Variable Duration of Decaffeinated Green Tea Extract Ingestion on Exercise Metabolism

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[1]Human Performance Laboratory, School of Sport and Exercise Sciences, University of Birmingham, Birmingham, UNITED KINGDOM; [2]Unilever R&D, Colworth Science Park, Sharnbrook, Bedfordshire, UNITED KINGDOM; [3]Unilever R&D, Vlaardingen, THE NETHERLANDS

ABSTRACT

RANDELL, R. K., A. B. HODGSON, S. B. LOTITO, D. M. JACOBS, M. ROWSON, D. J. MELA, and A. E. JEUKENDRUP. Variable Duration of Decaffeinated Green Tea Extract Ingestion on Exercise Metabolism. *Med. Sci. Sports Exerc.*, Vol. 46, No. 6, pp. 1185–1193, 2014. **Purpose**: The aim of this study was to investigate if the duration of decaffeinated green tea extract (dGTE) ingestion plays a role in augmenting fat oxidation rates during moderate-intensity exercise. **Methods**: In a crossover, placebo-controlled design, 19 healthy males (mean ± SD; age = 21 ± 2 yr, weight = 75.0 ± 7.0 kg, body mass index = 23.2 ± 2.2 kg·m^{-2}, maximal oxygen consumption [$\dot{V}O_{2max}$] = 55.4 ± 4.6 mL·kg^{-1}·min^{-1}) ingested dGTE and placebo (PLA) for 28 d, separated by a 28-d washout period. On the first day (dGTE 1 or PLA 1) and after 7 d (dGTE 7 or PLA 7) and 28 d (dGTE 28 or PLA 28), participants completed a 30-min cycle exercise bout (50% W_{max}), 2 h 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, glycerol, and epigallocatechin gallate. **Results**: The ingestion of dGTE did not significantly change whole-body fat oxidation rates during exercise on day 1, 7, or 28 compared with PLA. There were also no changes in plasma concentrations of fatty acids and glycerol at rest and during exercise as a result of dGTE ingestion at any time point compared with PLA. Plasma epigallocatechin gallate concentrations, immediately before the exercise bout, in the three dGTE trials were elevated compared with PLA but not different between 1, 7, and 28 d. **Conclusion**: In contrast to previous reports, we found that the 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

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 ~60% $\dot{V}O_{2max}$), fat oxidation increases in absolute terms (g·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).

Several 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) because of the postharvest 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 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 Hodgson et al. (9).

To gain an insight into the mechanisms, studies have investigated the effects of acute GTE ingestion on substrate

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metabolism (3,6,8,29,33). Under exercise conditions, Venables et al. (33) found that the ingestion of decaffeinated GTE (dGTE; total: 890 ± 13 mg catechin including 366 ± 5 mg EGCG) in the 24 h before an exercise bout increased fat oxidation during a 30-min steady-state cycle (60% $\dot{V}O_{2max}$) 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% $\dot{V}O_{2max}$) before and after 1 or 7 d ingestion of a caffeinated GTE beverage (~1200 mg of catechins per day plus 240 mg of caffeine per day). In this study, fat oxidation rates were unchanged after 1 d of GTE ingestion (22). The ingestion of GTE for 7 d significantly increased plasma fatty acid (FA) 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 that have investigated the substrate-enhancing effects of GTE during exercise after acute ingestion.

In rodents, it has been found that the chronic (8–15 wk) ingestion of GTE can significantly increase in fat oxidation rates during exercise compared with an exercise only control (18,19,27). It is believed that chronic ingestion may cause augmentations in fat oxidation through an up-regulation of mitochondrial proteins. This has been demonstrated in animal studies performed under resting (17,25) and exercise conditions (18), in which an increased expression of oxidative enzymes was observed after 8–10 wk of EGCG ingestion. For example, Murase et al. (18) found that after a 10-wk exercise training period, mice that consumed high doses of EGCG (0.2% and 0.5% of diet) had significantly greater expression of FA translocase (FAT)/CD36 and medium-chain acyl-CoA dehydrogenase mRNA compared with 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 nonphysically active humans, two studies have observed a significant increase in fat oxidation rates during moderate-intensity exercise after an 8- to 10-wk period of GTE ingestion (12,21). Although changes in skeletal muscle oxidative enzyme expression were not measured, these findings suggest that the chronic ingestion of GTE may result in an up-regulation of fat oxidation during exercise in healthy untrained adults.

Although many studies have investigated the independent effects of acute and chronic GTE ingestion, there is no study that 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 the 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 the long-term effects of dGTE ingestion on whole-body fat oxidation rates during moderate-intensity exercise (50% W_{max}). Substrate metabolism was measured after a single dose (dGTE 1) and after 7 d (dGTE 7) and 28 d (dGTE 28) of ingestion in physically active healthy males. We hypothesized that the ingestion of dGTE will alter fat oxidation during a 30-min steady-state exercise bout compared with placebo at all time points. Furthermore, we hypothesized that dGTE 28 will result in greater alterations of fat oxidation compared with 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 three to five times per week for 30–90 min and caffeine intake of ≤ 400 mg·d^{-1} (approximately less than four 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 crossover, 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 dropout.

Twenty individuals were recruited; one volunteer dropped out of the study after the first exercise test because of illness not related to the intervention. Therefore, 19 healthy lean males (mean $\pm$ SD; age = 21 ± 2 yr, weight = 75.0 ± 7.0 kg, body mass index = 23.2 ± 2.2 kg·m^{-2}, maximal oxygen consumption ($\dot{V}O_{2max}$) = 55.4 ± 4.6 mL·kg^{-1}·min^{-1}) completed the study.

Preliminary Testing

At least 1 wk before 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 ($\dot{V}O_{2max}$ test) to establish maximal oxygen uptake ($\dot{V}O_{2max}$) and maximal power output (W_{max}). A 20-min steady-state cycle (steady state) at 50% W_{max} (55% $\dot{V}O_{2max}$) 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 in the next section.

$\dot{V}O_{2max}$ **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, and 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 ($\dot{V}O_2$ and $\dot{V}CO_2$) were collected using an online gas analyzer (Oxycon Pro; Jaeger, Wuerzburg, Germany). $\dot{V}O_2$ was considered maximal, and the test was stopped if two of the four following criteria were met: 1) if $\dot{V}O_2$ did not increase even when workload increased, 2) an RER of >1.05, 3) a heart rate within 10 beats per min of age-predicted maximal heart rate, 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 RS800CX; Polar Electro Ltd., Warwick, UK), and W_{max} was calculated using the following equation (16):

$$W_{max} = W_{out} + 35(t/180),$$

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 workload for all subsequent experimental trials (50% W_{max}).

Steady state. After 15 min of rest, participants completed a steady-state cycle. This involved participants cycling for 20 min at a predetermined exercise intensity of 50% W_{max} (55% $\dot{V}O_{2max}$; calculated from the $\dot{V}O_{2max}$ test). To ensure the correct intensity was set (W), a 4-min measurement of $\dot{V}O_2$ was obtained, using an online gas analyzer (Oxycon Pro; Jaeger), every 5 min. If the recorded $\dot{V}O_2$ values did not equate to 55% $\dot{V}O_{2max}$ ($\pm$ >5%), the resistance on the cycle ergometer was adjusted accordingly.

General Study Design

In a double-blind, crossover, counterbalanced design, participants completed two 28-d periods of dGTE and placebo ingestion, separated by a 28-d washout period. On the first day (day 1) of supplementation (dGTE 1 or PLA 1), 2 h after ingestion, participants completed a 30-min cycle exercise bout at 50% W_{max} (an exercise protocol used by Venables et al. [33]). Participants continued to ingest the assigned supplement, on a daily basis, for a total of 28 d. After the same protocol as day 1, additional exercise trials were completed on the morning after 7 d (dGTE 7 or PLA 7) and 28 d (dGTE 28 or PLA 28) of ingestion. All exercise trials were identical in design (Fig. 1).

Exercise Trial

All participants reported to the Human Performance Laboratory between 6:00 and 8:00 a.m., after a 10- to 12-h overnight fast. 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;
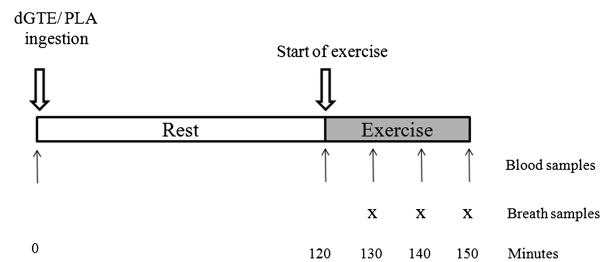

FIGURE 1—Exercise trial protocol. After the ingestion of either dGTE or PLA (first downward-pointed arrow), participants rested for 2 h (white box). The second downward-pointed arrow indicates the start of the exercise bout, which involved a 30-min steady-state cycle at ~50% W_{max} (gray box). Upward-pointed arrows represent blood samples and the start of each breath sample measurement are represented by X (collection time lasted 4 min).

Becton Dickinson) 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 h 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, UK) 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-h period and began a 30-min cycling exercise at 50% W_{max} (55% $\dot{V}O_{2max}$). Blood samples (15 mL) and 4-min respiratory breath samples (Oxycon Pro; Jaeger) were collected every 10 min during the exercise bout (Fig. 1). HR was recorded continuously using a Polar HR monitor (Polar RS800CX; Polar Electro Ltd.), and RPE was recorded every 10 min during the exercise bout.

Nutritional Intervention

The ingestion of dGTE or PLA began on the morning of the first exercise trial (dGTE 1 or PLA 1) for each supplementation period, 2 h before the exercise bout commenced. Participants continued to ingest the assigned supplement for a total of 28 d. On a daily basis, participants ingested four capsules per 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 1 h before 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 1 wk (28 in total).

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TABLE 1. Composition of dGTE and PLA capsules.

		dGTE	Placebo
Milligrams per capsule	EGCG	156 ± 3	—
	Total catechins	284 ± 6	—
	Caffeine	~3	—
	Cellulose	—	273 ± 25
Total (mg·d^{-1})	EGCG	624 ± 3	—
	Total catechins	1136 ± 24	—
	Caffeine	~11	—
	Cellulose	—	1049 ± 91

Values are presented as mean ± SD.

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 per 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-h 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 pretest day meal equated to a total of 2700 kcal. The participants differed slightly in body weight (range, 63–87 kg) and activity levels (30–90 min, three to five times a week). Although no exercise was performed in the 24 h before the exercise tests, we wanted to ensure that all participants were roughly in energy balance in this standardized 24-h period. Furthermore, during this 24-h 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 (Randox, England, UK) were analyzed on an ILAB 650 (Instrumentation Laboratory, Cheshire, UK).

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, 50,000 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. The reference material was pure EGCG (Sigma-Aldrich Company Ltd., Dorset, UK). 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 breath-by-breath measurements of $\dot{V}CO_2$ and $\dot{V}O_2$ (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, as follows:

$$\text{carbohydrate oxidation} = 4.210\dot{V}CO_2 - 2.962\dot{V}O_2$$

$$\text{fat oxidation} = 1.65\dot{V}O_2 - 1.701\dot{V}CO_2$$

Statstical Analysis

The statistical analysis was performed using SAS software version 9.2 (SAS Institute, Cary, NC), and $P < 0.05$ was used as the criterion for statistical significance. Data are expressed as mean ± SEM, 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 used as fixed effects in the model, with participant treated as a random effect. A repeated-measures covariance structure was used 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 used. The Tukey–Kramer method of adjustment for multiple comparisons was used to assess the treatment by supplementation interaction.

For resting blood metabolites, ANCOVA 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, were replaced by the average.

For analysis of FAs and glycerol, changes from preexercise 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). Participants were treated as a random effect. A

TABLE 2. $\dot{V}O_2$ and $\dot{V}CO_2$ (L·min^{-1}), energy expenditure (EE) (kJ·min^{-1}), and percent (%) contribution of fat and CHO to total EE during the 30-min steady-state exercise bout during all trials.

	dGTE 1	PLA 1	Diff	dGTE 7	PLA 7	Diff	dGTE 28	PLA 28	Diff
$\dot{V}O_2$ (L·min^{-1})	2.35 ± 0.05	2.34 ± 0.4	−0.01 ± 0.03	2.30 ± 0.05	2.32 ± 0.05	0.02 ± 0.02	2.30 ± 0.05	2.30 ± 0.5	0.00 ± 0.02
$\dot{V}CO_2$ (L·min^{-1})	2.03 ± 0.04	2.00 ± 0.03	−0.03 ± 0.02	1.98 ± 0.04	2.02 ± 0.04	0.03 ± 0.02	1.99 ± 0.04	2.00 ± 0.03	0.01 ± 0.03
% $\dot{V}O_{2max}$	56.1 ± 0.7	55.9 ± 0.8	−0.1 ± 0.8	55.8 ± 0.7	56.3 ± 0.7	0.5 ± 0.7	55.4 ± 0.7	55.5 ± 0.7	0.0 ± 0.6
EE (kJ·min^{-1})	48.6 ± 0.9	48.4 ± 0.9	−0.2 ± 0.7	47.6 ± 1.1	48.0 ± 1.0	0.5 ± 0.5	47.6 ± 1.0	47.6 ± 1.0	0.1 ± 0.5
% Fat oxidation	43.3 ± 2.5	47.1 ± 2.8	3.9 ± 2.1	44.1 ± 2.8	42.4 ± 2.6	−1.7 ± 2.1	43.5 ± 2.7	42.2 ± 2.9	−1.4 ± 2.6
% CHO oxidation	56.7 ± 2.5	52.9 ± 2.8	−3.9 ± 2.1	55.8 ± 2.8	57.6 ± 2.6	1.7 ± 2.1	56.5 ± 2.7	57.8 ± 2.9	1.4 ± 2.6

Data are presented as mean ± SEM. No significant differences were found in any of the measurements.

repeated-measures variance structure allowed for a possible correlation between observations on a particular visit.

The statistical analysis for plasma EGCG concentration was performed using the Statistical Package for the Social Sciences for Windows (version 19; SPSS Inc., Chicago, IL). Data are expressed as means ± SEM, unless otherwise stated. Differences in average plasma EGCG concentration, between dGTE 1 and PLA 1, dGTE 7 and PLA 7, and 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% $\dot{V}O_{2max}$. Measurements of mean (average of the three breath by breath recordings obtained every 10 min 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 with placebo, are shown in Figure 2. Average whole-body fat oxidation rates during the 30-min exercise bout was not statistically different after dGTE 1 (effect size [ES] = −0.3), dGTE 7 (ES = 0.10), and dGTE 28 (ES = 0.08) compared with placebo. Furthermore,

there was no statistical difference in CHO oxidation rates after the ingestion of dGTE at any of the measured time points. Therefore, the total energy expenditure and the relative contribution of fat and CHO oxidation to total energy expenditure were not statistically different in all three dGTE exercise trials compared with 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 with PLA 1. In addition, after the 7- and 28-d ingestion of dGTE, there was no statistically significant difference in plasma FA (ES = 0.20 and ES = 0.50 for 7 and 28 d, respectively) and glycerol (ES = 0.28 and ES = 0.44 for 7 and 28 d, 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 (Fig. 3). Furthermore, there was no statistical difference in plasma FAs (ES = 0.10 and ES = 0.16 for 7 and 28 d, respectively) and glycerol (ES = −0.21 and ES = 0.05 for 7 and 28 d, respectively) during exercise in the dGTE 7 and dGTE 28 compared with PLA 7 and PLA 28, respectively (Fig. 3).

Plasma EGCG. For the analysis of EGCG, outliers were determined as any data point that was ±2 SD away from the mean. Furthermore, it has been found that under basal fasted conditions, blood concentrations of EGCG range between 0 and 20 ng·mL^{-1} in healthy males (22). In the PLA 28 trial, two participants had plasma EGCG concentrations, which were significantly greater than 2 SD and >20 ng·mL^{-1}. Therefore, both these data points were removed from the final analysis of all blood and breath-by-breath variables.

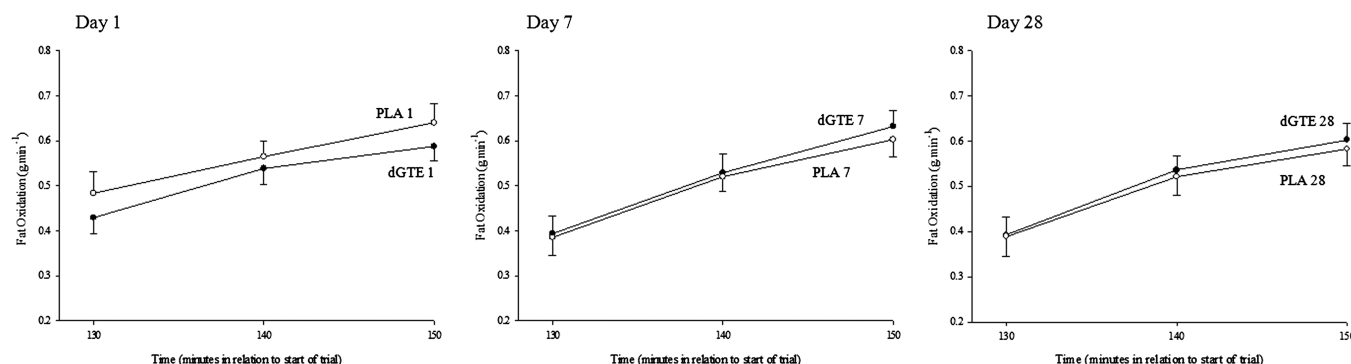**FIGURE 2—Mean ± SEM fat oxidation rates (g·min^{-1}), measured every 10 min during exercise on days 1, 7, and 28; placebo (*open circles*) and dGTE (*filled circles*).**

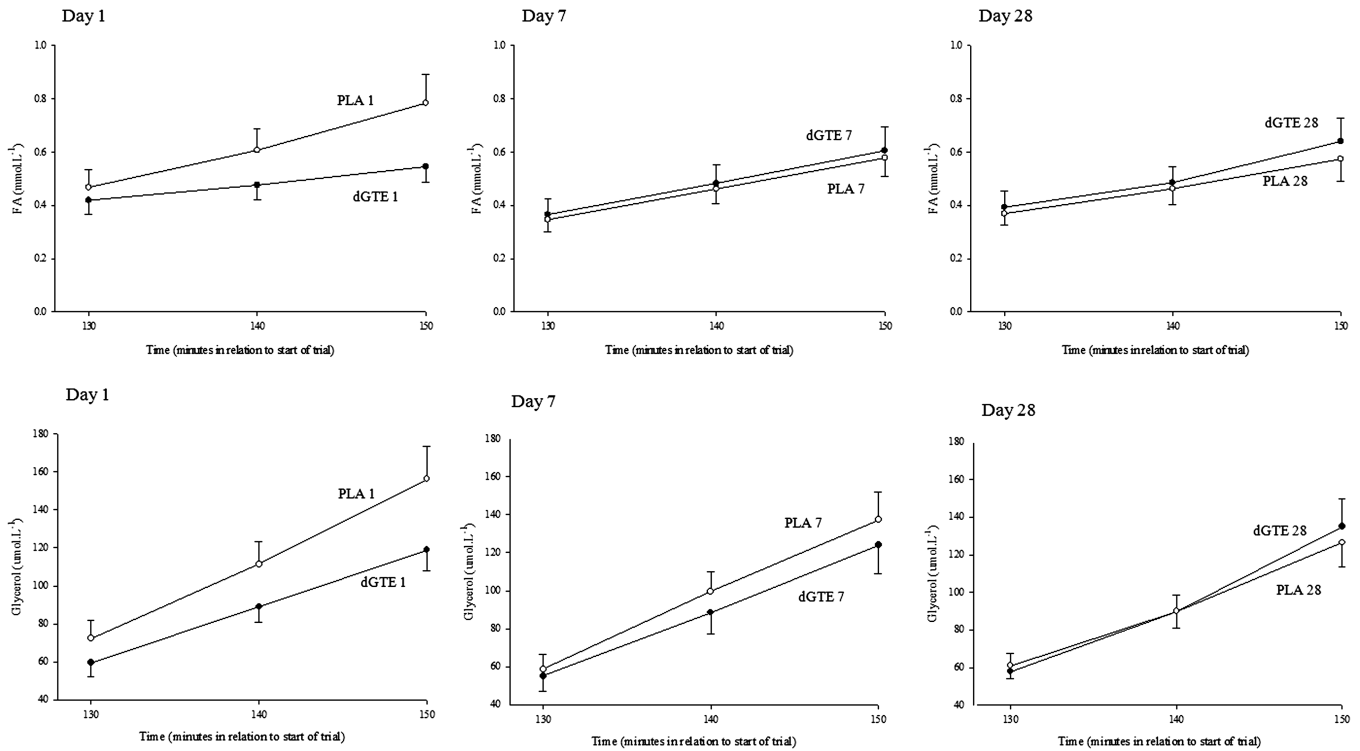**FIGURE 3—Mean ± SEM plasma FA (mmol·L^{-1}) (A) and glycerol (μmol·L^{-1}) (B) measured every 10 min during exercise on days 1, 7, and 28; placebo (*open circles*) and dGTE (*filled circles*). Time (min) is in relation to the start of the exercise test day. Data presented here is not normalized to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

Plasma EGCG concentration have been found to range from ~120 to 560 ng·mL^{-1} (average ~260 ng·mL^{-1}) 2 h after the consumption of GTE (~600 mg total catechins) (22). In addition, Chow et al. (5) found on average plasma EGCG to be ~800 ng·mL^{-1}, after the ingestion of 400 mg EGCG. In the present study, one participant's plasma EGCG concentration, 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 concentration was >2 SD 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 these data.

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

At t = 0, there was a significant difference in plasma EGCG in the dGTE 1 trial compared with dGTE 7 (P = 0.003) and dGTE 28 (P = 0.002) (Fig. 4B). This was expected as no
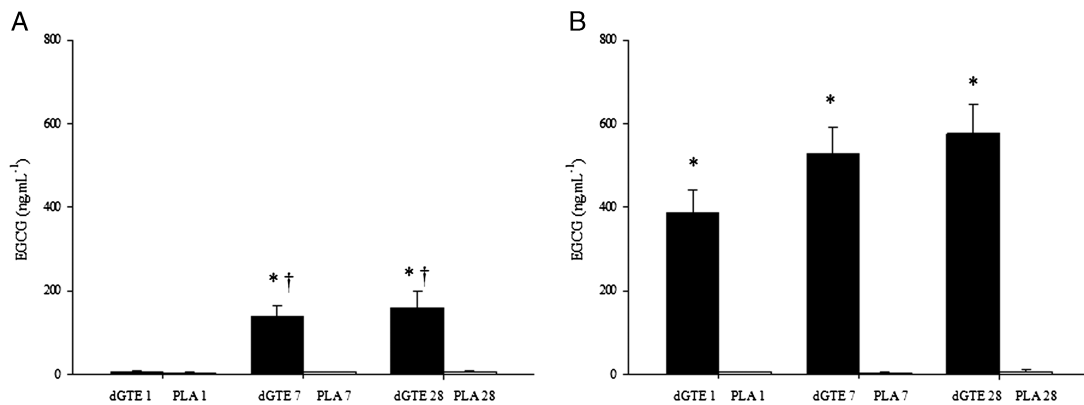

FIGURE 4—Mean ± SEM plasma EGCG (ng·mL^{-1}) at time point 0 (A; t = 0) and 2 h (B; t = 120) after the ingestion of dGTE (*black bars*) or PLA (*white bars*) during all trials. *Significant difference from placebo (P < 0.05). †Significant difference from dGTE 1 (P < 0.05).

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 (Fig. 4B).

DISCUSSION

In the present study, the 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 crossover design, the effects of dGTE ingestion on fat oxidation during exercise for a period of 28 d.

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 that the acute (24 h) ingestion of encapsulated dGTE increased fat oxidation rates by 17% during exercise compared with placebo. During this study (33), healthy physically active males ingested dGTE in the 24 h before and an additional dose ~1 h before the exercise bout. In the present study, our acute ingestion trial involved participants consuming a single bolus of dGTE before the exercise trial (dGTE 1). This difference in methodology could explain the inconsistencies in findings after acute GTE ingestion. Our EGCG data show elevated plasma levels in the fasted basal state after 7 d of dGTE ingestion. Furthermore, a recent study from our laboratory found elevated fasted plasma EGCG concentrations after consuming GTE in the previous 24 h (one dose consumed an hour before breakfast and an addition dose 1 h before dinner) (22). EGCG has a half-life of 8–10 h (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 d of dGTE feeding did not result in measureable changes in fat oxidation compared with placebo. In a recent study by Randell et al. (22), the ingestion of a caffeinated GTE beverage for 7 d did not alter substrate metabolism during a 60-min exercise bout, despite increases in plasmas FA concentrations. In addition, Eichenberger et al. (7), found no influence of the 3-wk ingestion of a caffeinated GTE (160 mg catechins per day) on any indices of fat metabolism during a 2-h exercise bout. Exercise may be a prerequisite for the green tea to exert its long-term effects. Only studies that have combined chronic GTE ingestion (8–10 wk) and an exercise training program, in untrained adults, have found an up-regulation 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, the ingestion of a caffeine-free GTE has been found to augment fat oxidation during exercise (33). More recently, the 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 up-regulation of fat metabolism. Therefore, in the present study, we administered a dGTE. However, independent of the 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 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 three to five times a week) with an average $\dot{V}O_{2max}$ of 55.2 mL·kg^{-1}·min^{-1} (ranging from $\dot{V}O_{2max}$: 49–64 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 $\dot{V}O_{2max}$) 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 $\dot{V}O_{2max}$ value above or below the mean of this population. The average $\dot{V}O_{2max}$ 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 $\dot{V}O_{2max}$ group ($-2.9\% \pm 11.9\%$, $12.0\% \pm 10.5\%$, and $16.0\% \pm 16.8\%$ for the three time points, respectively) than that observed in the high $\dot{V}O_{2max}$ group ($-6.6\% \pm 3.8\%$, $-2.6\% \pm 6.3\%$, $2.2\% \pm 5.7\%$ 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 $\dot{V}O_{2max}$ group compared with the high $\dot{V}O_{2max}$ group. Thus, $\dot{V}O_{2max}$ may be a predictor for the metabolic effects of GTE ingestion. It should also be acknowledged that $\dot{V}O_{2max}$ was only measured once (during the preliminary testing visit) in the present study. During the time course of the study (12 wk), $\dot{V}O_{2max}$ 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 $\dot{V}O_{2max}$ decreased). Taken together, future studies may specifically test the potency of GTE effects on participants differing in aerobic capacity ($\dot{V}O_{2max}$).

A steady-state exercise bout, at a fixed exercise intensity, has been used in all studies investigating the effects of GTE on substrate metabolism. However, this exercise protocol does not account for the large interindividual variation in fat

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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% to 84% $\dot{V}O_{2max}$ (mean 64% ± 4% $\dot{V}O_{2max}$). Furthermore, a large-scale study using 300 males and females found that FATMAX ranged from 25% to 77% $\dot{V}O_{2max}$ (mean 48% ± 1% $\dot{V}O_{2max}$) (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, 7, or 28 d of dGTE ingestion did not alter fat oxidation rates during exercise in physically active healthy males compared with 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 measureable change in fat metabolism, and any changes that may have occurred are small and inconsistent. However, we suggest that future studies should investigate the effects of aerobic capacity ($\dot{V}O_{2max}$) on GTE feeding and the potential individual differences in the exercise intensity at which GTE elicits maximal effects on augmenting fat oxidation.

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