Effects of Dosing Condition on the Oral Bioavailability of Green Tea Catechins after Single-Dose Administration of Polyphenon E in Healthy Individuals

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Abstract

Purpose: Green tea has been shown to exhibit cancer-preventive activities in preclinical studies. Its consumption has been associated with decreased risk of certain types of cancers in humans. The oral bioavailability of the major green tea constituents, green tea catechins, is low, resulting in systemic catechin levels in humans many fold less than the effective concentrations determined in *in vitro* systems. We conducted this clinical study to test the hypothesis that the oral bioavailability of green tea catechins can be enhanced when consumed in the absence of food.

Experimental Designs: Thirty healthy volunteers were randomly assigned to one of the following doses of Polyphenon E (a decaffeinated and defined green tea catechin mixture): 400, 800, or 1,200 mg, based on the epigallocatechin gallate content (10 subjects per dose group). After an overnight fast, study participants took a single dose of Polyphenon E with or without a light breakfast, which consisted of one or two 4-oz muffins and a glass of water. Following a 1-week washout period, subjects were crossed over to take the same dose of Polyphenon E under the opposite fasting/fed condition. Tea catechin concentrations in plasma and urine samples collected after dosing were determined by high-pressure liquid chromatography analysis.

Results: Consistent with previous reports, epigallocatechin gallate and epicatechin gallate were present in plasma mostly as the free form, whereas epicatechin and epigallocatechin were mostly present as the glucuronide and sulfate conjugates. There was >3.5-fold increase in the average maximum plasma concentration of free epigallocatechin gallate when Polyphenon E was taken in the fasting condition than when taken with food. The dosing condition led to a similar change in plasma-free epigallocatechin and epicatechin gallate levels. Taking Polyphenon E in the fasting state did not have a significant effect on the plasma levels of total (free and conjugated) epigallocatechin, but resulted in lower plasma levels of total epicatechin. Urinary epigallocatechin gallate and epicatechin gallate levels were very low or undetectable following Polyphenon E administration with either dosing condition. Taking Polyphenon E under the fasting state resulted in a significant decrease in the urinary recovery of total epigallocatechin and epicatechin. Polyphenon E administered as a single dose over the dose range studied was generally well-tolerated by the study participants. Mild and transient nausea was noted in some of the study participants and was seen most often at the highest study agent dose (1,200 mg epigallocatechin gallate) and in the fasting condition.

Conclusions: We conclude that greater oral bioavailability of free catechins can be achieved by taking the Polyphenon E capsules on an empty stomach after an overnight fast. Polyphenon E up to a dose that contains 800 mg epigallocatechin gallate is well-tolerated when taken under the fasting condition. This dosing condition is also expected to optimize the biological effects of tea catechins.

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Tea (*Camellia sinensis*) is one of the most consumed beverages in the world, especially in Asian countries. The relationship between tea consumption and cancer has been a subject of research interest for many investigators in the past decade. Several recent manuscripts have thoroughly reviewed and summarized epidemiologic and experimental studies on tea and cancer prevention (1–6). Because the highly polymerized components in black tea are not well-characterized, experimental studies demonstrating the chemopreventive effects of tea have been conducted primarily with green tea. The evidence obtained *in vitro* and from animal studies *in vivo* concerning the

potentially protective effects of green tea or green tea components is compelling. Green tea, green tea extract, green tea polyphenols, and epigallocatechin gallate (a major green tea component) have been shown to inhibit carcinogenesis induced by a wide variety of carcinogens in rodent cancer models. Cancer chemopreventive activity has been shown in the following target organs: colon, duodenum, esophagus, forestomach, large intestine, liver, lung, mammary glands, skin, and prostate.

The epidemiologic evidence on the protective effect of green tea consumption against the development of human cancers is not conclusive. Some studies suggested that green tea consumption could reduce the risk of certain cancers (7–9); such a protective effect has not been observed in other studies (10, 11). The inconsistent epidemiologic findings may be attributed to confounding variables such as individualized differences in tea preparation and consumption patterns, variability associated with tea production, variability in the bioavailability of the active green tea constituents, concomitant use of tobacco and alcohol, and individualized differences in lifestyle. Controlled prospective human intervention trials are clearly necessary to evaluate the chemopreventive activity of green tea or green tea constituents.

We have recently determined the clinical pharmacokinetics of green tea catechins following single and multiple dose administration of a defined green tea catechin extract (Polyphenon E) and epigallocatechin gallate (12, 13). The oral bioavailability of tea catechins was found to be low in humans, resulting in plasma concentrations 5 to 50 times less than concentrations shown to exert biological activities in *in vitro* systems (14–16). A number of factors may affect the oral bioavailability of green tea catechins and subsequently their biological responses. In a small pilot study, free epigallocatechin gallate plasma concentrations determined at a single time point (90 minutes post-dose) after ingestion of 3, 5, or 7 capsules of Sunphenon DCF-1 (corresponding to 225, 375, and 525 mg epigallocatechin gallate) was 300, 1,970, and 2,020 ng/mL, respectively (17). These levels were significantly higher than those reported by Yang et al. (18) and by us (12, 13). Upon further comparison of the design of these studies, we hypothesized that the dosing condition could have a significant impact on the systemic availability of green tea catechins. We conducted this clinical study to test the hypothesis that the oral bioavailability of green tea catechins can be enhanced when consumed in the absence of food. The information generated from this study is important for the design of future intervention trials, interpretation of epidemiologic findings, and extrapolation of animal data to human situations.

Materials and Methods

Study drugs. Polyphenon E capsules were supplied by the Chemoprevention Agent Development Research Group, National Cancer Institute (Bethesda, MD). Each capsule contained 416.7 mg of Polyphenon E (200 mg epigallocatechin gallate, 48.5 mg epigallocatechin, 34.2 mg epicatechin, 20 mg epicatechin gallate, and other tea catechins), 28.8 mg pregelatinized starch, 2.25 mg colloidal silicon dioxide, and 2.25 mg magnesium stearate in size 0 gelatin capsules. The study medications were stored at room temperature and protected from environmental extremes.

Participants. Thirty nonsmoking healthy men and women ≥18 years of age participated in the study. The participants had normal liver and renal function. Participants were excluded if they were pregnant, had cancers of any type within the past 5 years, had severe metabolic disorders or other serious acute or chronic diseases, had consumed tea regularly, or had participated in other clinical research studies within the past 3 months. The study was approved by the University of Arizona Human Subjects Committee. Written informed consent was obtained from all participants.

Study design. During the initial clinic visit, study participants completed a medical history form and underwent a brief physical examination. A fasting blood sample was collected and subjected to a complete blood count with differential leukocyte count and a comprehensive blood chemistry analysis. Eligible subjects were randomly assigned to one of the Polyphenon E doses (400, 800, and 1,200 mg, based on epigallocatechin gallate content) and were required to refrain from the ingestion of tea, apples, chocolate, and their products 2 weeks prior to the first pharmacokinetic study day and until the end of the second pharmacokinetic study day. The night prior to the first pharmacokinetic study day, subjects fasted after midnight except for water. On the morning of the first pharmacokinetic study day, each subject was randomly assigned to receive Polyphenon E with or without breakfast. Breakfast consisted of one or two 4-oz muffins and a glass of water. Study participants had the option of selecting from three varieties of Otis Spunkmeyer brand muffins: cheese streusel, wild blueberry, and banana nut flavors. The muffins contain 420 to 480 calories each, with 48 to 60 g of carbohydrates, 20 to 24 g of fat, and 6 g of protein. Blood samples were collected prior to and at 0.5, 1, 2, 4, 6, 8, 10, and 24 hours after Polyphenon E administration. A sandwich lunch was provided to all study subjects following the 4-hour blood collection. Urine samples were collected prior to and up to 24 hours (divided into two intervals: 0-8 and 8-24 hours post-dose) after dosing. Study subjects left the clinic after the 10-hour blood collection and continued their urine collection at home. Study subjects brought back the overnight urine collection the next morning and had a 24-hour blood collection. There was no restriction on the dinner with the exception of items to be refrained from the entire study period. Following a 1-week wash-out period, study subjects fasted overnight and were crossed-over to receive the same dose of Polyphenon E under the other fasting/fed condition. A complete blood count and comprehensive blood chemistry analysis was repeated after the subject completed the cross-over portion of the study.

Sample collection and processing. Blood samples were collected into Vacutainer tubes containing sodium heparin. Within 30 minutes of collection, tubes were centrifuged for 10 minutes at 2,000 rpm. After centrifugation, plasma was mixed with ascorbate-EDTA solution [0.4 mol/L NaH_2PO_4 buffer containing 20% ascorbic acid and 0.1% EDTA (pH 3.6)] in a fixed volume ratio and stored at −80°C until sample analysis. Urine specimen container was pre-added with 1.38 g NaH_2PO_4, 1 g ascorbic acid, and 5 mg EDTA to prevent degradation of tea catechins. Urine specimen was kept cold by storing the specimen container in a cooler chest with freezer packs during each collection period. The total volume of each urine sample was measured. An aliquot of urine sample was mixed with the ascorbate-EDTA solution and stored at −80°C until sample analysis.

Tea polyphenol concentration measurements. Epigallocatechin gallate, epicatechin, epigallocatechin, epicatechin gallate concentrations in plasma and urine samples were determined using a published method (18) with minor modifications. In brief, for determination of free green tea catechins, plasma or urine samples were extracted with ethyl acetate. The ethyl acetate layer was mixed with a small aliquot of 0.1% ascorbic acid before drying by vacuum centrifugation. The dried residue was redissolved in 15% acetonitrile and injected onto high-pressure liquid chromatography. For determination of the total of free and glucuronic acid/sulfate conjugates of tea catechins, plasma or urine samples were mixed with an aliquot of β-glucuronidase and sulfatase in the presence of ascorbate-EDTA solution. Following

Table 1. Subject demographic data by dose levels

	Polyphenon E (400 mg epigallocatechin gallate)	Polyphenon E (800 mg epigallocatechin gallate)	Polyphenon E (1,200 mg epigallocatechin gallate)
Number of subjects	10	10	10
Number of males	4	2	1
Mean age in years (range)	42 (20-56)	39 (21-51)	45 (22-66)
Height (in.)*	67 ± 3	66 ± 4	64 ± 5
Weight (lbs)*	165 ± 45	179 ± 52	157 ± 46

*Mean ± one SD.

pretreatment, the samples were extracted as described above for the free catechins.

The high-pressure liquid chromatography system consisted of an ESA Model 465 refrigerated autosampler, an ESA Model 580 two-pump solvent delivery system, an ESA 5500 Coulochem electrode array system, and a Supelcosil C_{18} reversed-phase column (150 × 4.6 mm; particle size, 5 μm; Supelco, Inc., Bellefonte, PA). The autosampler and column temperatures were maintained at 6°C and 35°C, respectively. This assay employed a gradient of two mobile phases. Buffer A consisted of 30 mmol/L NaH_2PO_4 buffer, acetonitrile, and tetrahydrofuran in the volume ratio of 98.13:1.75:0.12 (pH 3.35). Buffer B consisted of 15 mmol/L NaH_2PO_4 buffer, acetonitrile, and tetrahydrofuran in the volume ratio of 41.5:58.5:12.5 (pH 3.45). The flow rate was maintained at 1 mL/minute. The column was eluted with 96% buffer A and 4% buffer B from 0 to 7 minutes. Then the linear gradient was changed progressively to 17% buffer B at 25 minutes, 28% at 31 minutes, 33% at 37 minutes, and 98% at 38 minutes. It was maintained at 98% from 38 to 43 minutes and finally changed back to 4% buffer B at 44 minutes for the analysis of the next sample. The eluent was monitored by the Coulochem electrode array system with potential settings at −10, 150, 300, and 500 mV.

Data analysis. The following pharmacokinetic variables of free epigallocatechin gallate were estimated using the WINNONLIN program (version 4.0.1) with the noncompartment approach: time to reach the maximum plasma drug concentration (T_{max}), maximum plasma drug concentration (C_{max}), area under the plasma drug concentration-time profile (AUC), systemic clearance/bioavailability (CL/F), apparent volume of distribution/bioavailability (Vd/F), and elimination half-life ($t_{1/2}$). The C_{max} of free epigallocatechin, epicatechin, and epicatechin gallate, and of total catechins (free and conjugated) was obtained by visual inspection of the concentration-time data.

The amount of total epigallocatechin and epicatechin excreted in the urine for each collection interval was calculated by the product of urinary catechin concentration and urine volume. The total amount excreted over 24 hours after dosing was obtained by adding the amount excreted over 0 to 8 and 8 to 24 hours after dosing.

The distribution of pharmacokinetic variable data was normalized by logarithmic transformation prior to statistical analyses. The primary analyses are to determine whether the pharmacokinetics of tea catechins are different between fasting versus fed states. Pharmacokinetic variables such as AUC, C_{max}, T_{max}, half-life, CL/F, Vd/F, and total amount excreted over 24 hours were compared between the fasting and fed conditions by standard cross-over statistical analyses. The pkcross routine in Stata 8.0 (StataCorp 2003) was used. In this software, the default parameterization estimates overall mean, period effects, treatment effects, and sequence effects, assuming no carryover effects. A $P < 0.05$ was considered statistically significant. In secondary analysis, epigallocatechin gallate pharmacokinetic variables such as CL/F, Vd/F, dose-normalized AUC, and dose-normalized C_{max} were compared among different dose levels using one-way ANOVA followed by post hoc t tests corrected for multiple comparisons. For the post hoc t test, a $P < 0.0167$ was considered statistically significant.

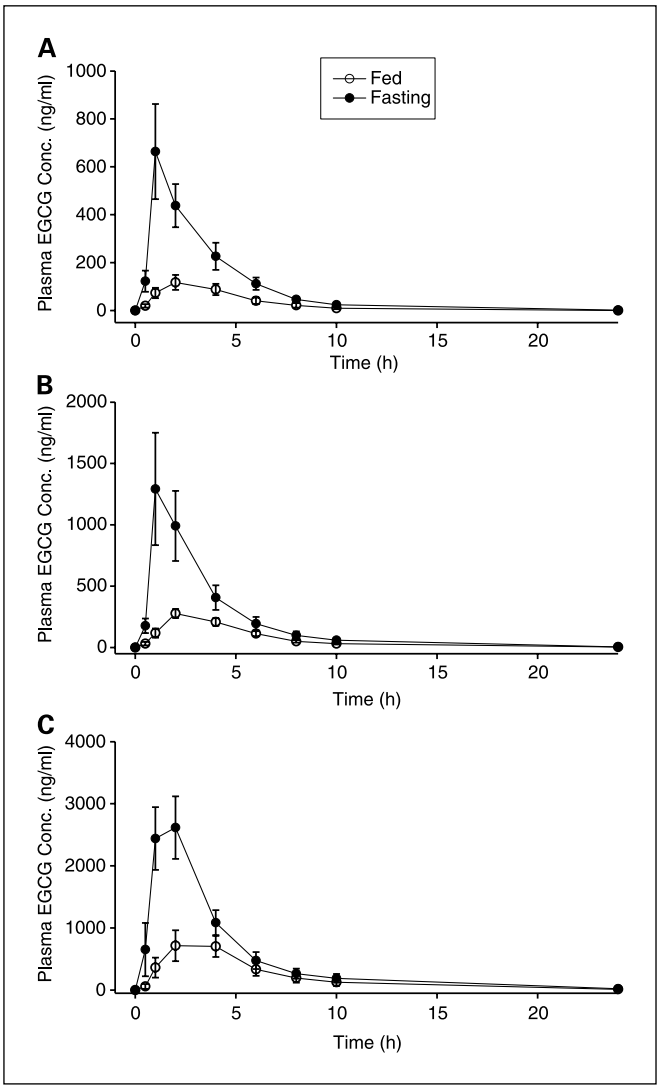

Fig. 1. Average plasma free epigallocatechin gallate concentration versus time profiles after oral administration of Polyphenon E in the absence or presence of food. Points, mean; bars, SE. *A,* Polyphenon E dose containing 400 mg epigallocatechin gallate; *B,* Polyphenon E dose containing 800 mg epigallocatechin gallate; *C,* Polyphenon E dose containing 1,200 mg epigallocatechin gallate.

Table 2. Pharmacokinetic variables of free epigallocatechin gallate after oral administration of Polyphenon E in the absence or presence of food

Pharmacokinetic variables	Polyphenon E (400 mg epigallocatechin gallate)		Polyphenon E (800 mg epigallocatechin gallate)		Polyphenon E (1,200 mg epigallocatechin gallate)	
	Fed	Fasting	Fed	Fasting	Fed	Fasting
AUC (min·μg/mL)	36.70 ± 24.66*	126.96 ± 47.02†	90.89 ± 37.39	254.48 ± 214.21†	299.40 ± 285.86	685.53 ± 394.33†
C_{max} (ng/mL)	141.8 ± 89.1	798.7 ± 573.1†	294.0 ± 113.5	1,522.4 ± 1,357.8†	923.6 ± 755.3	3,371.6 ± 1,651.2†
T_{max} (min)	122.90 ± 83.70	93.90 ± 58.99‡	154.90 ± 78.31	83.30 ± 31.04‡	175.10 ± 74.58	90.60 ± 28.37‡
CL/F (L/min)	14.53 ± 7.84	3.64 ± 1.55†	11.63 ± 9.25	4.80 ± 2.69†	7.39 ± 4.86	2.37 ± 1.32†
Vd/F (L)	2,872.8 ± 2,672.8	912.4 ± 695.6†	3,112.9 ± 2,810.8	946.6 ± 558.3†	2,825.1 ± 2,257.7	664.0 ± 223.5†
$t_{1/2}$ (min)	145.2 ± 129.1	170.5 ± 104.6	220.9 ± 209.3	156.5 ± 77.5	254.9 ± 59.9§	228.4 ± 75.3

*Mean ± one SD.
†Significantly different from the fed condition, $P < 0.0001$ when data were pooled from all doses.
‡Significantly different from the fed condition, $P < 0.0005$ when data were pooled from all doses.
§Significantly different from 400 mg dose of the same dosing condition, $P < 0.005$.

Results

Table 1 summarizes the demographic data of the study participants. A total of 30 subjects (10/dose group) completed the study. There were no significant differences in the average age, weight, and height among dose groups. There were between one and four male participants in each dose level. Figure 1A-C illustrate the average plasma-free epigallocatechin gallate concentration-time profiles after Polyphenon E administration in the absence or presence of food. After dosing, plasma epigallocatechin gallate levels increased toward a peak and declined rapidly as a function of time. A significant increase in plasma epigallocatechin gallate levels was noted when Polyphenon E was taken on an empty stomach following an overnight fast in comparison with that taken with food. The effect of dosing condition on plasma epigallocatechin gallate levels is consistently observed across all three dose levels.

Table 2 summarizes the average pharmacokinetic variables of free epigallocatechin gallate after taking Polyphenon E on an empty stomach or with food. The epigallocatechin gallate AUC obtained without food was significantly higher than that obtained with food for all three doses (126.96 ± 47.02 versus 36.70 ± 24.66 minutes·μg/mL at the 400 mg dose level; 254.48 ± 214.21 versus 90.89 ± 37.39 minutes·μg/mL at the 800 mg dose level; 685.53 ± 394.33 versus 299.40 ± 285.86 minutes·μg/mL at the 1,200 mg dose level; $P < 0.0001$ when data were pooled from all three doses). Similarly, the epigallocatechin gallate C_{max} obtained without food was significantly higher than that obtained with food (798.7 ± 573.1 versus 141.8 ± 89.1 ng/mL at the 400 mg dose level: 1,522.4 ± 1,357.8 versus 294.0 ± 113.5 ng/mL at the 800 mg dose level; 3,371.6 ± 1,651.2 versus 923.6 ± 755.3 ng/mL at the 1,200 mg dose level; $P < 0.0001$ when data were pooled from all three doses). The dosing condition also has a significant effect on CL/F and Vd/F, mostly due to an increase in the oral bioavailability (F) of epigallocatechin gallate in the fasting condition.

Table 3 summarizes the maximum plasma concentrations of free catechins after oral administration of Polyphenon E under the fasting and fed states. As observed in our previous studies (12, 13), free epicatechin levels were very low or undetectable in plasma samples. Similar to changes observed for plasma free epigallocatechin gallate, plasma free epigallocatechin and epicatechin gallate C_{max} levels in the fasting condition were significantly higher than those in the fed condition.

Table 3. Maximum plasma free catechin concentrations (ng/mL) after oral administration of Polyphenon E in the absence or presence of food

Catechin	Polyphenon E (400 mg epigallocatechin gallate)		Polyphenon E (800 mg epigallocatechin gallate)		Polyphenon E (1,200 mg epigallocatechin gallate)	
	Fed	Fasting	Fed	Fasting	Fed	Fasting
Epigallocatechin	15.9 ± 16.4*	39.7 ± 44.6†	34.1 ± 20.3	74.3 ± 70.0†	42.9 ± 36.7	131.0 ± 94.2†
Epicatechin	1.6 ± 3.9	5.6 ± 7.8	2.8 ± 5.0	1.9 ± 3.4	10.1 ± 15.3	16.6 ± 30.0
Epigallocatechin gallate	141.8 ± 89.1	798.7 ± 573.1‡	294.0 ± 113.5	1,522.4 ± 1,357.8‡	923.6 ± 755.3	3,371.6 ± 1,651.2‡
Epicatechin gallate	15.7 ± 8.8	87.6 ± 62.0‡	33.9 ± 11.0	174.4 ± 141.1‡	112.9 ± 88.2	382.6 ± 176.0‡

*Mean ± one SD.
†Significantly different from the fed condition, $P < 0.001$ when data were pooled from all doses.
‡Significantly different from the fed condition, $P < 0.0001$ when data were pooled from all doses.

Table 4. Maximum plasma total catechin concentrations (ng/mL) after oral administration of Polyphenon E in the absence or presence of food

Catechin	Polyphenon E (400 mg epigallocatechin gallate)		Polyphenon E (800 mg epigallocatechin gallate)		Polyphenon E (1,200 mg epigallocatechin gallate)	
	Fed	Fasting	Fed	Fasting	Fed	Fasting
Epigallocatechin	128.6 ± 74.9*	81.8 ± 67.3	177.1 ± 86.2	135.5 ± 83.6	200.3 ± 115.1	323.4 ± 231.1
Epicatechin	156.3 ± 98.1	94.7 ± 63.3†	234.0 ± 122.7	149.8 ± 79.7†	346.2 ± 218.0	272.3 ± 173.6†
Epigallocatechin gallate	174.4 ± 80.6	754.9 ± 399.9‡	360.1 ± 136.9	1,622.7 ± 1,501.4‡	871.9 ± 625.3	3,988.4 ± 2,068.2‡
Epicatechin gallate	21.2 ± 9.0	86.3 ± 48.8‡	48.7 ± 17.5	197.5 ± 179.3‡	117.5 ± 91.5	450.7 ± 218.7‡

*Mean ± one SD.
†Significantly different from the fed condition, $P < 0.005$ when data were pooled from all doses.
‡Significantly different from the fed condition, $P < 0.0001$ when data were pooled from all doses.

Table 4 summarizes the maximum plasma concentrations of total catechins after oral administration of Polyphenon E in the absence or presence of food. Total catechin levels were determined after plasma samples were treated with β-glucuronidase and sulfatase. Differences between total and free catechin levels represent the concentrations of conjugated catechins. Comparing the total and free catechin C_{max} data (Tables 3 and 4) suggests that epigallocatechin and epicatechin were mostly present in plasma as the conjugated forms and epigallocatechin gallate and epicatechin gallate were mostly present as the free form. The fasting effect on the C_{max} of total epigallocatechin gallate and epicatechin gallate is consistent with that of free epigallocatechin gallate and epicatechin gallate. Taking Polyphenon E under the fasting state did not have a significant effect on the plasma levels of total epigallocatechin, but significantly decreased plasma levels of total epicatechin ($P < 0.005$).

The urine samples collected for this study have been analyzed for total catechin levels after subjecting the samples to glucuronidase and sulfatase treatment. Similar to what was observed previously (12), urinary epigallocatechin gallate and epicatechin gallate levels were very low or undetectable following Polyphenon E administration. Table 5 summarizes the amount of total epigallocatechin and epicatechin recovered in the urine over 24 hours after oral administration of Polyphenon E. Taking Polyphenon E on an empty stomach resulted in less amounts of total epigallocatechin and epicatechin recovered in the urine than those recovered after taking Polyphenon E with food.

Table 6 lists adverse events deemed possibly or probably related to study agent because of temporal proximity. All doses of Polyphenon E used in this trial were generally well-tolerated. The incidence of gastrointestinal adverse events, particularly nausea, increased at the higher doses and under fasting conditions. The headaches experienced by a number of participants were thought to be related to the absence of habitual caffeine beverage consumption on the long pharmacokinetic study days.

Discussion

The oral bioavailability of tea catechins has been found to be low in rodents. Chen et al. (19) reported that <2% of the epigallocatechin gallate dose given orally was available in the systemic blood in rats. An oral bioavailability of <13% was recently reported for epigallocatechin gallate in mice (20). The oral bioavailability of tea catechins has not been determined in humans because of the lack of i.v. formulations. We have recently determined the clinical pharmacokinetics of green tea catechins following single and multiple dose administration of Polyphenon E and epigallocatechin gallate (12, 13). The CL/F and Vd/F of epigallocatechin gallate in humans were found to be around 6 to 14.6 L/minute and 1,000 to 4,800 L, respectively. These large CL/F and Vd/F values suggest that the

Table 5. Amounts of total catechin (mg) recovered in the urine over 24 hours after oral administration of Polyphenon E in the absence or presence of food

Catechin	Polyphenon E (400 mg epigallocatechin gallate)		Polyphenon E (800 mg epigallocatechin gallate)		Polyphenon E (1,200 mg epigallocatechin gallate)	
	Fed	Fasting	Fed	Fasting	Fed	Fasting
Epigallocatechin	4.85 ± 1.83*	2.67 ± 1.25†	5.54 ± 1.59	4.49 ± 3.15†	6.48 ± 4.10	5.39 ± 3.09†
Epicatechin	4.62 ± 1.76	2.64 ± 1.13‡	6.49 ± 2.28	5.26 ± 3.54‡	9.20 ± 6.25	6.44 ± 4.11‡

*Mean ± 1 SD.
†Significantly different from the fed condition, $P < 0.005$ when data were pooled from all doses.
‡Significantly different from the fed condition, $P < 0.0005$ when data were pooled from all doses.

Table 6. Summary of incidence of reported adverse events possibly or probably related to Polyphenon E administration, occurring within 24 hours of Polyphenon E administration

Side effect	National Cancer Institute grade	Dose and dosing condition					
		400 mg		800 mg		1,200 mg	
		Fed (n = 10)	Fasting (n = 10)	Fed (n = 10)	Fasting (n = 10)	Fed (n = 10)	Fasting (n = 10)
Gastrointestinal							
Nausea	1	0	0	2	1	2	7
	2	0	1	0	1	1	1
Dyspepsia	1	0	0	1	1	1	0
	2	0	0	0	0	0	0
Diarrhea	1	0	0	0	1	0	1
	2	0	0	0	0	0	0
Other (gas, eructation)	1	1	0	0	0	0	1
	2	0	0	0	0	0	0
Pain							
Abdominal pain	1	1	0	0	0	0	2
	2	0	0	0	1	1	0
Headache	1	0	1	2	2	1	0
	2	0	0	0	0	0	0
Neurology							
Dizziness	1	0	0	1	0	0	0
	2	0	0	0	0	0	0
Dermatology							
Rash	1	0	0	0	0	0	0
	2	0	0	0	0	0	1

oral bioavailability (F) of tea catechins in humans is also low. Because of low oral bioavailability, plasma tea catechin concentrations determined in humans after oral administration of green tea extract or green tea catechins were 5 to 50 times less than the concentrations shown to exert biological activities in *in vitro* systems (14–16).

Because a significant fraction of the orally administered green tea catechins is not absorbed or is eliminated presystemically, small changes in factors limiting the systemic availability of green tea catechins could have a significant impact on their oral bioavailability. We have shown in this study that taking Polyphenon E on an empty stomach after an overnight fast resulted in a dramatic increase in the blood levels of free epigallocatechin gallate, epigallocatechin, and epicatechin gallate. Nevertheless, taking Polyphenon E under fasting conditions resulted in a decrease in the blood and urine levels of total epigallocatechin and epicatechin. Because epigallocatechin and epicatechin are mostly present as the conjugated form, a decrease in the total catechin concentration and an increase in the free catechin concentration suggests that less epigallocatechin and epicatechin conjugates are formed and bioavailable in the fasting condition. Formation of conjugated metabolites of soy polyphenols, daidzein and genistein, has been shown to be modulated in rodents after an acute fast (21). It has been postulated that an acute fast depletes precursors for the glucuronidation reaction (22, 23). It is plausible that less epigallocatechin and epicatechin undergo presystemic glucuronidation and/or sulfation reactions in the fasting condition, resulting in more free catechins escaping the presystemic loss and being available in the systemic blood. Unlike epigallocatechin and epicatechin, epigallocatechin gallate and epicatechin gallate were mostly present as the free form in plasma and in low quantities in urine. It is not known whether an overnight fast will have a similar effect on the conjugation of epigallocatechin gallate and epicatechin gallate.

An additional factor that could affect the oral bioavailability of green tea catechins is luminal catechin degradation. Green tea catechins have been shown to be stable in acidic conditions, but degrade more rapidly at pH levels above 6.5 (24, 25). Thirty-eight percent of epigallocatechin gallate was found to remain intact when incubated at 37°C (pH 7.4) for 5 minutes (24) and minimal amounts of epigallocatechin gallate remained when incubated for 3 hours (pH 7.4; ref. 25). Following a meal, the gastric pH increases to a peak range (pH 5.8-6.7) from an acidic range (pH 1.1-1.6; ref. 26). The presence of food also delays the gastric emptying rate. Based on these physiologic changes and the *in vitro* stability data, it is possible that green tea catechins may be more stable in a fasted stomach than a fed stomach, which could contribute to the enhanced oral bioavailability observed in the fasting condition.

Food has also been shown to decrease the intestinal absorption of pharmaceutical drugs through irreversible interactions between drugs and dietary components or reversible interactions but exhibiting an absorption window in the proximal small intestine (27), a decrease in drug dissolution rate as a result of elevation of the luminal viscosity

(28), and interactions between drugs and bile acids secreted following food ingestion (29). These factors could also contribute to the dramatic increase in the oral bioavailability of green tea catechins observed in the fasting condition.

As shown in Tables 2-4, there is a large subject-to-subject variability in the pharmacokinetics of green tea catechins. This variability has been previously observed by us (12, 13) and others (18) and is consistent with compounds that undergo extensive presystemic elimination. The appropriate sample size for this study was calculated during the clinical protocol development process with the consideration of this variability. A sample size of 30 was found to result in 80% power to observe a 20% difference in epigallocatechin gallate AUC between dosing conditions based on a 5% level of significance and a cross-over design. The higher percentage of difference observed in this study suggests a higher statistical power.

All doses of Polyphenon E used in this trial were generally well-tolerated after single-dose administration. No significant differences were observed in the adverse events reported for the 400 and 800 mg dose levels between the dosing conditions. Mild and transient nausea was noted in some of the study participants and was seen most often at the highest study agent dose (1,200 mg epigallocatechin gallate) and in the fasting condition. Because of this adverse event, taking Polyphenon E at the 1,200 mg dose in the fasting condition is not considered feasible for chronic use.

It is not known whether taking Polyphenon E with a meal with a composition different from that used in our study would allow for better gastrointestinal tolerance at higher doses but not impair the oral bioavailability of green tea catechins. A recent study has assessed the impact of different macronutrients on flavanol (epicatechin + catechin) absorption from sugar-free, flavanol-rich cocoa (30). It was found that flavanol absorption is increased significantly by concurrent consumption of carbohydrate-rich meals, including sugar, bread, and grapefruit juice (~140% increase in AUC values). Lipid and protein-rich meals such as butter, milk, and steak had minimal effects on flavanol absorption. The muffins provided in our study are rich in carbohydrate and fat content. Because carbohydrate-rich meals already provided facilitated flavanol absorption when compared with lipid and protein rich meals (30), it is expected that lipid- and protein-rich meals would also reduce the oral bioavailability of green tea catechins in comparison with the fasting condition.

We conclude that greater oral bioavailability of free catechins can be achieved by taking Polyphenon E on an empty stomach after an overnight fast. Polyphenon E up to a dose that contains 800 mg epigallocatechin gallate is well-tolerated when taken under the fasting condition. This dosing condition is also expected to optimize the biological effects of tea catechins.

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