FA (mmol·L⁻¹) (A) and glycerol (µmol·L⁻¹) (B) measured every 10 min during exercise on Day 1, Day 7 and Day 28; placebo (open circles) and dGTE (filled circles). Time (minutes) are in relation to the start of the exercise test day. Data presented here is not normalized to t=120min.

Figure 4. Mean (± SEM) plasma EGCG (ng·mL⁻¹) at time point 0 (A; t=0) and two hours (B; t=120) following ingestion of dGTE (black bars) or PLA (white bars) during all trials. * represents a significant difference from placebo (p <0.05). † represents a significant difference from dGTE 1 (p <0.05).
Figure 1.

![Diagram showing a timeline of dGTE/PLA ingestion, rest, and exercise phases with blood and breath samples at specified times.]

- **dGTE/PLA ingestion**
- **Start of exercise**
- **Rest**
- **Exercise**
  - Blood samples at 0, 120, 130, 140, 150 minutes
  - Breath samples at 0, 120, 130, 140, 150 minutes
Figure 2

Day 1

Day 7

Day 28

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Figure 4

(A) 

(B)
Table 1: Composition of dGTE and PLA capsules. Values are means (±SD).

<table>
<thead>
<tr>
<th></th>
<th>dGTE</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGCG mg/ capsule</td>
<td>156 ± 3</td>
<td>-</td>
</tr>
<tr>
<td>Total Catechins</td>
<td>284 ± 6</td>
<td>-</td>
</tr>
<tr>
<td>Caffeine ~</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>Cellulose</td>
<td>-</td>
<td>273 ± 25</td>
</tr>
<tr>
<td>EGCG Total mg/ day</td>
<td>624 ± 3</td>
<td>-</td>
</tr>
<tr>
<td>Total Catechins</td>
<td>1136 ± 24</td>
<td>-</td>
</tr>
<tr>
<td>Caffeine ~</td>
<td>11</td>
<td>-</td>
</tr>
<tr>
<td>Cellulose</td>
<td>-</td>
<td>1049 ± 91</td>
</tr>
</tbody>
</table>
Table 2. Mean (±SEM) $VO_2$ and $VCO_2$ (L·min$^{-1}$), Energy Expenditure (EE) (kJ·min$^{-1}$) and percent (%) contribution of fat and CHO to total EE, over the 30-min steady state exercise bout, during all trials. No significant differences were found in any of the measurements.

<table>
<thead>
<tr>
<th></th>
<th>dGTE 1</th>
<th>PLA 1</th>
<th>Diff</th>
<th>dGTE 7</th>
<th>PLA 7</th>
<th>Diff</th>
<th>dGTE 28</th>
<th>PLA 28</th>
<th>Diff</th>
</tr>
</thead>
<tbody>
<tr>
<td>$VO_2$ (L·min$^{-1}$)</td>
<td>2.35 (0.05)</td>
<td>2.34 (0.4)</td>
<td>-0.01 (0.03)</td>
<td>2.30 (0.05)</td>
<td>2.32 (0.05)</td>
<td>0.02 (0.02)</td>
<td>2.30 (0.05)</td>
<td>2.30 (0.5)</td>
<td>0.00 (0.02)</td>
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<tr>
<td>$VCO_2$ (L·min$^{-1}$)</td>
<td>2.03 (0.04)</td>
<td>2.00 (0.03)</td>
<td>-0.03 (0.02)</td>
<td>1.98 (0.04)</td>
<td>2.02 (0.04)</td>
<td>0.03 (0.02)</td>
<td>1.99 (0.04)</td>
<td>2.00 (0.03)</td>
<td>0.01 (0.03)</td>
</tr>
<tr>
<td>% $VO_2$max</td>
<td>56.1 (0.7)</td>
<td>55.9 (0.8)</td>
<td>-0.1 (0.8)</td>
<td>55.8 (0.7)</td>
<td>56.3 (0.7)</td>
<td>0.5 (0.7)</td>
<td>55.4 (0.7)</td>
<td>55.5 (0.7)</td>
<td>0.0 (0.6)</td>
</tr>
<tr>
<td>EE (kJ·min$^{-1}$)</td>
<td>48.6 (0.9)</td>
<td>48.4 (0.9)</td>
<td>-0.2 (0.7)</td>
<td>47.6 (1.1)</td>
<td>48.0 (1.0)</td>
<td>0.5 (0.5)</td>
<td>47.6 (1.0)</td>
<td>47.6 (1.0)</td>
<td>0.1 (0.5)</td>
</tr>
<tr>
<td>% Fat Oxidation</td>
<td>43.3 (2.5)</td>
<td>47.1 (2.8)</td>
<td>3.9 (2.1)</td>
<td>44.1 (2.8)</td>
<td>42.4 (2.6)</td>
<td>-1.7 (2.1)</td>
<td>43.5 (2.7)</td>
<td>42.2 (2.9)</td>
<td>-1.4 (2.6)</td>
</tr>
<tr>
<td>% CHO Oxidation</td>
<td>56.7 (2.5)</td>
<td>52.9 (2.8)</td>
<td>-3.9 (2.1)</td>
<td>55.8 (2.8)</td>
<td>57.6 (2.6)</td>
<td>1.7 (2.1)</td>
<td>56.5 (2.7)</td>
<td>57.8 (2.9)</td>
<td>1.4 (2.6)</td>
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