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# Bioavailability and antioxidant effect of epigallocatechin gallate administered in purified form versus as green tea extract in healthy individuals☆

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**Abstract**

Tea polyphenols have strong in vitro antioxidant activity. Due to their limited bioavailability, however, their contribution to in vivo antioxidant activity may depend on the form of administration. A human intervention study was performed to evaluate the bioavailability and antioxidant capacity of (−)-epigallocatechin-3-gallate (EGCG) administered as a single large dose in the form of either purified EGCG or as green tea extract (Polyphenon E). Plasma concentrations of tea polyphenols were determined by high-performance liquid chromatography (HPLC) analysis combined with coulometric array electrochemical detection (ECD). We found no differences in plasma EGCG concentrations and trolox equivalents determined by the trolox equivalent antioxidant capacity assay after administration of either form of EGCG. However, we found that the plasma antioxidant activity was significantly affected by changes in the plasma urate concentration, which may have interfered with the effect of tea polyphenols on the antioxidant activity. In addition, lymphocyte 8-hydroxydeoxyguanosine to deoxyguanosine (8-OHdG/$10^6$dG) ratios were determined by HPLC with ECD. The 8-OHdG/$10^6$dG ratios did not change significantly during the 24 h following both EGCG interventions but correlated significantly within individuals determined during the two interventions separated by 1 week. In summary, changes in plasma uric acid due to dietary intake were significantly correlated to the plasma antioxidant activity and exerted a stronger influence on the plasma antioxidant activity compared with the EGCG intervention. In future studies of dietary effects on the plasma antioxidant capacity, changes in plasma uric acid will need to be closely monitored.
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*Keywords:* Tea polyphenols; Bioavailability; Antioxidant activity; TEAC; Uric acid; 8-Hydroxydeoxyguanosine/deoxyguanosine ratio

## 1. Introduction

Tea (*Camellia sinensis*) in the form of green or black tea is one of the most consumed beverages in the world [1]. At the same time, the market for green tea supplements is growing. Tea and tea flavonoid consumption has been linked to lower incidences of chronic diseases such as cardiovascular disease and cancer [2]. The health benefits associated with tea consumption have been attributed in part to the antioxidant and free radical scavenging activity of the four principal and most abundant tea flavanols: (−)-epigallocatechin (EGC); (−)-epicatechin (EC); (−)-epigallocatechin-3-gallate (EGCG); and (−)-epicatechin-3-gallate (ECG) [3]. It has been shown in different cell lines and animal models that tea flavanols also inhibit cell proliferation, induce cell cycle arrest and apoptosis, stimulate angiogenesis and affect cell signaling pathways [4]. The antioxidant activity of tea flavanols has been clearly

*Abbreviations:* EGC, (−)-epigallocatechin; EC, (−)-epicatechin; EGCG, (−)-epigallocatechin-3-gallate; ECG, (−)-epicatechin-3-gallate; C-ECD, coulometric array electrochemical detection; HPLC, high-performance liquid chromatography; TEAC, trolox equivalent antioxidant capacity; 8-OhdG, 8-hydroxydeoxyguanosine; dG, deoxyguanosine.

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demonstrated in in vitro experiments. As reviewed by Rietveld and Wiseman [3] and Higdon and Frei [5], most in vivo studies demonstrated an increase in the plasma antioxidant activity after the consumption of tea or tea polyphenols. The typical increase in the plasma antioxidant activity ranged from 2% to 15% [5]. This might be due to the limited bioavailability of tea flavanols and the fact that the human blood has excellent physiological antioxidant protection based on its content of ascorbic acid, uric acid, glutathione and others [6]. For example, a study by Van Amelsvoort et al. [7] demonstrated a similar pattern in changes of plasma uric acid and plasma antioxidant activities after tea polyphenol intake [7]. In addition, it has been demonstrated that the concentration of some of the plasma antioxidant compounds depends on the dietary intake of macronutrients [8].

The high-performance liquid chromatography (HPLC) detection in human lymphocytes of the DNA oxidation product 8-hydroxydeoxyguanosine (8-OHdG) in ratio to its nonoxidized parent compound has been shown to be a reliable marker of oxidative damage [9]. The 8-OHdG in lymphocyte DNA from men and women in five countries showed a striking elevation of damage in men of Northern European countries relative to men of Southern European countries [10]. A correlation was observed between 24-h urinary 8-OHdG and 8-OHdG in lymphocytes measured by HPLC [9]. In a 4-month tea intervention with four cups of green tea, urinary 8-OHdG levels decreased significantly in smokers [11]. No pharmacokinetic data of lymphocyte 8-OHdG/$10^6$dG ratios after short-term tea interventions are available. Our study is the first to measure changes in lymphocyte DNA oxidative damage for 24 h after a single tea flavanol intervention.

Tea flavanols are mainly absorbed into circulation from the small intestine. The limited bioavailability of tea flavanols is dependent on their high molecular weight and effective molecular size due to the large number of hydrogen bond-donating hydroxyl groups, which form a large hydration shell [12]. Depending on the type of transport mechanism, the absorption and biologic activity of EGCG might be affected by the form of administration (i.e., in purified form or as mixed green tea extract).

The purpose of this study was to compare the absorptive pharmacokinetics of tea flavanols from a mixed green tea extract [Polyphenon E (Poly E)] versus those from purified EGCG and determine the effect of the interventions on the plasma antioxidant defense and lymphocyte oxidative DNA damage. In a crossover design, a single oral dose of either the green tea extract Poly E (618 mg of EGCG, 168 mg of EGC, 166 mg of EC and 77 mg of ECG) or purified EGCG (580 mg) was administered to 20 healthy participants and pharmacokinetic parameters of tea flavanols in plasma and urine were determined by HPLC analysis. Plasma antioxidant activity was determined using the trolox equivalent antioxidant capacity (TEAC) assay. Lymphocyte oxidative DNA damage was determined by HPLC measuring the ratio of 8-OHdG/$10^6$dG ratio in lymphocyte DNA.

## 2. Methods and materials

### 2.1. Chemical products

EGCG, EGC, EC, ECG, β-D-glucuronidase type X-A from *Escherichia coli* and arylsulfatase type VIII from abalone entrails were purchased from Sigma-Aldrich (St. Louis, MO, USA). Bulk Poly E and EGCG were provided by the Food Research Laboratories (Mitsui Nori, Fujieda City, Japan). Poly E and EGCG were formulated in capsules by Pharmavite (Valencia, CA, USA). EGCG capsules contained 145 mg of EGCG and Poly E capsules contained 154 mg of EGCG, 42 mg of EGC, 41.5 mg of EC, 19 mg of ECG and 4 mg of caffeine each. The capsules were stored at room temperature, protected from environmental extremes. HPLC-grade acetonitrile, citric acid and ammonium acetate were purchased from Fisher Scientific (Pittsburgh, PA, USA). ABTS (2,2′ azinobis(3-ethylbenzothiazoline-6-sulphonic acid diammonium salt), manganese dioxide, trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), magnesium chloride, Tris–HCL, sodium acetate, diethylenetriamine pentaacetic acid (DTPA) and deoxyguanosine (dG) were purchased from Sigma-Aldrich. Phosphodiesterases (PDEs) I from snake venom (*Crotalus adamanteus*) and II from calf spleen were purchased from Worthington Biochemical (Lakewood, NJ, USA). 8-OHdG was purchased from Calbiochem (San Diego, CA, USA). DNase I from bovine pancreas and nuclease P1 (NP1) from Penicillium citrinum were purchased from Roche Diagnostics (Indianapolis, IN, USA). All reagents were of highest commercial quality (Fisher Scientific, Pittsburgh, PA, USA).

### 2.2. Study design

All study participants were required to refrain from the ingestion of tea or tea products for 7 days prior to the first intervention and until the end of the second intervention. On the pharmacokinetic study day, study participants arrived after an overnight fast early in the morning and were provided with a light breakfast of toast with butter and jelly or cereal and low-fat milk. After 20 min, baseline blood was drawn and participants swallowed four capsules of either EGCG or Poly E according to the randomization schedule provided by the UCLA Biostatistics Unit. Participants were allowed unlimited water intake throughout the intervention. Blood samples were collected at 1, 2, 4, 6, 8 and 24 h after supplement administration. Participants self-collected urine during the following time intervals: baseline, 0–4, 4–8 and 8–24 h. Participants received a low-flavonoid lunch and dinner.

### 2.3. Sample collection and processing

Once collected, blood samples were kept on ice and centrifuged at 4°C within 1 h of collection. After centrifu-

gation, plasma was aliquoted into cryotubes. A total of 40 μl of 20% ascorbic acid–0.1% EDTA solution (0.4 mM $NaH_2PO_4$, pH 3.6) was added to two aliquots of 800 μl of plasma. Buffy coat was removed and treated with ammonium chloride/EDTA (0.9%/0.1 mM) to lyse the remaining red cells. This process was repeated twice and white cells were taken up in 1 ml of PBS and frozen at −70°C until analysis. Urine was aliquoted and two aliquots of 1.3 ml were mixed with 30 μl of 20% ascorbic acid–0.1% EDTA solution. All samples were stored in −70°C until analysis.

### 2.4. Analysis of tea flavanols in plasma with HPLC and coulometric array electrochemical detection

Plasma flavanol analysis was performed using a modification of the method by Lee et al. [13]. Briefly, 200 μl of thawed plasma was mixed with 12 μl of 10% ascorbic acid–40 mM $NaH_2PO_4$–0.1% EDTA, 20 μl of 50 mM sodium phosphate (pH 7.4), 20 μl of 2.318 μmol/L catechin gallate internal standard, 500 U of β-D-glucuronidase type X-A from *E. coli* (Sigma, St. Louis, MO, USA) and 4 U of sulfatase type VIII from abalone entrails (Sigma). The mixture was incubated at 37°C for 45 min followed by two extractions using 2 ml of ethyl acetate. A total of 10 μl of 0.2% ascorbic acid–0.005% EDTA was added to the pooled supernatant followed by vacuum concentration for 2 h at low heat with a Savant SC-100 Speed-Vac system (Savant Instruments, Farmingdale, NY, USA). The samples were reconstituted in 200 μl of Mobile Phase A and 20 μl each injected into the HPLC column. The HPLC–electrochemical detection (ECD) system consisted of an Agilent Technologies 1100 quaternary pump and temperature-regulated autosampler controlled by Chemstation Software 9.01 (Agilent Technology, Wilmington, DE, USA), an ESA 5600A Coularrray electrochemical detector (ESA, Bedford, MA, USA), a $C_{18}$ Alltima guard column, 7.5 mm×4.6 mm, particle size of 5 μm (Alltech, Deerfield, IL, USA) and a $C_{18}$ Alltima column, 53 mm×7 mm, particle size of 5 μm (Alltech). Standard solutions of EGC, EC, EGCG, ECG and CG were prepared in 75 mM citric acid/25 mM ammonium acetate:acetonitrile (90:10 vol/vol) solution and stored at −70°C. Recovery of flavanols from plasma was determined in three different concentrations with each batch of plasma samples (Table 2). The column was eluted at room temperature with a linear gradient from 100% Mobile Phase A [75 mM citric acid/25 mM ammonium acetate] to 90% Mobile Phase A and 10% Mobile Phase B [75 mM citric acid/25 mM ammonium acetate:acetonitrile (50:50)] in 4 min at a flow rate of 1 ml/min. The gradient was linearly changed further to 70% A/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 there for analysis of the next sample. The eluent was monitored by ECD with potential settings at −90, −10, 70, 150, 230, 310, 400 and 480 mV. The dominant channel was at 230 mV.

### 2.5. Plasma TEAC assay

The TEAC assay was performed according to Miller et al. [14]. $ABTS^{\cdot+}$ radical cations were prepared by adding solid manganese dioxide to a 5 mM aqueous stock solution of ABTS. The $ABTS^{\cdot+}$ cations were passed through a Whatman No. 1 filter paper and a PVDF syringe filter. The concentration was adjusted with 75 mM Na/K phosphate buffer (pH 7) to an absorbance of 0.7 at 734 nm and preincubated at 30°C prior to use. Fresh $ABTS^{\cdot+}$ cation solution was prepared daily. Trolox was used as an antioxidant standard. Plasma samples were diluted 1:30 in Na/K phosphate buffer (75 mM, pH 7). A total of 200 μl of $ABTS^{\cdot+}$ radical cation solution was mixed with 20 μl of diluted plasma in 96-well plates and absorbance was read after 75 min. Samples were analyzed in triplicate determinations. A fresh trolox standard curve was prepared with each batch of plasma analysis and micromolar trolox equivalents (TEs) were calculated.

### 2.6. Lymphocyte DNA ratio of 8-OHdG/$10^6$dG determination by HPLC

DNA was extracted from lymphocytes of approximately 8 ml of whole blood using a DNA isolation kit from Roche Diagnostics. DNA was dissolved in AE buffer (Qiagen, Valencia, CA, USA) and digestion was performed according to Huang et al. [15]. The following incubations were performed: DNAse I, 30 min at 37°C; NP1, 60 min at 37°C; AP, 30 min at 37°C; and PDE I and PDE II, 30 min at 37 °C. The incubation mixture was filtered through a Millipore ultrafree 0.5 filter. The iron chelator DTPA was added to the DNA hydrolysates to prevent artifactual oxidation. A total of 20 μl hydrolysate was analyzed by HPLC. The HPLC–ECD system consisted of an Agilent Technologies 1100 binary pump, autosampler and variable wavelength detector controlled by Chemstation Software 7.01 (Agilent Technology), an ESA Coulochem II electrochemical detector (ESA), a $C_{18}$ Alltima guard column, 7.5 mm×4.6 mm, particle size of 5 μm (Alltech) and a YMC, ODS-AQ column, 4.6×15 cm, 120 Å, S-5 (Waters, Milford, MA, USA). The mobile phase consisted of 8% aqueous methanol containing 50 mM sodium acetate buffer (pH 5.2). Elution was isocratic at a flow rate of 0.8 ml/min. The dG concentration was monitored based on absorbance

Table 1
Pharmacokinetic parameters such as AUC, maximum concentration ($C_{max}$) and time of maximum uptake ($T_{max}$) for plasma EGCG after purified EGCG and Poly E intake[a]

| Intervention | Purified EGCG | Poly E |
|---|---|---|
| AUC (μmol*h/L) | 3.4±1.5[a] | 3.4±1.4[a] |
| $C_{max}$ (μmol/L) | 0.7±0.4[a] | 0.5±0.2[a] |
| $T_{max}$ (h) | 2.5±1.1[a] | 2.6±1.2[a] |

[a] Values are mean±S.E.M. from 20 participants. Values with different superscript letters are significantly different $P<.005$; SAS mixed-model procedure).

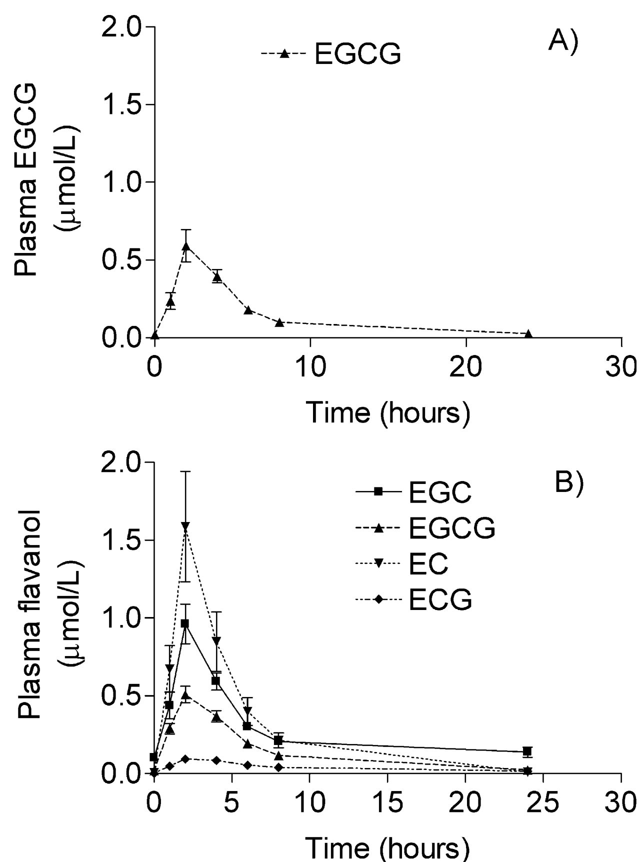


Fig. 1. Pharmacokinetics of plasma polyphenol concentrations after (A) EGCG and (B) Poly E interventions. Values are mean±S.E.M. of 20 participants.

(245 nm) and 8-OHdG based on the electrochemical reading (400 mV). Levels were quantified using the standard curves of each compound. The degree of DNA damage was expressed as 8-OHdG per $10^6$ dG.

### 2.7. Plasma uric acid analysis

Plasma uric acid was determined using an Amplex Red assay kit from Molecular Probes (Eugene, OR, USA). This assay uses the conversion of uric acid to allantoin and hydrogen peroxide, which in turn reacts stoichiometrically with Amplex Red reagent to generate the red-fluorescent oxidation product resorufin. The formation of resorufin was measured spectrophotometrically at 560 nm.

Table 2
Comparison of the overall effect of the 8-OHdG/$10^6$dG ratio, plasma uric acid and plasma TE after purified EGCG and Poly E intake calculated as AUC[a]

| Intervention | AUC (μmol*h/L) | |
|---|---|---|
| | Purified EGCG | Poly E |
| 8-OHdG/$10^6$dG ratio | 181±29[a] | 181±23[a] |
| Plasma uric acid | 6195±1443[a] | 6125±1408[a] |
| Plasma TE | 3438±217[a] | 3435±186[a] |

[a] Values are mean±S.D. from 20 participants. Values with different superscript letters are significantly different ($P$<.005; SAS mixed-model procedure).

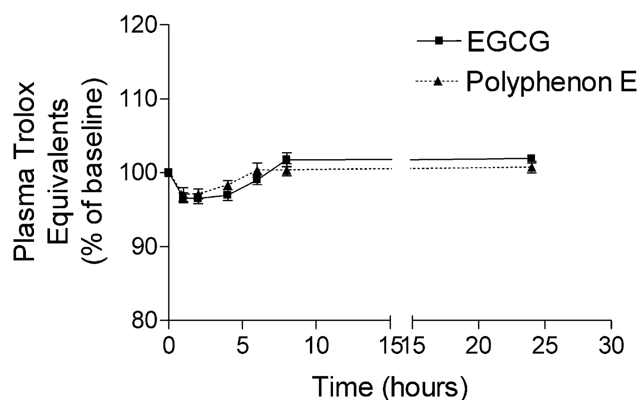


Fig. 2. Pharmacokinetics of plasma TEs after EGCG and Poly E interventions. Values are mean±S.E.M. of 20 participants. The AUC for each treatment was assessed using the trapezoidal rule and no difference between the two interventions was found. To indicate that no plasma analyses have been performed between 8 and 24 h, the $x$-axis has been interrupted.

### 2.8. Statistical analysis

Statistical analysis for a general 2×2 crossover design was carried out. Treatment effect, treatment by sequence effect and carry-over effect were included in the model. No carry-over effect was observed. The areas under the plasma concentration–time (0–24 h) curve and TEAC curve were estimated using the linear trapezoidal rule. The peak plasma concentration ($C_{max}$) and the time-to-peak concentration were recorded as observed. Models were constructed using the SAS procedure PROC MIXED. Change of plasma TEAC and uric acid concentration between different time points was analyzed using the paired $t$ test. All tests were two sided with a significance level of .05. The Spearman

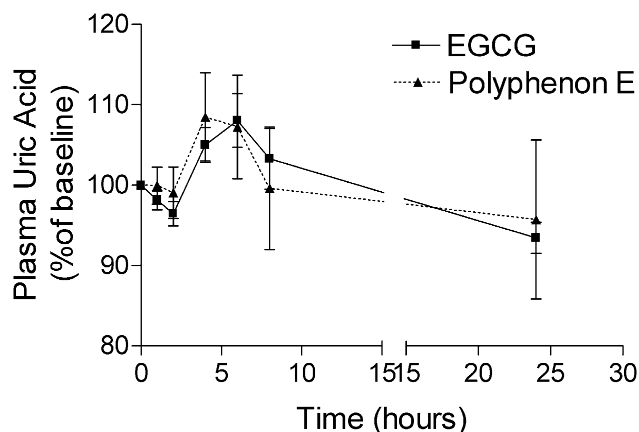


Fig. 3. Pharmacokinetics of plasma uric acid concentrations after EGCG and Poly E interventions. Values are mean±S.E.M. of 20 participants. To indicate that no plasma analyses have been performed between 8 and 24 h, the $x$-axis has been interrupted.

Table 3
Spearman correlation coefficients and $P$ values of plasma uric acid concentration to plasma antioxidant activity by treatment and time

| Hours | 0 | 1 | 2 | 4 | 6 | 8 | 24 |
|---|---|---|---|---|---|---|---|
| Purified EGCG | .62; $P$=.0044 | .52; $P$=.0223 | .56; $P$=.0116 | .53; $P$=.0188 | .56; $P$=.0134 | .55; $P$=.0150 | .64; $P$=.0058 |
| Poly E | .68; $P$=.0015 | .64; $P$=.0029 | .71; $P$=.0007 | .74; $P$=.0002 | .39; $P$=.0952 | .59; $P$=.0083 | .57; $P$=.0104 |

The correlation coefficient in the presence of repeated measures was estimated using Hamlett et al.'s method [16].

correlation coefficient and significance of correlation were calculated using Hamlett et al.'s method [16].

## 3. Results

### 3.1. Plasma pharmacokinetics of flavanol concentrations

The amount of brewed tea and green tea supplement administered was determined to provide the same EGCG content. Therefore, the total flavanol content (EGC+EC+EGCG+ECG) in the mixed tea extract was 177% compared with the purified EGCG intervention. After the consumption of this single large dose of EGCG either in purified form or as mixed tea extract (Poly E), the concentration of total (unconjugated, sulfated and glucuronidated) tea flavanols was quantified by HPLC with coulometric array ECD (C-ECD). The pharmacokinetic parameters for plasma EGCG concentrations are summarized in Table 1. There was no statistical difference between the maximal concentration ($C_{\max}$), area under the curve (AUC) and time of maximal plasma concentration ($T_{\max}$) for plasma EGCG between the two interventions. Maximum plasma total flavanol (EGC+EC+EGCG+ECG) concentration after Poly E intake was 3.2 μmol/L compared with 0.5 μmol/L after purified EGCG intake (Fig. 1). No significant amounts of other polyphenols (EGC, EC, ECG) were detected in the plasma collected after purified EGCG intake.

### 3.2. Plasma antioxidant capacity and plasma urate concentration after purified EGCG and Poly E interventions

After both interventions, the plasma antioxidant activity decreased at 2 h as determined using the TEAC assay. The plasma antioxidant activity returned to baseline levels after approximately 6 h and remained there up to 24 h. There was no significant difference in the plasma antioxidant activity and uric acid concentration between the two interventions with purified EGCG versus Poly E intake (Table 2; Figs. 2 and 3). The plasma antioxidant activity and plasma urate content were correlated significantly (Spearman correlation coefficient, .56; Table 3; Fig. 4). From 0 to 8 h, the plasma urate pharmacokinetic alterations followed a similar pattern compared with the plasma antioxidant capacity of initial decrease followed by an increase (Figs. 2 and 3). At 6 h, plasma uric acid concentration increased above baseline, whereas the plasma antioxidant activity remained at baseline. From 8 to 24 h, the mean plasma uric acid concentration decreased significantly while the plasma antioxidant activity remained at baseline level.

### 3.3. Lymphocyte oxidative DNA damage determined by the HPLC measurement of DNA 8-OHdG/10$^6$dG ratio

Oxidative DNA damage was determined using the 8-OHdG/10$^6$dG ratio by HPLC with C-ECD. As demonstrated in Fig. 5, there was no significant change overall (0 to 24 h) based on calculation of the AUC in the 8-OHdG/10$^6$dG ratio following either EGCG or Poly E intake (Table 2). Interindividual variation of the 8-OHdG/10$^6$dG ratio ranged from 2.8 to 10.7. Ratios determined in lymphocytes collected from the same participants during the first and second interventions showed significant correlation (Fig. 6). The 8-OHdG/10$^6$dG ratios determined in our study were slightly higher compared with the median value (4.24) determined by 11 laboratories participating in an interlaboratory validation study [17]. A minor artificial

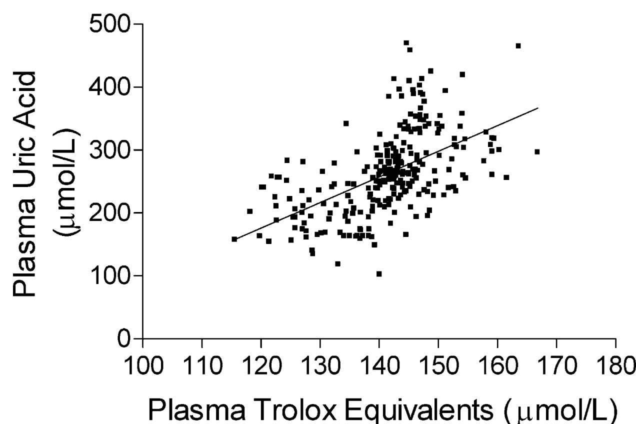


Fig. 4. Correlation of plasma uric acid concentration to plasma antioxidant activity expressed in TEs for 20 participants. Spearman correlation coefficient was .56.

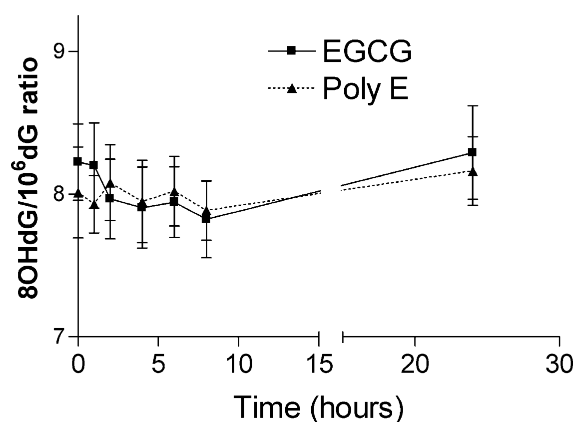


Fig. 5. Pharmacokinetics of oxidative DNA damage in buffy coat. Values are mean±S.E.M. of 20 participants.

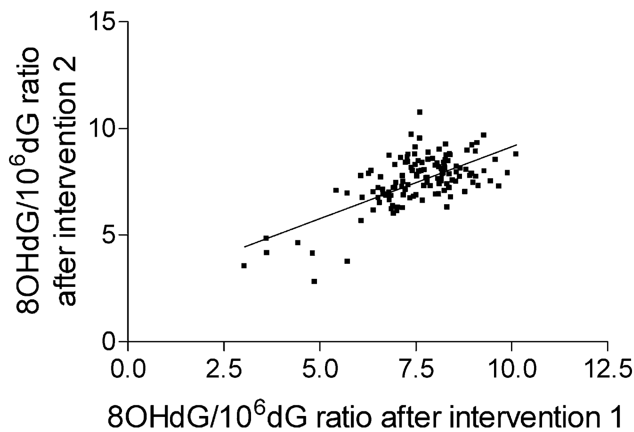


Fig. 6. Correlation of lymphocyte 8-OHdG/$10^6$dG ratio within individuals of samples collected during Intervention 1 compared with Intervention 2 of 20 participants. Spearman correlation coefficient was .663 with $P$<.0001.

increase in lymphocyte 8-OHdG/$10^6$dG ratios could have occurred during storage of samples for several months at −80°C between blood collection and analysis.

## 4. Discussion

In this intervention study, we investigated whether the bioavailability and antioxidant activity of EGCG are affected by the form of administration. A single large dose of EGCG in the form of either purified EGCG alone or the same amount of EGCG in the form of a green tea extract was administered to healthy human participants. We detected no difference between the EGCG plasma concentration after intervention with purified EGCG compared with that after intervention with green tea extract. We concluded that there was no competition from other tea flavanols on EGCG transport across the intestinal wall for the administration of 600 mg of EGCG. This confirms findings by Chow et al. [18], who also found no difference in the pharmacokinetic characteristics of EGCG administered as a single dose of either EGCG or Poly E from 200 to 800 mg. We further investigated the hypothesis that the administration of a complete green tea extract (1029 mg of total flavanols containing 618 mg of EGCG) will increase the plasma antioxidant activity and decrease oxidative DNA damage compared with the administration of 580 mg of purified EGCG alone. We observed no difference in the plasma antioxidant activity following the two interventions. Surprisingly, we observed a decrease in plasma antioxidant activity instead of an increase after EGCG administration. Our previous in vitro study demonstrated that a plasma concentration higher than 5 μmol/L of one or a combination of the four main tea flavanols (EGC, EC, EGCG or ECG) was required to increase the antioxidant activity in buffer or plasma above baseline using the TEAC assay [19]. Therefore, the maximum plasma concentration of total tea flavanols of 3.2 μmol/L following the current intervention is below the plasma concentration necessary to expect an increase in the antioxidant activity. To interpret the actual decrease in antioxidant activity, we determined the concentration of uric acid, a major contributor to the antioxidant protection in plasma. A recent study by Lotito and Frei [8] demonstrated that the plasma antioxidant capacity is under the influence of dietary changes. Their dietary intervention of apples, fructose–water or bagels altered plasma uric acid concentrations and antioxidant activity. The consumption of apples or fructose–water was associated with an increase in plasma urate concentration and simultaneous increase in antioxidant capacity [8]. The authors suggested that increased fructose intake stimulated the AMP deaminase, leading in turn to increased urate formation. Feeding bagels had the opposite effect of decreasing the antioxidant activity. In our study, the decrease in plasma antioxidant activity was also significantly correlated with decreased plasma urate concentrations. We suggest that in our study, the consumption of the small high-carbohydrate breakfast led to decreased plasma uric acid concentration and in turn to decreased plasma antioxidant activity. A similar correlation between the plasma antioxidant activity and plasma uric acid content was demonstrated by Van Amelsvoort et al. [7] after the administration of 1.5 mmol of EGC or ECG. In that study, the tea polyphenols were administered to participants after an overnight fast in combination with a standard breakfast. The results demonstrated an initial increase followed by a decrease of the plasma antioxidant activity and plasma uric acid concentration [7].

At the 24-h time point, we observed a difference in the pharmacokinetic changes over time between the means of plasma antioxidant activity and plasma uric acid concentrations. While the mean of plasma antioxidant activity remained at baseline, the mean of plasma uric acid concentrations was decreased compared to the 8-h value. We propose that other contributors to the antioxidant activity such as ascorbic acid, glutathione or metabolites of tea flavanols of intestinal origin including 4-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid, 3-methoxy-4-hydroxyhippuric acid and vanillic acid might serve as factors at the 24-h time point [20]. Formation and absorption of these metabolites are delayed compared with the parent compound and most likely will fall into this period (8–24 h) [21]. We have been able to demonstrate that some of these potential tea flavanol metabolites have in vitro antioxidant activity [22].

In our study, the TEAC assay was used to measure the plasma antioxidant activity. The ratio of uric acid to trolox equivalent was about 0.5 as determined in plasma (Fig. 4). The same ratio determined in buffer was 1.6 (data not shown). This indicates that the TEAC assay may be less sensitive when used for plasma analyses. As shown in the review of the effect of tea on ex vivo antioxidant activity by Hidgon and Frei [5], studies using the ferric reducing ability of plasma (FRAP) assay most often detected an increase in plasma antioxidant activity. A recent study by Widlansky et al. [23], however, did not find a significant increase in

plasma antioxidant activity determined by either the ORAC or the FRAP assay.

Increased oxidative DNA damage has been associated with carcinogenesis [11]. Nutritional antioxidants play a major role in the protection of cells from oxidative damage. The third goal of this intervention study was to follow the alteration in lymphocyte 8-OHdG/$10^6$dG ratio in healthy participants for 24 h after a single large dose of EGCG from either a purified source or Poly E. The results showed no significant change in 8-OHdG/$10^6$dG ratios during the 24-h blood collection with either intervention. However, a significant correlation was found in 8-OHdG/$10^6$dG ratios within participants from samples collected for the two interventions separated by 1 week. This demonstrates that there are consistent interindividual differences in endogenous oxidative damage that might be dependent on variations in antioxidant capacity, difference in basic endogenous oxidative stress levels or DNA repair enzyme activities.

In summary, we conclude that changes in plasma uric acid due to dietary intake were significantly correlated to the plasma antioxidant activity and exerted a stronger influence on the plasma antioxidant activity compared with the EGCG intervention. In future studies of dietary effects on antioxidant capacity in plasma changes in plasma, uric acid will need to be closely monitored.

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