Bioavailability and antioxidant activity of tea flavanols after consumption of green tea, black tea, or a green tea extract supplement

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ABSTRACT

Background: Green and black tea polyphenols have been extensively studied as cancer chemopreventive agents. Many in vitro experiments have supported their strong antioxidant activity. Additional in vivo studies are needed to examine the pharmacokinetic relation of absorption and antioxidant activity of tea polyphenols administered in the form of green or black tea or tea extract supplements.

Objective: The purpose of this study was to compare the pharmacokinetic disposition of tea polyphenols and their effect on the antioxidant capacity in plasma 8 h after a bolus consumption of either green tea, black tea, or a green tea extract supplement.

Design: Thirty healthy subjects were randomly assigned to 3 different sequences of green tea, black tea, or a green tea extract supplement in a 3 × 3 crossover design with a 1-wk washout period in between treatments.

Results: Flavanol absorption was enhanced when tea polyphenols were administered as a green tea supplement in capsule form and led to a small but significant increase in plasma antioxidant activity compared with when tea polyphenols were consumed as black tea or green tea. All 3 interventions provided similar amounts of (−)-epigallocatechin-3-gallate.

Conclusions: Our observations suggest that green tea extract supplements retain the beneficial effects of green and black tea and may be used in future chemoprevention studies to provide a large dose of tea polyphenols without the side effects of caffeine associated with green and black tea beverages. Am J Clin Nutr 2004;80:1558–64.

KEY WORDS Bioavailability, pharmacokinetics, black tea, green tea, antioxidant capacity, tea flavanols, trolox equivalent antioxidant capacity

INTRODUCTION

Green tea (GT) and black tea (BT) are both derived from the tea leaves of *Camellia sinensis*. GT is manufactured by drying the leaves of *C. sinensis*. The manufacture of BT requires an additional fermentation step, which generates theaflavins and thearubigins (1). Theaflavins and thearubigins are oligomeric polyphenolic compounds synthesized from monomeric tea flavanol units (Figure 1). Tea flavanols are regarded as the biologically active constituents of tea (2). The main flavanols found in GT, and to a lesser extent in BT, are (−)-epigallocatechin-3-gallate (EGCG), (−)-epigallocatechin (EGC), (−)-epicatechin-3-gallate (ECG), and (−)-epicatechin (EC). EGCG is the most abundant and widely studied tea polyphenol (2). EGCG and ECG have the highest radical scavenging activity (3). Because of the worldwide popularity of tea and because of the absence of toxicity as a natural dietary agent, tea is an excellent candidate for dietary cancer prevention (4). Decaffeinated GT supplements are available to provide large doses of polyphenols without the unwanted side effects of caffeine. Caffeine, however, might contribute to the anticarcinogenic effect of tea, as shown in several animal models (5). Studies in cell lines and animal models have clearly shown that tea flavanols have antiproliferative, antiangiogenic, and anticarcinogenic activities (6). However, efforts to translate in vitro and animal studies to human interventions have met with limited success (7). They are hampered in part by the artificial nature of in vitro studies, which include the exposure of cells to supraphysiologic concentrations of tea catechins and a lack of information on the bioavailability of tea flavanols. Limited data on the bioavailability of green and BT flavanols from green and BT solids or GT extract supplements (GTS) is available from separate laboratories (8, 9) but has not been investigated in the same study participants under conditions providing equal amounts of EGCG.

The in vitro antioxidant potential of different teas and tea flavanols has been evaluated extensively (3, 10). Data from in vivo studies investigating the antioxidant activity of tea showed mixed results depending on the methods applied and study design (11, 12). Most of the studies were either long term (3 wk) or short term (up to 3 h) and were conducted in fasting participants.

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2 Supported by NIH grants R03 CA91163, 5P01 CA42710, and M01-RR00865. Pharmanex (Provo, UT) provided the green tea extract, Uncle Lee’s Tea (El Monte, CA) provided the green tea, and R.C. Bigelow, Inc (Fairfield, CT), provided the Darjeeling blend black tea.

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It was the objective of this study to determine the pharmacokinetics of plasma concentrations of tea flavanols and their effect on the antioxidant capacity of plasma after a bolus consumption of GT, BT, or GTS for 8 h.

SUBJECTS AND METHODS

Study design

Thirty healthy subjects (14 men and 16 women) between 20 and 39 y of age were recruited. Subjects were asked to abstain from consuming tea, tea-related products, vitamins, minerals, and dietary or herbal supplements throughout the study period. Heavy tea drinkers and users of hormone therapy or antibiotics were excluded. All subjects were screened with a complete medical history and signed the informed consent form. A randomized 3 × 3 crossover design with a 1-wk washout period was used for the intervention study. The subjects were randomly assigned to 3 different sequences of GT, BT, or GTS at the University of California, Los Angeles (UCLA), General Clinical Research Center (GCRC). Participants arrived fasting and were provided with a low-flavonoid breakfast. After breakfast, baseline blood samples were drawn, and the subjects were given 30 min to consume the tea or tea supplement. Subjects were allowed unlimited water intake and were provided with a low-flavonoid lunch and dinner. Blood was collected 1, 2, 4, 6, and 8 h after the tea or tea capsule consumption. The protocol was approved by the Institutional Review Board for the Protection of Human Subjects in Research at UCLA.

Chemicals and solvents

EGCG, EGC, EC, ECG, \( \beta \)-glucuronidase type VIII from Escherichia coli, arylsulfatase type VIII from abalone entrails, 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), manganese dioxide, and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich (St Louis). Green tea (Uncle Lee’s Tea, El Monte, CA) and BT bags (Bigelow Darjeeling Blend; R.C. Bigelow Inc, Fairfield, CT) from one production lot was generously provided by each tea company. GTS was donated by Pharmanex (Provo, UT). HPLC-grade acetonitrile, citric acid, and ammonium acetate were purchased from Fisher Scientific (Pittsburgh).

Tea preparation

Tea flavanol, theaflavin, and caffeine concentrations were measured in the tea beverages, which were prepared in the same way as in a previous study that used HPLC analysis (3). For the intervention study, hot BT and GT were prepared by trained staff at the UCLA GCRC. Four bags of BT or 3 bags of GT were added to 426 mL boiling water and steeped for 5 min. The number of tea bags was chosen to match the EGCG content. Tea was served immediately with or without sugar or 45 mL milk.

Sample collection and storage

The blood samples were collected immediately after each draw at the UCLA GCRC and transported to the UCLA Center for Human Nutrition on ice for processing and storage. Urine volumes were measured and recorded. Blood in EDTA vacuum tubes were centrifuged at 4 °C at 2060 \( \times \) g for 10 min in a GS-6R centrifuge (Beckman Instruments, Palo Alto, CA); 0.8-mL plasma aliquots were stored at \(-70^\circ\)C without supplement or with 80 \( \mu \)L of 20% ascorbic acid—0.1% EDTA solution (0.4 mmol/L NaH2PO4, pH 3.6).

Flavanol analysis in plasma and urine

A modification of the method by Lee et al (13) was used. Briefly, 200 \( \mu \)L plasma or urine was mixed with 12 \( \mu \)L of 10% ascorbic acid:40 mmol/L NaH2PO4;0.1% EDTA, 20 \( \mu \)L of 50 mmol sodium phosphate/L (pH 7.4), 20 \( \mu \)L of 2.3 \( \mu \)mol catechin gallate/L as internal standard, 500 units of \( \beta \)-glucuronidase type X from Escherichia coli (Sigma Chemical Co, St Louis), and 4 units of sulfatase type VIII from abalone entrails (Sigma Chemical Co). The mixture was incubated at 37 \( ^\circ \)C for 45 min. For plasma extraction the reaction was stopped by the addition of...
2 mL ethyl acetate followed by vigorous shaking for 20 min and centrifugation at 4 °C at 2000 × g for 5 min. The supernatant fraction was transferred to a new borosilicate tube, and the ethyl acetate extraction was repeated. For urine extraction, the reaction was stopped by adding 400 μL sodium acetate (0.5 mol/L, pH 5). The urine sample was purified by using a BondElute LRC solid-phase extraction (SPE) column (100 mg, C18; Varian, Inc, Palo Alto, CA). After the SPE column was washed with 2 mL sodium acetate (0.05 mol/L, pH 6.5), flavanols were eluted with 4 mL ethyl acetate; 10 μL 0.2% ascorbic acid:0.005% EDTA was added to the pooled supernatant fraction (plasma extraction) or SPE eluent (urine extraction) and vacuum concentrated for 2 h at low heat. Samples were reconstituted in 200 μL of 75 mmol citric acid/L/25 mmol ammonium acetate/L:acetonitrile (75:25, by vol), vortexed vigorously for 15 min, sonicated for 10 min, and centrifuged (14000 × g, 10 min, 4 °C), and 20 μL of the supernatant fraction was injected into the HPLC column. The HPLC-electrochemical detection system consisted of an Agilent 1100 Series quaternary pump solvent delivery system (Agilent Technology, San Diego), an Agilent 1100 temperature regulated autosampler, an Agilent 1100 Chemstation Software 9.01, an ESA 5600A coulometric array electrochemical detector (ESA, Bedford, MA), a C18 Altima guard column (7.5 mm × 4.6 mm, particle size of 5 μm; Alltech, Deerfield, IL), and a C18 Altima column (53 mm × 7 mm, particle size of 5 μm; Alltech). Standard flavanol solutions (18–59 μmol/L) and internal standard catechin gallate were prepared in a 75 mmol citric acid/L/25 mmol ammonium acetate/L:acetonitrile (90:10, by vol) solution and stored at −70 °C. The column was eluted at 25 °C with a linear gradient from 100% buffer A (75 mmol citric acid/L and 25 mmol ammonium acetate/L) to 90% buffer A and 10% buffer B (75 mmol/L citric acid and 25 mmol/L ammonium acetate:acetonitrile, 50:50) in 4 min at a flow rate of 1 mL/min. The gradient was linearly changed from 90% buffer A and 10% buffer B to 70% A and 30% B (4–12 min), 66%A/34%B (12–17 min), 63%A/37%B (17–20 min), 57%A/43%B (20–29 min), 100% B (29–33 min), and 100% A (33–35 min) and maintained at 100% A until analysis of the next sample. The eluent was monitored by HPLC-electrochemical detection with potential settings at −90, −10, 70, 150, 230, 310, 400, and 480 mV. The dominant channel was 230 mV. Recovery of flavanols from plasma was determined in 3 different concentrations with each batch of plasma samples. The minimal detection limit was 0.02, 0.001, 0.004, and 0.001 μmol/L in plasma and 0.1, 0.02, 0.1, and 0.004 μmol/L in urine for EGC, EC, EGCG and ECG, respectively.

### Plasma trolox equivalent antioxidant capacity assay

The trolox equivalent antioxidant capacity (TEAC) assay was performed according to Miller et al (14). ABTS + radical cations were prepared by adding solid manganese dioxide to a 5 mmol/L aqueous stock solution of ABTS + (14). The ABTS + cations were passed through a Whatman no. 1 filter paper and a PVDF syringe filter. The concentration was adjusted with a sodium phosphate buffer (75 mmol/L, pH 7) to an absorbance of 0.7 at 750 nm and preincubated at 30 °C before use. Fresh ABTS + cation solution was prepared daily. Trolox was used as an antioxidant standard. Plasma samples were diluted 1:20 in sodium phosphate buffer (75 mmol/L, pH 7). An ABTS + radical cation solution (200 μL) was mixed with 20 μL diluted plasma in 96 well plates and absorbance was read after 5–75 min in the Thermomax microplate reader ( Molecular Devices, Sunnyvale, CA).

Samples were analyzed in triplicate determinations. A fresh Trolox standard curve was prepared with each batch of plasma analysis, and trolox equivalents (TE) in μmol/L were derived from the standard curve at 5 and 75 min incubation.

## Statistics

Statistical analysis for a general 3 × 3 crossover design was carried out. Treatment effects, treatment-by-sequence effects, and carryover effects were included in the model. The peak plasma concentrations (Cmax) and the time to peak concentration (tmax) were recorded as observed. The area under the plasma concentration-time (0–8 h) curve (AUC) was estimated by using the linear trapezoidal rule. Because no carryover effects were identified, this study was treated as a repeated measurement study with 3 sequences, 3 periods, and 3 interventions (GT, BT, and GTS). The mixed model was used to compare the AUC, Cmax, and Tmax values between the 3 interventions. The change in plasma TE from baseline to 8 h was analyzed by using the AUC. Pairwise comparisons were carried out, and the P values were adjusted by using Tukey’s method. All statistical analyses were performed by using SAS version 8 (SAS Institute Inc, Cary, NC) (15), and all tests were two-sided and had an significance level of < 0.05.

### RESULTS

#### Plasma pharmacokinetic profile of tea flavanols

After a bolus consumption of GT, BT, or GTS, the concentration of total (unconjugated, sulfated, and glucoronidated) tea flavanols was quantified by HPLC with coulometric array electrochemical detection. The major tea flavanols detected in plasma were EGC, EC, EGCG, and ECG. The extraction method showed a recovery of 90–109%.

The content of flavanols, gallic acid, total theaflavin, and caffeine of the 3 tea products used in the intervention are shown in Table 1. Standardizing the EGCG content in the tea preparations affected the contents of the other tea flavanols. Green tea contained 2.5 and 11 times the amount of EGCG than of BT and GTS, respectively, and BT contained 4 times that of EGCG than of GTS (Table 1). The EC content was not different between BT and GTS, whereas GT contained twice as much EC than BT and GTS. There was no difference in ECG between GT, BT, and GTS.

The pharmacokinetic parameters are summarized in Table 2. The GT preparation provided the highest dose of EGCG and EC. Cmax and AUC values for plasma EGCG concentrations were significantly higher after GT consumption than after BT and GTS consumption. In addition, plasma EC Cmax was significantly higher after GT consumption than after BT and GTS consumption. BT contained 4 times as much EGCG compared with GTS, but plasma Cmax and AUC were only twice the concentration after BT consumption than after GTS consumption (Table 2). Although there was no difference in EC consumption, GTS showed elevated Cmax and AUC values (P < 0.0001) for plasma EC compared with BT (Table 2). The same trend was also apparent for EGCG and ECG. GTS consumption resulted in 60% and 123% increases in EGCG’s Cmax compared with the GT and BT interventions, respectively, and 90% and 123% increases in ECG’s Cmax compared with GT and BT, respectively (Table 2).

These results strongly suggest that when the same amounts of tea flavanols were administered in the form of tea brewed or as GTS,
the GTS was more effective in elevating serum flavanol concentrations. The total amount of EGC, EGCG, EC, and ECG provided by the GT, BT, and GTS were 679.0 ± 9.4, 496.5 ± 3.3, and 386.5 ± 3.2 mg, respectively. Consistent with the consumption dose, the highest total plasma flavanol concentration was attained with GT consumption (1.23 ± 0.09 μmol/L) (Figure 2).

However, the total plasma flavanol concentrations did not correlate with initial intakes of BT and GTS. The GTS intervention resulted in a higher total plasma flavanol concentration (0.66 ± 0.05 μmol/L) than did the BT intervention (0.47 ± 0.04 μmol/L), even though the BT contained 28.5% more flavanols (Figure 2).

After GTS consumption, t\(\text{max}\) was extended by ≈1 h for all 4 tea flavanols compared with after BT and GT consumption (\(P < 0.05\); Table 2). The urinary flavanol excretion, expressed as a percentage of flavanol consumption, for EGCG and EC was 0.2–1.3%, whereas for EGCG and ECG it was 0.01–0.06% (Table 3). The percentage excretions for EGC and EC were significantly higher after GTS than after GT and BT (Table 3).

In vitro trolox equivalent antioxidant capacity assay values of flavanols in buffer or plasma

The conventional TEAC assay is performed by incubating the sample with ABTS•⁺ radicals for 1 min. Since we adapted the assay to a 96 well plate for technical reasons, we extended the initial
We also continued reading the absorbance every 15 min for 75 min. The effect of time on the suppression of the absorbance of the ABTS$^{•-}$ radical by a pooled plasma or trolox (20 μmol/L) sample incubated for 5–60 min is shown in Figure 3. The decrease in the absorbance of the ABTS$^{•-}$ radical by reduction of the cation radical is equivalent to the antioxidant capacity. The suppression of the absorbance of the ABTS$^{•-}$ radical was completed after 5 min of incubation with trolox, whereas after incubation with the pooled plasma, the absorbance declined slowly and at 60 min 15% of the original absorbance was left (Figure 3). The determination of the antioxidant capacity of flavanols at different concentrations added to buffer or to pooled plasma is shown in Figure 4. Because of the instability of the flavanol standards at pH 7, five-minute incubation data are shown (Figure 4). The plasma antioxidant activity was increased at flavanol concentrations starting at 7.5 μmol/L. EGCG and ECG showed higher antioxidant activity than did EGC and EC. The increase was linear with increasing flavanol concentrations up to 30 μmol/L (Figure 4). The same rate of increase in buffer and pooled plasma was observed with increasing flavanol concentrations. The TE value of all 4 flavanols mixed together was equivalent to the calculated sum of TE of the individual flavanols up to 15 μmol/L (Figure 4).

![FIGURE 3.](image)

**FIGURE 3.** Effect of time (0–60 min) on the suppression of the absorbance of the radical cation 2,2’-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt by a pooled plasma or trolox (20 μmol/L) sample. Values are the mean (±SEM) of 3 determinations per time point. Antioxidant activity was determined by using the trolox equivalent antioxidant capacity assay.

![FIGURE 4.](image)

**FIGURE 4.** Trolox equivalent (TE) values of pooled plasma to which different concentrations of (−)-epigallocatechin (EGC), (−)-epicatechin (EC), (−)-epigallocatechin-3-gallate (EGCG), and (−)-epicatechin-3-gallate (ECG), or a mixture of the 4 flavanols were added (A) or to which the same concentrations of the flavanols were added (B). Values are the mean (±SEM) of 3 determinations per time point. Antioxidant activity was determined by using the trolox equivalent antioxidant capacity assay, which measured the reduction in the radical cation 2,2’-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt.

### TABLE 3

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<th>Plasma</th>
<th>GTS</th>
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<tr>
<td></td>
<td>GT</td>
<td>BT</td>
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<tr>
<td>% of intake</td>
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<tr>
<td>EGC</td>
<td>0.36 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.26 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>EC</td>
<td>0.56 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.23 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>EGCG</td>
<td>0.07 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.08 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>ECG</td>
<td>0.12 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.11 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
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<sup>1</sup> All values are x ± SEM; n = 28–30 participants in a randomized crossover design. Means in a row with different superscript letters are significantly different, P < 0.05 (mixed-model ANOVA and Tukey’s method).
Pharmacokinetics of TE after GT, BT, and GTS consumption

The change in plasma antioxidant capacity after the consumption of GT, BT, and GTS was evaluated by using the mixed model with baseline adjustment as the AUC of TE values from 0 to 8 h after the bolus tea consumption. As shown in Table 4, when plasma samples were incubated with the ABTS·•⁺ radicals for 5 min, the AUC for TE was significantly higher after GTS consumption than after BT and GT consumption. When incubated for 75 min, the AUC for TE was significantly higher after GTS than after BT only.

DISCUSSION

The main objective of this study was to compare the pharmacokinetics and effects on plasma antioxidant activity of tea flavanols after a bolus consumption of GT, BT, or GTS. The study was conducted with the use of GT, BT, and GTS matched for their EGCG contents. GT and GTS generally contain higher amounts of EGCG than BT (3). However, Darjeeling and Ceylon BTs contain unusually high amounts of EGCG (16). In the current study, we used the Darjeeling Blend of BT to match the EGCG content of the GT and GTS. This tea, however, also contained a typical amount of total theaflavins to be classified as BT.

The maximum plasma flavanol concentrations determined in this study compared well with data generated by other investigators (8). Confirming previous studies, we also detected a lower plasma bioavailability of EGCG than of EGC and EC (8). Furthermore, we were able to detect and quantify plasma concentrations of ECG. Our initial attempts to identify ECG in plasma were hindered by its low plasma concentration, which was masked by interfering peaks in the voltogram of the electrochemical detector. However, our method was optimized to separate the ECG peak. The ECG peak was verified by comparison with an ECG standard with the use of liquid chromatography/electrospray ionization mass spectrometry (data not shown). When the same dose of flavanols was administered through GT, BT, or GTS, the GTS resulted in significantly greater increases in $C_{\text{max}}$ and AUC values. $t_{\text{max}}$ was always extended by $>1$ h for all 4 flavanols after GTS consumption compared with after GT and BT consumption. In addition, the percentage of EGC and EC intakes excreted in the urine was significantly higher after GTS consumption than after GT and BT consumption. These results strongly suggest that the absorption of polyphenols from a GTS, delivered in a standard gelatin capsule, was delayed but was higher than after GT or BT administration. It can be postulated that GTS increases the bioavailability of tea flavanols at the absorption phase. More studies are needed to understand this phenomenon.

After GTS consumption, the AUC of plasma antioxidant activity over 8 h was significantly increased by a small amount (2–3%) compared with after GT and BT consumption when determined in the traditional TEAC assay. The in vitro spiking of a pooled plasma sample showed that a plasma polyphenol concentration of 7.5 μmol/L was necessary to cause an increase in the antioxidant activity. The mean maximal plasma concentration of total flavanols was $\approx$1.2 μmol/L. This showed that the increase in plasma flavanol concentration was not high enough to cause an increase in the antioxidant capacity. This small increase in plasma antioxidant activity might be due to flavanol metabolites or degradation products. Meng et al (17) have shown that tea catechins are metabolized to ring-fission products (valerolactones) and methylated catechins. These ring-fission products can also be further metabolized in the colon to simple phenolic acids such as 4-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid, 3-methoxy-4-hydroxyhippuric acid, and vanillic acid, which have been detected in human urine after tea consumption (18). Some phenolic acids showed considerable in vitro antioxidant activity (data not published). In addition dimerization of EGCG may increase antioxidant capacity, because it has been shown that dimerization of EGCG at an alkaline pH will lead to new dimerization products (theasinensin A and D) with an antioxidant activity 2–3-fold that of EGCG (19).

We conclude that polyphenols administered in the form of GTS showed enhanced bioavailability compared with that of GT or BT, which led to a small but significant increase in antioxidant capacity. Flavanol metabolite formation may have contributed to the antioxidant effect because of the lack of correlation between
plasma flavanol concentrations and TE values; these substances deserve further study.

The study was conceived by SMH, VLWG, and DH. GDT and RRM coordinated the trial. YN, NHL, and SMH performed the analytic analyses. HW performed the statistical analyses. NHL and SMH prepared the manuscript. At the time of the study, none of the authors had any advisory board affiliations.

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