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Analysis of Tea Catechins in Human Plasma by High-Performance Liquid Chromatography with Solid-Phase Extraction

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Accurate monitoring of tea catechins in biological samples might provide a means of better evaluation of their benefits. The aim of the present study was to develop a rapid method for extracting tea catechins from human plasma samples with a solid-phase extraction technique and to subsequently measure their concentrations using an HPLC system. A human plasma sample spiked with known concentrations of the analyte standards was passed through a Waters Oasis HLB cartridge. After repeated washing, tea catechins were eluted with 70% dimethylformamide containing 0.1% phosphoric acid, and the resulting eluate was injected into an HPLC system. Analytes were separated on a reverse-phase C_{18} column using an isocratic mobile phase and detected electrochemically. The coefficient of variation for inter- and intraday reproducibility was less than 5.0% and 6.4%, respectively. Linearity was established for the concentration range of 0.01−1.0 μM. The method was successfully applied to measure tea catechin concentrations in the plasma of two healthy subjects who received a single ingestion of a green tea beverage. The proposed method enables the rapid and accurate quantitation of plasma tea catechins and might prove useful for the evaluation of beneficial health effects of tea consumption.

KEYWORDS: Catechins; solid-phase extraction; human plasma.

INTRODUCTION

Green tea is a popular beverage commonly consumed in Asian countries. Although green tea contains a number of bioactive constituents, it is particularly rich in polyphenol compounds. Green tea is produced by steaming or firing fresh leaves at high temperatures, thereby inactivating the oxidizing enzymes and keeping the polyphenol constituents intact (*1*). The characteristic polyphenols found in green tea predominantly consist of (−)-epigallocatechin (EGC), (−)-epicatechin (EC), (−)-epigallocatechin gallate (EGCG), and (−)-epicatechin gallate (ECG) (Figure 1). In the manufacturing process of canned and bottled tea beverages, approximately one-half of the original tea catechins are converted into their corresponding epimers such as (−)-gallocatechin (GC), (−)-catechin (C), (−)-gallocatechin gallate (GCG), and (−)-catechin gallate (CG) by heat treatment (*2*−*4*). In the past decade, the market for ready-to-drink green tea beverages has grown rapidly in Japan. Consumption of canned or bottled green tea beverages consequently has led to the intake of eight different kinds of tea catechins.

Green tea has attracted much attention for its beneficial health effects, particularly with respect to its potential for preventing cancer and cardiovascular diseases (*5*−*7*). As we gain a better understanding of how these catechins in green tea affect health, their metabolic fate in the body system is the issue that inevitably arises. In vivo activity of tea catechins is determined by effective concentrations at target sites and thus by intestinal absorption; by metabolism; and by excretion into bile, urine, and feces. Researchers have investigated the kinetics and extent of intestinal absorption of tea catechins by measuring plasma concentrations and/or urinary excretion after oral ingestion of whole green tea infusion, green tea extract, and tea catechins in their pure form (*8*, *9*).

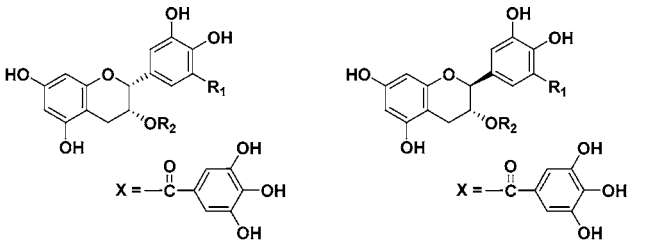

Figure 1. Chemical structures of tea catechins.

Several high-performance liquid chromatographic (HPLC) methods have been published regarding the analysis of tea catechins in blood plasma. Most of these methods involve the extraction of tea catechins from plasma or tissue homogenates with ethyl acetate (*10*−*15*), acetonitrile (*16*), or methanol (*17*),

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followed by an HPLC separation coupled with electrochemical detection. This liquid−liquid extraction is the most common method for obtaining tea catechins from biological samples, but it requires time-consuming steps. Accordingly, the development of a simple and accurate methodology to analyze tea catechins in biological samples would be welcomed. The solid-phase extraction (SPE) technique is widely used for sample cleanup preparation before HPLC analysis. Compared to the classic liquid−liquid extraction, the SPE method is less time-consuming, is easier to perform, and uses less solvent. In some articles, the SPE technique has been adopted for a part of the selective cleanup procedure of tea catechins (*16*, *18*). However, such methodologies still consist of a primary deproteinization process and a secondary step for further cleanup with SPE. Direct application of plasma samples to SPE cartridges will be obviously ideal and lead to further time savings. The objectives of this research were to develop a method for the determination of plasma tea catechins using an HPLC system along with the SPE extraction technique and to apply this method to pilot pharmacokinetic investigations of orally administered green tea beverage.

MATERIALS AND METHODS

Reagents and Materials. EGC, EC, EGCG, and ECG and their corresponding epimers such as GC, C, GCG, and CG, the purities of which were at least 95%, were purchased from Funakoshi Co., Ltd. (Tokyo, Japan). Ethyl gallate as an internal standard (IS), L-ascorbic acid, ethylenediaminetetraacetic acid disodium salt dehydrate (Na_2-EDTA), *n*,*n*-dimethyl formamide (DMF), and HPLC-grade acetonitrile were obtained from Wako Pure Chemical Industry, Ltd. (Osaka, Japan). The hydrolyzing enzymes, β-glucuronidase type X−A and sulfatase type VIII, were from Sigma-Aldrich Co. (St. Louis, MO). Water was purified using a Milli-Q system (Millipore, Bedford, MA). All other reagents were of analytical or guaranteed reagent grade.

The solid-phase extraction cartridges used were Oasis HLB (30 mg/mL) and Sep-Pak C_{18} (100 mg/mL) supplied by Waters Corporation (Milford, MA), with the aid of a Supelco vacuum manifold station (Bellefonte, PA).

Standard Solutions. A stock solution of a mixture of tea catechins (10 μM) was prepared in 0.1 M sodium phosphate buffer containing 0.5 mM Na_2EDTA (pH 3.5) and stored at −70 °C until use. Appropriate dilutions of the stock solution of tea catechins were made with the same buffer to prepare the working solution. The stock solution of IS (1 mM) was also further diluted with the same buffer to give the working solution containing 5 μM of ethyl gallate. Calibration standard samples were prepared in 200 μL of blank plasma by spiking with 10 μL of the working solutions to obtain a concentration range of 0.005−1.0 μM. Quality control concentrations represented 0.05 and 0.2 μM of each tea catechin in plasma samples for the purpose of the validity check of the method. The enzymatic deconjugation step by β-glucuronidase and sulfatase was not included in the assay validation.

SPE of Tea Catechins from Plasma Samples. Ten microliters of 5 μM IS was added to 200 μL of the thawed plasma samples containing 10 μL of 10% (w/v) L-ascorbic acid. Plasma samples were diluted by adding 0.7 mL of 0.1 M phosphate buffer containing 0.5 mM Na_2-EDTA (pH 3.5), and they were directly applied to the Oasis HLB cartridges, that had been preconditioned successively with 1 mL of water, 1 mL of 70% (v/v) DMF containing 0.1% (v/v) phosphoric acid, and 1 mL of water, and allowed to run through. The cartridges were washed with 2 mL of water and 1 mL of 30% (v/v) methanol. Tea catechins were then eluted with 0.7 mL of 70% (v/v) DMF containing 0.1% (v/v) phosphoric acid. After filtration with an Ekicrodisk type 3CR 0.45-μm filter (Pall Corporation, Tokyo, Japan), 20 μL of the resulting filtrate was injected into the HPLC system.

In the case of Sep-Pak C_{18}, this disposable cartridge was preconditioned by rinsing in order with 1 mL of water, 1 mL of methanol, and 1 mL of water. The plasma sample spiked with tea catechins was diluted with 0.7 mL of 0.1 M phosphate buffer containing 0.5 mM Na_2EDTA (pH 3.5) and then applied to this SPE cartridge. After being washed with 2 mL of water, catechins in the cartridge were collected in another tube by eluting with 1 mL of methanol. The resulting eluate was concentrated under reduced pressure, and the residue was redissolved in 0.7 mL of the mobile phase of HPLC. After passage through an Ekicrodisk type 3 0.45-μm filter (Pall Corporation, Tokyo, Japan), 20 μL of the resulting filtrate was applied to an HPLC system.

HPLC Instruments and Conditions. The HPLC system consists of a model PX-8010 controlled dual pump (Tosoh Co., Tokyo, Japan), a model AS-8010 auto-injector (Tosoh, Co.), a model CO-8010 column oven (Tosoh, Co.), a coulochem II electrochemical detector (ESA Inc., Bedford, MA) with a model 5020 guard cell and a model 5010 analytical cell, and a computer equipped with VStation version 1.6 chromatography data integration system (GL Science Inc., Tokyo, Japan).

The HPLC analytical conditions with electrochemical detection were basically similar to those in the previous report by Umegaki et al. (*16*). Eight kinds of tea catechins were separated on a 250 × 4.6 mm i.d., 3 μm, Capcell Pak type AQ reverse-phase C_{18} column equipped with a 10 × 4.0 mm i.d., Capcell Pak type AQ guard cartridge (Shiseido, Co., Tokyo, Japan). Isocratic elution was employed using 0.1 M sodium phosphate buffer containing 0.5 mM Na_2EDTA (pH 3.5)/acetonitrile (85/15, v/v) at a flow rate of 0.8 mL/min. The column temperature was maintained at 40 °C. The applied voltage of the analytical cell was set at −200 mV for electrode 1 and +200 mV for electrode 2, and that of guard cell was +250 mV. Electrochemical data from electrode 2 were collected, and the approximate basal cell current was 2 nA.

Pilot Human Studies. The study involved the participation of two healthy male volunteers (subject 1, age 33, weight 82 kg, height 173 cm; subject 2, age 34, weight 64 kg, height 167 cm), who were fully informed about all procedures before their written informed consent was obtained. The clinical aspects of this study were proposed in accordance with the Helsinki Declaration of 1964 as revised in 1989 and were approved by the Ethical Committee of ITO EN, Ltd. (Tokyo, Japan). Subjects were instructed to abstain from green tea and related beverages for 1 day before the experiment. On the experimental day, a blood sample was collected under overnight-fasted conditions. The subjects then ingested 190 mL of the canned green tea beverage containing 52 mg of EGC, 102 mg of GC, 16 mg of EC, 26 mg of C, 63 mg of EGCG, 69 mg of GCG, 11 mg of ECG, and 10 mg of CG, and subsequently, blood samples were collected at 0, 1, 2, 4, and 6 h post-ingestion in heparinized tubes. Blood samples were immediately centrifuged at 1500*g* for 15 min at 4 °C to separate the plasma. Two hundred microliters of the plasma was mixed with 10 μL of 10% (w/v) L-ascorbic acid as a preservative solution in a disposable tube and stored at −70 °C. The enzymatic method used for liberating free catechins from their conjugated forms by β-glucuronidase and sulfatase was that described by Lee et al. (*10*). Briefly, the thawed plasma was mixed with 10 μL of a mixture of β-glucuronidase (250 units) and sulfatase (20 units), and the reaction mixture was incubated at 37 °C for 45 min. Then, 700 μL of 0.1 M sodium phosphate buffer (pH 3.5) containing 0.5 mM Na_2EDTA and 10 μL of 10 μM IS were added to the reaction mixture. The resulting solution was subjected to the SPE procedure as described above. From the time-course profiles of plasma concentration of each tea catechin, the area under the curves (AUC) was calculated by the linear trapezoidal rule using GraphPad Prism for Windows version 4.0 (GraphPad Software, San Diego, CA).

RESULTS AND DISCUSSION

HPLC Chromatogram. Separations achieved using the experimental conditions of this assay for tea catechins are illustrated in **Figure 2**A. Selectivity was indicated by the absence of any endogenous interference at the retention time of each peak, as evaluated by chromatograms of control human plasma and plasma spiked with eight tea catechins.

Method for Development of SPE. In relation to the efficiency of the SPE system, a series of commercial SPE cartridges in combination with different elution solvents was

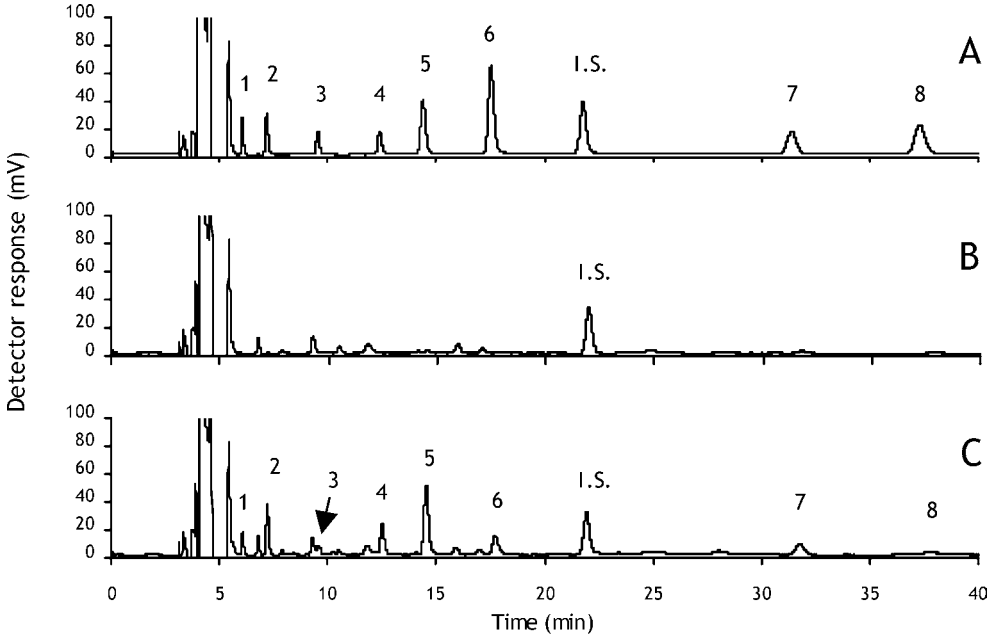

Figure 2. Typical chromatograms of (A) eight tea catechin standards at the concentration of 0.2 μM, (B) plasma extract obtained before green tea ingestion, (C) plasma extract from the same person 2 h after green tea ingestion. HPLC conditions as described in the text. Peaks in order of elution: (1) (–)-gallocatechin, (2) (–)-epigallocatechin, (3) (–)-catechin, (4) (–)-epicatechin, (5) (–)-epigallocatechin gallate, (6) (–)-gallocatechin gallate, ethyl gallate as internal standard, (7) (–)-epicatechin gallate, (8) (–)-catechin gallate.

Table 1. Percentage Recoveries of Tea Catechins and Their Epimers by Solid-Phase Extraction ($n = 3$)

SPE product	elution solvent	recoveries from spiked plasma (%)							
		EGC	GC	EC	C	EGCG	GCG	ECG	CG
Sep-Pak C_{18}	methanol	72 ± 0	64 ± 2	84 ± 1	76 ± 1	44 ± 2	57 ± 3	43 ± 2	67 ± 7
Oasis HLB	methanol	62 ± 15	60 ± 10	89 ± 3	83 ± 2	13 ± 0	5 ± 1	17 ± 2	12 ± 2
	70% DMF	86 ± 1	95 ± 2	97 ± 2	90 ± 2	99 ± 4	95 ± 2	94 ± 2	86 ± 0

tested to ascertain the optimum conditions. A few silica-based reverse-phase supports were tested for efficient cleanup of the sample and quantitative recovery of the analytes. The diluted blank plasma samples spiked with standard tea catechins were directly applied to the SPE cartridges and followed by elution with some organic modifiers. Plasma proteins could be removed from the SPE cartridges by repeated washing. Recoveries of tea catechins were assessed by comparing the peak areas from plasma samples spiked with known amounts of tea catechins and IS, processed according to the described method, versus direct injections of a standard solution of tea catechins. The results demonstrated that the use of Sep-Pack C_{18} cartridge with methanol elution generally led to unsatisfactory recoveries of tea catechins with a galloyl moiety such as EGCG, GCG, ECG, and CG (**Table 1**). This is because a portion of such tea catechins passed through without adsorption to the SPE cartridges because of their strong interactions with plasma proteins. The Oasis HLB is known to be advantageous for compounds having catechol structures and is a recently applied tool for the preparation of polyphenolic compounds (*19*−*22*). In the case of Oasis HLB cartridges and methanol as the elution solvent, recoveries of EGCG, GCG, ECG, and CG were extremely low, owing to specific affinities of tea catechins having a galloyl moiety to be strongly retained by the resins. The issue was resolved with the correct choice of elution solvent. Figure 3 indicates that the recovery of each tea catechin rose with increasing DMF concentration in the eluting solution and reached a plateau level at over 70% (v/v) DMF. Consequently, this method employed 70% (v/v) DMF containing 0.1% (v/v) phosphoric acid as the elution solvent.

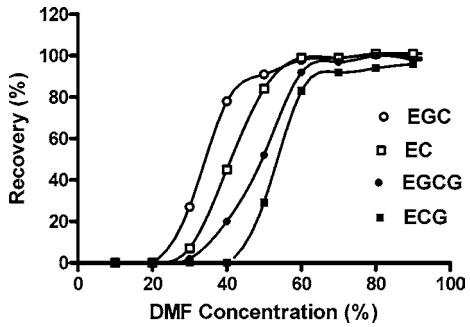

Figure 3. Optimum concentration of dimethyl formamide (DMF) to elute tea catechins using Oasis HLB cartridge. Each point represents the mean of duplicate measurements.

Standard Curves and Method Validation. The calibration curves consisted of seven or eight concentration points (0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, and 1.0 μM) of each tea catechin. Calibration data were acquired by plotting the ratios of the peak areas of different concentrations of tea catechins to that of IS. The least-squares method was used to calculate the regression equations. Calibration curves showed excellent linearity in the concentration range of 0.005−1.0 μM for EGCG and GCG and 0.01−1.0 μM for the rest of the species analyzed, with correlation coefficients higher than 0.9990. Extraction from plasma samples spiked with 0.2 μM of each catechin according to the above procedure confirmed satisfactory recovery values better than 86% (**Table 1**). Reproducibility was evaluated by analyzing samples at the concentration of 0.05 or 0.2 μM of each tea catechin on the same day in seven replicates (intraday

Table 2. Plasma Parameters of Tea Catechins after Ingestion of Green Tea Beverage[a]

	tea catechins ingested (mg)	subject 1			subject 2		
		T_{max} (h)	C_{max} (μM)	AUC_{0-6h} (μM·h)	T_{max} (h)	C_{max} (μM)	AUC_{0-6h} (μM·h)
EGC	53	2	0.21	0.76	2	0.27	1.20
GC	102	2	0.14	0.40	2	0.13	0.46
EC	16	2	0.23	0.68	2	0.28	1.05
C	26	2	0.02	0.04	2	0.05	0.18
EGCG	63	2	0.38	1.01	2	0.28	1.11
GCG	69	2	0.06	0.21	4	0.06	0.24
ECG	11	2	0.15	0.37	2	0.11	0.35
CG	10	2	0.02	0.08	2	0.02	0.05

[a] Plasma samples from two healthy volunteers who consumed green tea beverage were treated with the mixture of β-glucuronidase/sulfatase and extracted using Oasis HLB cartridges. Plasma tea catechins were determined by a reverse-phase HPLC along with electrochemical detection.

reproducibility) and five separate days in triplicate (interday reproducibility). The relative standard deviations of the analytical method were less than 5.0% and 6.4% for the inter- and intraday reproducibility, respectively. The detection limit of tea catechins (defined at a signal-to-noise ratio of about 3) ranged from 1 to 5 nM in 200 μL of plasma.

Human Trial. The method established above was used to measure tea catechin concentrations in plasma from two healthy subjects who received a single ingestion of 190 mL of green tea beverage containing eight kinds of tea catechins under fasted conditions, and blood samples were collected before and 1, 2, 4, and 6 h after ingestion. **Figure 2B**,**C** shows the chromatographic profiles of plasma extract obtained from subject 1 before and 2 h after ingesting green tea beverage. The major tea catechins were detected in the plasma after it was treated with hydrolyzing enzymes of β-glucuronidase and sulfatase to convert them into their free forms. As can be seen, some distinct peaks of tea catechins were indicated in the HPLC chromatogram of plasma obtained after green tea ingestion. The times to reach the maximum plasma levels (T_{max}) of tea catechins were about 2 h after ingestion (except for GCG in subject 2) (**Table 2**), suggesting that tea catechins might be rapidly absorbed from the upper portion of the digestive tract and distributed in the plasma. The maximum plasma concentrations (C_{max}) of EGCG in the two subjects were 0.38 and 0.28 μM following intake of 63 mg of EGCG via green tea beverage. A recent review article by Manach et al. (*8*) converted the already published pharmacokinetic results of EGCG into a single 50-mg dose equivalent, indicating that C_{max} of EGCG was 0.12 ± 0.03 μM (range = 0.03−0.38 μM). Thus, the results in the present study were in accordance with previous findings. The green tea beverage also contained almost the same amount of GCG (69 mg), whereas plasma GCG exhibited extremely low levels. For both subjects, the AUC values of GCG over 6 h were approximately one-fifth those of EGCG. Similar results were observed between original tea catechins (EGC, EC, and ECG) and their corresponding epimers (GC, C, and CG). Baba et al. (*23*) found that the bioavailability of EC was higher than that of (+)-catechin in rats and suggested that chemical features such as solubility and lipophilicity of each substance might be involved in the results. Because the present study was clearly a small-size pilot study, further studies with larger sample sizes are required to explain whether the stereochemical features of tea catechins influenced their bioavailability for absorption, metabolism, and disposition in humans.

Concluding Remarks. This new approach also provides much better reproducibility and is more time saving without requiring deproteinization of plasma proteins before application to the SPE cartridge and further evaporation before injection into the HPLC system. Particularly with simultaneous determinations of eight kinds of tea catechins in human plasma, this analytical procedure might be useful for the overall evaluation of beneficial health effects from the consumption of green tea beverage.

ABBREVIATIONS USED

EGC, (−)-epigallocatechin; GC, (−)-gallocatechin; EC, (−)-epicatechin; C, (−)-catechin; EGCG, (−)-epigallocatechin gallate; GCG, (−)-gallocatechin gallate; ECG, (−)-epicatechin gallate; CG, (−)-catechin gallate; SPE, solid-phase extraction; IS, internal standard; AUC, area under the curve; T_{max}, time to reach the maximum concentration; C_{max}, maximum concentration.

Supporting Information Available: Intra- and interday variance and linearity of tea catechin assay in spiked human plasma. This material is available free of charge via the Internet at http://pubs.acs.org.

LITERATURE CITED

(1) Graham, H. N. Green tea composition, consumption, and polyphenol chemistry. *Prev. Med.* **1992**, *21*, 334−350.
(2) Wang, H.; Helliwell, K. Epimerization of catechins in green tea infusions. *Food Chem.* **2000**, *70*, 337−344.
(3) Ito, R.; Yamamoto, A.; Kodama, S.; Kato, K.; Yoshimura, Y.; Matsunaga, A.; Nakazawa, H. A study on the change of enantiomeric purity of catechins in green tea infusion. *Food Chem.* **2003**, *83*, 563−568.
(4) Suzuki, M.; Sano, M.; Yoshida, R.; Degawa, M.; Miyase, T.; Maeda-Yamamoto, M. Epimerization of tea catechins and O-methylated derivatives of (−)-epigallocatechin-3-O-gallate: Relationship between epimerization and chemical structure. *J Agric. Food Chem.* **2003**, *51*, 510−514.
(5) Higdon, J. V.; Frei, B. Tea catechins and polyphenols: Health effects, metabolism, and antioxidant functions. *Crit. Rev. Food Sci. Nutr.* **2003**, *43*, 89−143.
(6) McKay, D. L.; Blumberg, J. B. The role of tea in human health: An update. *J. Am. Coll. Nutr.* **2002**, *21*, 1−13.
(7) Yang, C. S.; Landau, J. M. Effects of tea consumption on nutrition and health. *J. Nutr.* **2000**, *130*, 2409−2412.
(8) Manach, C.; Williamson, G.; Morand, C.; Scalbert, A.; Rémésy, C. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *Am. J. Clin. Nutr.* **2005**, *81*, 230S−42S.
(9) Kroon, P. A.; Clifford, M. N.; Crozier, A.; Day, A. J.; Donovan, J. L.; Manach, C.; Williamson, G. How should we assess the effects of exposure to dietary polyphenols in vitro? *Am. J. Clin. Nutr.* **2004**, *80*, 15−21.
(10) Lee, M.-J.; Wang X.-Y.; Li, H.; Chen, L.; Sun, Y.; Gobbo, S.; Balentine, D. A.; Yang, C. S. Analysis of plasma and urinary tea polyphenols in human subjects. *Cancer Epidemiol. Biomark. Prev.* **1995**, *4*, 393−399.
(11) Maiani, G.; Serafini, M.; Salucci, M.; Azzini, E.; Ferreo-Luzzi, A. Application of a new high-performance liquid chromatographic method for measuring selected polyphenols in human plasma. *J. Chromatogr. B* **1997**, *692*, 311−317.
(12) Pietta, P.; Simonetti, P.; Gardana, C.; Brusamolino, A.; Morazzoni, P.; Bombardelli, E. Relationship between rate and extent of catechin absorption and plasma antioxidant status. *Biochem. Mol. Biol. Int.* **1998**, *46*, 895−903.

(13) Lee, M.-J.; Prabhu, S.; Meng, X.; Li, C.; Yang, C. S. An improved method for the determination of green and black tea polyphenols in biomatrices by high-performance liquid chromatography with coulometric array detection. *Anal. Biochem.* **2000**, *279*, 164−169.

(14) van Amelsvoort, J. M. M.; van het Hof, K. H.; Mathot, J. N. J. J.; Mulder, T. P. J.; Wiersma, A.; Tijnerg, L. B. M. Plasma concentrations of individual tea catechins after a single oral dose in humans. *Xenobiotica* **2001**, *31*, 891−901.

(15) Kotani, A.; Miyashita, N.; Kusu, F. Determination of catechins in human plasma after commercial canned green tea ingestion by high-performance liquid chromatography with electrochemical detection using a microbore column. *J. Chromatogr. B* **2003**, *788*, 269−275.

(16) Umegaki, K.; Sugisawa, A.; Yamada, K.; Higuchi, M. Analytical method of measuring tea catechins in human plasma by solid-phase extraction and HPLC with electrochemical detection. *J. Nutr. Sci. Vitaminol.* **2001**, *47*, 402−408.

(17) Nakagawa, K.; Miyazawa, T. Chemiluminescence-high-performance liquid chromatographic determination of tea catechin, (−)-epigallocatechin 3-gallate, at picomole levels in rat and human plasma. *Anal. Biochem.* **1997**, *248*, 41−49.

(18) Unno, T.; Kondo, K.; Itakura, H.; Takeo, T. Analysis of (−)-epigallocatechin gallate in human serum obtained after ingesting green tea. *Biosci. Biotechnol. Biochem.* **1996**, *60*, 2066−2068.

(19) Chu, K. O.; Wang, C. C.; Chu, C. Y.; Rogers, M. S.; Choy, K. W.; Pang, C. P. Determination of catechins and catechin gallates in tissues by liquid chromatography with coulometric array detection and selective solid-phase extraction. *J. Chromatogr. B* **2004**, *810*, 187−195.

(20) Ishii, K.; Furuta, T.; Kasuya, Y. High-performance liquid chromatographic determination of quercetin in human plasma and urine utilizing solid-phase extraction and ultraviolet detection. *J. Chromatogr. B* **2003**, *794*, 49−56.

(21) Unno, T.; Tamemoto, K.; Yayabe, F.; Kakuda, T. Urinary excretion of 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone, a ring-fission metabolite of (−)-epicatechin, in rats and its in vitro antioxidant activity. *J. Agric. Food Chem.* **2003**, *51*, 6893−6898.

(22) Vaidyanathan, J. B.; Wakke, T. Glucuronidation and sulfation of the tea flavonoid (−)-epicatechin by the human and rat enzymes. *Drug Metab. Dispos.* **2002**, *30*, 897−903.

(23) Baba, S.; Osakabe, N.; Natsume, M.; Muto, Y.; Takizawa, T.; Terao J. In vivo comparison of the bioavailability of (+)-catechin, (−)-epicatechin and their mixture in orally administered rats. *J. Nutr.* **2001**, *131*, 2885−2891.

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