Bioavailability of Polyphenon E Flavan-3-ols in Humans with an Ileostomy1–4

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Abstract
To investigate the degree of absorption of flavan-3-ols in the small intestine, human subjects with an ileostomy ingested 200 mg of Polyphenon E, a green tea extract, after which ileal fluid and urine, collected over a 24-h period, were analyzed by high-performance liquid chromatography with photodiode array and mass spectrometric detection. The data obtained indicated that although ~40% of flavan-3-ol intake is recovered in ileal fluid, substantial quantities are absorbed in the small intestine. Moreover, 14 urinary metabolites, comprising sulfates, glucuronide, and methylated derivatives, were identified and quantified. All were metabolites of (epi)catechin or (epi)gallocatechin, representing 47 ± 2% and 26 ± 9%, respectively, of the ingested parent compound. These high recoveries indicate that these flavan-3-ols absorbed in the small intestine are much more bioavailable than most dietary flavonoids. No 3-O-galloylated flavan-3-ols or their metabolites were detected in urine. The absence of urinary flavan-3-ol metabolites after ingestion of 200 mg of (–)-epigallocatechin gallate indicates that there is no removal of the 3-O-galloyl group in vivo, and hence, this does not account for the high urinary recovery of (epi)gallocatechin metabolites after ingestion of Polyphenon E. Increasing the intake of Polyphenon E, by feeding doses of 200, 500, and 1500 mg, led to increased urinary excretion of (epi)catechin metabolites but not metabolites of (epi)gallocatechin. Coingestion of 200 mg of Polyphenon E with bread, cheese, or glucose did not significantly modify the absorption, metabolism, and excretion of flavan-3-ols. It does not necessarily follow, however, that the same would occur when flavan-3-ols are ingested with more complex food matrices. J. Nutr. 138: 1535S–1542S, 2008.

Introduction
Tea, produced principally from infusions of dried leaves of Camellia sinensis, is, after water, the most widely consumed beverage in the world. Epidemiological studies have linked tea consumption, notably green tea, with reduced incidence of cardiovascular disease (1–3) and cancer (2). However, some other studies and meta-analyses have found inconclusive relations between tea consumption and health effects (4–7). Protective effects of green tea have been attributed to their polyphenol content, the most abundant compounds being a group of 8 flavan-3-ols (catechins) (Fig. 1) (8), which are known to be potent antioxidants. The main flavan-3-ol and the most potent antioxidant in most green teas is (–)-epigallocatechin-3-gallate (8,9), although in some less common teas (–)-epicatechin gallate predominates (10).

Numerous studies have assessed the potential health benefits of green tea catechins using a variety of in vivo and in vitro models. Ingestion of green tea has been shown to increase antioxidant status of plasma in humans (11,12). Green tea catechins may reduce the risk of cardiovascular disease because of their hypolipemic (13–17), antiatherosclerotic (18), and antihypertensive effects (19). They also have antidiabetic (13,20), antiangiogenic (21), antiobesity (21–23) and antiinflammatory properties (24,25). In addition, flavan-3-ols have shown beneficial effects on human vascular function (26) and possibly osteoarthritis (27).

Green tea and catechins also have anticancerogenic and antimutagenic effects (28,29), have a chemopreventive action on lung tumorigenesis (30) and prostate (31) and breast cancer (32), and induce apoptosis of carcinoma cells (33). They have also
been reported to exert antiviral, antibacterial (34), and neuroprotective effects (35,36) as well as having a capacity to prevent gastrointestinal disorders (37) and improve muscular function in dystrophy models (38).

After ingestion of green tea, flavan-3-ols are absorbed and metabolized and appear in blood and urine (39–41). Flavan-3-ols, including (−)-epigallocatechin-3-gallate, have been detected in blood as intact forms and metabolites and reach micromolar concentrations in plasma (42–44). Absorption appears to take place in the small intestine, with unabsorbed compounds reaching the colon, where they undergo extensive bacterial degradation (45,46).

Despite a wealth of data on bioavailability of green tea flavan-3-ols (47), little is known about their absorption in the small intestine and how it can be affected by the presence of other food components, the so-called matrix effect. Human subjects with an ileostomy provide a useful model for studying the absorption of micronutrients as analysis of ileal fluid after supplementation provides information of their fate in the small intestine without the complications of interactions with the colonic microflora.

In this study, the intestinal fate of flavan-3-ols after ingestion of different doses of a green tea extract was investigated, along with the effect of the food matrix on absorption and metabolism.

Materials and Methods

Materials. Mitsui Norin supplied a green tea extract, Polyphenon E, as well as (−)-epigallocatechin-3-gallate extracted from green tea and standards for (+)-catechin, (−)-epicatechin, (+)-gallocatechin, (−)-epigallocatechin, (+)-gallocatechin gallate, (−)-epigallocatechin gallate, (+)-catechin gallate, and (−)-epicatechin gallate. Ethyl gallate was obtained from Sigma. HPLC solvents were supplied by Rathburn Chemicals. Gelatin capsules were obtained from Agar Scientific. White bread, glucose tablets, and full-fat cheese were purchased from a local supermarket.

Feeding protocol. The study protocol was approved by the Glasgow Royal Infirmary Research Ethics Committee. Five ileostomy volunteers (3 male and 2 female) were recruited and gave their written informed consent. Ileostomy subjects had undergone a total colostomy but had a normal small intestine and were otherwise healthy. All subjects followed a low-flavonoid diet for 2 d before the study by avoiding all fruits and vegetables, alcoholic beverages, cocoa, coffee, and tea. After an overnight fast, the volunteers consumed a capsule containing 200 mg of Polyphenon E on its own or with either 2 slices of white bread, 3 glucose tablets (10.2 g), or 3 portions of a full-fat cheese (49.8 g of “Laughing Cow” cheese, 45% fat). In the study on the effect of dose, volunteers consumed capsules containing either 200, 500, or 1500 mg of Polyphenon E. In a third feed, volunteers consumed a 200-mg capsule of (−)-epigallocatechin gallate that contained 3% (−)-epigallocatechin as an impurity. Ileal fluid and urine were collected before the intake of the capsules (time 0 h) and over a 24-h period after supplementation. After the volumes of ileal fluid and urine that were collected had been recorded, aliquots were acidified to pH 3 with 50% aqueous formic acid before being stored at −80°C.

Sample preparation. Polyphenon E was dissolved at 10 g/L of methanol, after which 10-μL aliquots were analyzed in triplicate by HPLC with photodiode array and mass spectrometric detection (HPLC-PDA-MS). Urine samples were defrosted, thoroughly mixed, and centrifuged at 16,100 × g for 20 min at 4°C. Triplicate 100-μL aliquots of the supernatant were then analyzed by HPLC-PDA-MS.

The extraction of ileal fluid was carried out as follows: 2.5-g aliquots were mixed with 10 mL of a 50% aqueous methanol solution containing 1% formic acid, and 20 mmol/L diethyldithiocarbamate and 2 mg ethyl gallate as an internal standard were added. The mixture was blended for 1 min using an Ultra Turrax homogenizer (T-25, IKA-WERKE). Samples were further extracted for 30 min using an orbital shaker (IKA-WERKE) before being centrifuged at 4900 × g for 20 min at 4°C. The resulting supernatant was collected and the pellets reextracted using methanol containing 1% formic acid and 20 mM diethyldithiocarbamate using the same protocol. The 2 supernatants were mixed and reduced to dryness in vacuo. Samples were resuspended in 1 mL of 10% aqueous methanol containing 0.1% formic acid and centrifuged at 16,100 × g for 20 min at 4°C. Triplicate 20-μL samples of the supernatant were analyzed by HPLC-PDA-MS.

HPLC-PDA-MS. Samples were analyzed on a Surveyor HPLC system comprising of a HPLC pump, PDA detector, scanning from 250 to 700 nm, and an autosampler cooled to 4°C (Thermo Finnigan). Separation was carried out using a 250 × 4.6 mm i.d. 4 μm Synergi RP-Max column (Phenomenex) maintained at 40°C and eluted at a flow rate of 1 mL/min with either a gradient over 30 min of 5–25% acetonitrile in 0.1% aqueous formic acid (analysis of ileal fluid and Polyphenon E) or a gradient over 45 min of 5–30% acetonitrile in 0.1% aqueous formic acid (analysis of urine). After passing through the flow cell of the PDA detector, the column eluate was split, and 0.3 mL/min was directed to a LCQ Duo ion trap mass spectrometer adapted for MS² analysis and fitted with an electrospray interface (Thermo Finnigan) operating in negative ion mode. Analyses were initially carried out using full-scan, data-dependent MS² scanning from m/z 100 to 2000. Compound identities were confirmed by MS³ consecutive reaction monitoring, collision energy 35%. For instance, identification of methyl-(epi)catechin sulfate metabolites involved isolation and fragmentation of the m/z 383 ion. The resulting methyl-(epi)catechin ion at m/z 303 (80 amu loss of a sulfate moiety) was then selected for further fragmentation. The resulting MS³ profile was then compared with the fragmentation of authentic
Flavan-3-ols in ileal fluid and Polyphenon E were quantified using absorption at 280 nm by reference to standard calibration curves. Quantification of flavan-3-ol metabolites in urine was carried out using selected ion monitoring (SIM). The conditions used were as outlined above except that the mass spectrometer was set up to monitor specific ions. For quantifying (epi)gallocatechin glucuronide, methyl-(epi)gallocatechin glucuronide, and methyl-(epi)gallocatechin sulfate, the parent ions were m/z 481, m/z 495, and m/z 399, respectively. No standards of these compounds were available, so (–)-epigallocatechin was used as a calibration standard. For quantifying (epi)catechin glucuronide, (epi)catechin sulfate, and methyl-(epi)catechin sulfate, the parent ions were m/z 465, m/z 369, and m/z 383, respectively. No standards of these compounds were available, so (–)-epicatechin was used as a calibration standard.

Statistical analysis. Values in the text and tables are means ± SEM. Statistical differences are assessed by ANOVA for repeated measures followed by the Tukey-Kramer test using Statview 5 software (SAS Institute). Differences were considered significant at P < 0.05.

Results

Quantification of flavan-3-ols in Polyphenon E. HPLC-PDA-MS\textsuperscript{3} analysis showed that 200 mg of Polyphenon E contained a total of 452 ± 3 μmol (n = 3) of monomeric flavan-3-ols. (–)-Epigallocatechin-3-gallate was the major component (310 ± 4 μmol) comprising 69% of the total flavan-3-ols, followed by (–)-epicatechin (71 ± 1 μmol), (–)-epicatechin-3-gallate (32 ± 2 μmol), and (–)-epigallocatechin (18 ± 2 μmol). Their (+)-epimers are also present but in relatively small amounts with a total of 21.6 μmol (see Supplemental Data). The notable difference between the flavan-3-ol content of Polyphenon E and green tea infusions is that tea contains much higher levels of (–)-epigallocatechin as well as substantial amounts of gallic acid, chlorogenic acids, flavonols, and caffeine (8).

Identification of flavan-3-ols in ileostomy fluid. The identification of flavan-3-ols in ileal fluid was carried out using HPLC-PDA-MS\textsuperscript{3}. Ileal fluid collected after the ingestion of 200 mg of Polyphenon E contained only (–)-epicatechin, (–)-epicatechin gallate, (–)-epigallocatechin gallate, and (+)-gallocatechin gallate in amounts quantifiable by HPLC-PDA at 280 nm. MS also detected trace amounts of flavan-3-ol metabolites, principally sulfated derivatives, but in amounts that were below the limits of quantification by PDA detection.

Identification of flavan-3-ol metabolites in urine. Urine samples collected after ingestion of 200 mg of Polyphenon E were also analyzed by HPLC-PDA-MS\textsuperscript{3}. None of the original Polyphenon E flavan-3-ols was present, but 14 metabolites of (epi)catechin and (epi)gallocatechin were detected. Without reference compounds, the relative portion of (+)- and (–)-enantiomers of the various metabolites could not be determined and might be different from the proportions present in the ingested flavan-3-ol supplement (48,49). It was therefore not possible to distinguish between (–)-epicatechin and (+)-catechin metabolites and also (–)-epigallocatechin and (+)-gallocatechin derivatives. No metabolites of either (epi)catechin gallate or (epi)gallocatechin gallate were detected. The identification of the complex array of glucuronide, sulfate, and methyl metabolite isomers of (epi)catechin and (epi)gallocatechin (Fig. 2) was based on the following criteria (also see online material).

Peak 1 (t\textsubscript{R} = 14.1 min) had a negatively charged molecular ion ([M-H]\textsuperscript{−}) at m/z 481, which on MS\textsuperscript{2} produced a fragment ion corresponding to (epi)gallocatechin at m/z 305. The 176 amu loss equates with cleavage of a glucuronide moiety. Moreover, the MS\textsuperscript{3} fragmentation of m/z 305 ions produced daughter ions at m/z 261, 221, 179, and 125 in keeping with the presence of (epi)gallocatechin. This peak is, therefore, identified as an (epi)gallocatechin glucuronide.

Peak 2 (t\textsubscript{R} = 17.3 min) had a [M-H]\textsuperscript{−} at m/z 495, and MS\textsuperscript{2} yielded an ion at m/z 319. This 176 amu loss indicates cleavage of a glucuronyl unit. MS\textsuperscript{3} of the ion at m/z 319 yielded ions that corresponded to a methyl-(epi)gallocatechin, with a fragment at m/z 137 specific of methylation on the position 4′ of the B ring (50). This MS fragment pattern indicates that peak 2 is a 4′-methyl-(epi)gallocatechin glucuronide.

Peak 3 (t\textsubscript{R} = 21.0 min) had a [M-H]\textsuperscript{−} at m/z 465, which on loss of 176 amu (cleavage of a glucuronyl unit) yielded a MS\textsuperscript{2} ion at m/z 289. MS\textsuperscript{3} of the m/z 289 ion yielded fragments characteristic of (epi)catechin, indicating that this peak is an (epi)catechin glucuronide, possibly (–)-epicatechin-3′-glucuronide (Fig. 3), which has been identified in urine collected after oral ingestion of (–)-epicatechin by humans (51).

Peaks 4, 6, and 9 (t\textsubscript{R} = 26.9, 27.5, and 31.4 min, respectively) all had a [M-H]\textsuperscript{−} at m/z 369 that yielded a MS\textsuperscript{2} ion at m/z 289. This 80 amu loss indicates cleavage of a SO\textsubscript{3} unit. MS\textsuperscript{3} of the m/z 289 ion provided a mass spectrum corresponding to that of (epi)catechin. This indicates that peaks 4, 6, and 9 are (epi)catechin sulfates.

Peaks 5 and 7 (t\textsubscript{R} = 27.4 and 28.5 min) both had a [M-H]\textsuperscript{−} at m/z 399 that, with an 80 amu loss (cleavage of a SO\textsubscript{3} unit) yielded a MS\textsuperscript{2} ion at m/z 319, which on MS\textsuperscript{3} produced a fragment ion at m/z 319 consistent with a methyl-(epi)gallocatechin. Peaks 5 and 7 are, therefore, identified as methyl-(epi)gallocatechin sulfates.

Peaks 8, 10, 11, 12, 13, and 14 (t\textsubscript{R} = 30.9, 33.7, 35.6, 37.2, 39.3, and 41.4 min, respectively) all had a m/z 383 [M-H]\textsuperscript{−} that, on MS\textsuperscript{2}, produced a m/z 303 fragment indicative of the cleavage of a SO\textsubscript{3} unit. MS\textsuperscript{3} of the m/z 303 ion yielded a mass spectrum corresponding to that of a methyl-(epi)catechin. Moreover, with peaks 13 and 14, MS\textsuperscript{3} of m/z 303 ion produced an ion at m/z 137, specific for methylation on position 4′ of the B ring (50). Therefore, peaks 8, 10, 11, and 12 are methyl-(epi)catechin sulfates, where the position of methylation is unknown, whereas peaks 13 and 14 are 4′-methyl-(epi)catechin sulfates.

After the initial qualitative analysis, urine samples were analyzed by HPLC with MS in SIM mode, and typical HPLC-SIM traces that were obtained and used to quantify the 14 individual flavan-3-ol metabolites are illustrated in Figure 2.

Food matrix effects. In order to investigate potential matrix effects, 200 mg of Polyphenon E was ingested either on its own or with either bread, glucose tablets, or cheese, after which 24-h ileal fluid and urine were collected and analyzed quantitatively. The quantities of flavan-3-ols in ileal fluid are summarized in Table 1. The overall recoveries of flavan-3-ols ranged from 39% to 48% of intake; however, the average recovery of (–)-epicatechin and (–)-epigallocatechin was 27 ± 2% of intake compared with 60 ± 3% for (–)-epigallocatechin gallate and (+)-gallocatechin gallate. When incubated in vitro with gastric juice for 1 h and ileal fluid for 4 h at 37°C, there was ~90% recovery of Polyphenon E flavan-3-ols (data not shown). Thus, the differential recoveries in ileal fluid after ingestion of Polyphenon E indicate that (–)-epicatechin and (–)-epigallocatechin gallate may be absorbed in the small intestine more efficiently.
than (–)-epigallocatechin gallate and (+)-gallocatechin gallate. Statistical analysis showed no significant effects of either bread, glucose, or cheese on the recoveries of flavonols in ileal fluid, indicating that their absorption in the small intestine was not subject to a matrix effect (Table 1).

Levels of 14 individual flavan-3-ol metabolites in urine of ileostomy volunteers were analyzed by HPLC-SIM (Fig. 2), and the quantities of the 6 different types of metabolites are presented in Table 2. No unmetabolized flavan-3-ols were detected in any of the urine samples. When Polyphenon E was ingested by itself, a total of 42 ± 11 μmol of flavan-3-ol metabolites, equivalent to 9.2 ± 2.4% of intake, was excreted in urine 24 h postingestion. The majority of the excreted compounds were metabolites of (epi)catechin (36 ± 9 μmol, 47 ± 12% of intake), principally as sulfate and methyl sulfate derivatives. (Epi)gallocatechin metabolites were excreted in lower quantities (5.7 ± 1.9 μmol, 26 ± 9% of intake), mainly as the methyl-(epi)gallocatechin sulfates, peaks 6 and 7. No 3-O-galloylated flavan-3-ol metabolites were detected. Statistical analyses show no significant differences in metabolite excretion between ingestion of Polyphenon alone and with either bread, glucose, or cheese (Table 2).

Dose effects. After ingestion of either 200, 500, or 1500 mg (452, 1130, and 3390 μmol) of Polyphenon E, there were dose-dependent statistically significant increases in the overall levels of flavan-3-ol appearing in ileal fluid collected over a 24-h period (Table 3). However, when total flavan-3-ol levels were expressed as a percentage of intake, figures of 43 ± 11%, 50 ± 7%, and 73 ± 12%, were obtained, with only the latter value, for the highest dose, being significantly different (P < 0.001). When the levels of individual flavan-3-ols in the ileal fluid are considered, they followed a broadly similar profile except for a lower percentage recovery with the 1500-mg intake. The exception was (–)-epigallocatechin gallate, the major flavan-3-ol in Polyphenon E, which had an 87% recovery with the 1500-mg intake. These findings provide no evidence to suggest that the 3-O-galloyl flavonols undergo cleavage of the gallate moiety during passage through the small intestine.

The overall quantities of urinary flavan-3-ol metabolites increased significantly with amount of Polyphenon E ingested. When expressed as a percentage of intake, the recoveries were 9.3 ± 2.4%, 9.7 ± 2.7%, and 7.9 ± 0.8%. These values are not significantly different, although once again there are differences in the recoveries of metabolites of (epi)gallocatechin and
(epi)catechin (Table 4). The excretion of (epi)gallocatechin metabolites, 5.7 ± 1.9, 3.0 ± 0.8, and 5.3 ± 1.2 μmol changed little with increasing dose. However, when expressed as a percentage of (epi)gallocatechin intake, the 26 ± 9% obtained with the 200-mg dose fell to 5.5 ± 1.4% and 3.3 ± 0.6% at the 2 highest intakes. In contrast, the level of excretion of (epi)catechin metabolites, 47 ± 12%, 56 ± 14%, and 45 ± 4% of intake, did not decline significantly with increased dose. This indicates that at higher Polyphenon E intakes, the systems responsible for absorption, metabolism, and excretion of (epi)catechins, in contrast to those for (epi)gallocatechins, appear not to be subject to saturation effects.

**Discussion**

Flavan-3-ol levels, in ileal fluid collected 0–24 h after ingestion of 200 mg of Polyphenon E, when expressed as a percentage of intake, show the average recovery of (−)-epicatechin and (−)-epigallocatechin to be 27 ± 2% compared with 59 ± 3% for (−)-epicatechin gallate and (−)-epigallocatechin gallate. In vitro incubations in gastric juice and ileal fluid indicated that the Polyphenon E flavan-3-ols are stable in the stomach and small intestine, so the recoveries in ileal fluid imply that (−)-epicatechin and (−)-epigallocatechin are absorbed into the circulatory system from the small intestine more efficiently than their 3-O-gallloylated analogs. The data obtained with ileal fluid (Table 1) also indicate that in healthy humans with an intact colon, substantial quantities of Polyphenon E flavan-3-ols, principally (−)-epigallocatechin gallate, will pass from the small to the large intestine, where they will become subject to breakdown by colonic bacteria to produce phenolic acids such as di- and trihydroxyphenyl-γ-valerolactones (Fig. 3) (52–54).

Analysis of the urine of ileostomy volunteers showed that the predominant compounds to be excreted were sulfates of (epi)catechin and methyl-(epi)catechin (Table 2). No 3-O-gallloylated flavan-3-ols or their metabolites were detected. The overall urinary excretion of (epi)catechin metabolites was 36 ± 9 μmol, which is 47 ± 12% of the ingested (epi)catechin. Although (epi)gallocatechin metabolites were excreted in smaller quantities, 5.7 ± 1.9 μmol, this nonetheless represented 26 ± 9% of (epi)gallocatechin intake. Thus, both (epi)catechins and (epi)gallocatechins are highly bioavailable.

In keeping with recoveries after ingestion of Polyphenon E (Tables 3 and 4), feeds with (−)-epigallocatechin gallate revealed a 58 ± 9% recovery in ileal fluid and an absence of flavan-3-ol metabolites in urine. This indicates that (−)-epigallocatechin gallate does not undergo loss of the 3-O-galloyl moiety and conversion to (−)-epigallocatechin during passage through the body. In keeping with this finding, no 4-O-methyl-gallic acid

### Table 1: Quantification of flavan-3-ols in ileal fluid collected 0–24 h after ingestion of 200 mg of Polyphenon E either alone, with bread, with glucose, or with cheese

<table>
<thead>
<tr>
<th>Flavan-3-ols</th>
<th>Alone</th>
<th>With bread</th>
<th>With glucose</th>
<th>With cheese</th>
</tr>
</thead>
<tbody>
<tr>
<td>(−)-Epicatechin</td>
<td>15 ± 6 (21)</td>
<td>15 ± 5 (21)</td>
<td>15 ± 8 (21)</td>
<td>25 ± 14 (36)</td>
</tr>
<tr>
<td>(−)-Epigallocatechin gallate</td>
<td>160 ± 42 (62)</td>
<td>147 ± 65 (47)</td>
<td>183 ± 87 (69)</td>
<td>169 ± 50 (54)</td>
</tr>
<tr>
<td>(+)-Gallocatechin gallate</td>
<td>7.8 ± 0.6 (71)</td>
<td>5.8 ± 2.2 (53)</td>
<td>8.1 ± 4.9 (74)</td>
<td>8.1 ± 2.5 (74)</td>
</tr>
<tr>
<td>(−)-Epicatechin gallate</td>
<td>11 ± 4 (34)</td>
<td>8.8 ± 3.0 (28)</td>
<td>8.9 ± 4.2 (28)</td>
<td>8.3 ± 3.3 (26)</td>
</tr>
<tr>
<td>Total flavan-3-ols</td>
<td>194 ± 50 (43 ± 11)</td>
<td>177 ± 73 (39 ± 18)</td>
<td>215 ± 92 (48 ± 20)</td>
<td>210 ± 66 (46 ± 14)</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 5, and percentage of the ingested parent compound consumed.

### Table 2: Quantification of flavan-3-ol metabolites in urine of volunteers after ingestion of 200 mg (452 μmol) of Polyphenon E either alone, with bread, with glucose, or with cheese

<table>
<thead>
<tr>
<th>Flavan-3-ols (HPLC peak)</th>
<th>Alone</th>
<th>With bread</th>
<th>With glucose</th>
<th>With cheese</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Epigallocatechin metabolites)</td>
<td>μmol/24 h (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epigallocatechin glucuronide (1)</td>
<td>0.9 ± 0.4</td>
<td>1.5 ± 0.8</td>
<td>1.0 ± 0.6</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td>4' -Methyl-epigallocatechin glucuronide (2)</td>
<td>0.9 ± 0.2</td>
<td>1.2 ± 0.4</td>
<td>0.7 ± 0.1</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>Methyl-epigallocatechin sulfates (6,7)</td>
<td>3.9 ± 1.4</td>
<td>3.5 ± 1.4</td>
<td>8.7 ± 4.3</td>
<td>4.1 ± 2.5</td>
</tr>
<tr>
<td>Total epigallocatechin metabolites</td>
<td>5.7 ± 1.9 (26 ± 9)</td>
<td>6.2 ± 2.6 (28 ± 12)</td>
<td>10 ± 5 (45 ± 23)</td>
<td>5.3 ± 2.9 (24 ± 13)</td>
</tr>
<tr>
<td>(Epicatechin metabolites)</td>
<td>μmol/24 h (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(−)-Epicatechin-3'-glucuronide (3)</td>
<td>3.4 ± 0.8</td>
<td>3.6 ± 0.8</td>
<td>6.4 ± 2.5</td>
<td>2.6 ± 0.6</td>
</tr>
<tr>
<td>Epicatechin sulfates (4,5,9)</td>
<td>18 ± 5</td>
<td>9.6 ± 4.0</td>
<td>9.5 ± 4.2</td>
<td>9.8 ± 3.7</td>
</tr>
<tr>
<td>Methyl-epicatechin sulfates (8,10–14)</td>
<td>15 ± 3</td>
<td>15 ± 3</td>
<td>18 ± 4</td>
<td>15 ± 4</td>
</tr>
<tr>
<td>Total epicatechin metabolites</td>
<td>26 ± 9 (47 ± 12)</td>
<td>28 ± 8 (36 ± 10)</td>
<td>34 ± 11 (44 ± 14)</td>
<td>27 ± 8 (35 ± 10)</td>
</tr>
<tr>
<td>Total flavan-3-ol excretion</td>
<td>42 ± 11 (9.2 ± 2.4)</td>
<td>34 ± 11 (7.5 ± 2.4)</td>
<td>44 ± 16 (9.7 ± 3.5)</td>
<td>32 ± 11 (7.1 ± 2.4)</td>
</tr>
</tbody>
</table>

1 Values are means excreted over a 24-h period ± SEM, n = 5, and in parentheses as a percentage of the ingested parent compounds. For HPLC peaks, see Figure 2.
was detected in urine after ingestion of either Polyphenon E or (−)-epigallocatechin gallate. Urinary 4-O-methyl-gallic acid, a metabolite of gallic acid, is a marker of tea consumption (55), but unlike green tea and black tea (8), Polyphenon E does not contain gallic acid.

Although (−)-epigallocatechin gallate is not excreted in urine, several earlier studies have detected it in the bloodstream, principally in an unmetabolized form, with a peak plasma concentration \( C_{\text{max}} \) in the high nanomolar to low micromolar range after ingestion of Polyphenon E (39,41,43). This is very unusual from 2 perspectives. First, following absorption, flavonoids typically appear in the bloodstream as sulfate and glucuronide conjugates, and once absorbed, they are invariably excreted, sometimes after being subjected to further metabolism (56).

The absence of (−)-epigallocatechin gallate in human urine, despite reaching plasma \( C_{\text{max}} \) 1.44 h after ingestion of 200 mg of Polyphenon E, and having an elimination half-life of 1.9 h (41), is of interest. It is possible that the kidneys are unable to remove (−)-epigallocatechin gallate from the bloodstream, but if this is the case, there must be other mechanisms that result in the rapid decline after \( C_{\text{max}} \) has been reached. Studies in which tritium-labeled (−)-epigallocatechin gallate was injected intravenously into bile-duct-cannulated rats have shown that 57% of the injected radioactivity was excreted in bile within 4 h, and 77% within 48 h, compared with 2% in urine over the 48-h period (57). This involved extensive metabolism of the (−)-epigallocatechin gallate, as it was present in bile as sulfated and/or glucuronide conjugates of 4′-methyl- and 4′,4′-dimethyl-(−)-epigallocatechin gallate (Fig. 3). In an earlier study in which a relatively high dose of (−)-epigallocatechin gallate (≈400 mg/kg body weight, equivalent to a 70-kg, 170-cm-tall human ingesting 16.6 g of (−)-epigallocatechin gallate) was fed to rats by gavage, only ∼3% of intake was detected in bile, principally as metabolites (58). To what extent enterohepatic recirculation of (−)-epigallocatechin gallate metabolites occurs in humans has not been established. If it were a significant event in the present study, then (−)-epigallocatechin gallate metabolites should have been detected in substantial amounts in ileal fluid. This did not occur; although flavan-3-ol metabolites, principally sulfated derivatives, were detected by MS, they were in amounts that were below the limits of quantification by PDA detection and were very minor components compared with the unmetabolized flavan-3-ols. This is a topic that requires further investigation.

The feeding studies also revealed that not bread, glucose, or cheese affected the absorption of Polyphenon E flavan-3-ols in the small intestine or the urinary excretion of flavan-3-ol metabolites (Tables 1 and 2). It does not necessary follow, however, that the same would occur when flavan-3-ols are ingested with more complex food matrices.

The feeding studies in which volunteers with an ileostomy ingested either 200, 500, or 1500 mg of Polyphenon E revealed some interesting findings that complement earlier studies in which it was shown that increasing the dose of green tea or (−)-epigallocatechin gallate resulted in increased plasma \( C_{\text{max}} \) values (41,43,59–61). The data presented in Table 4 show that with increasing Polyphenon E intake, total flavan-3-ol metabolite excretion in urine increases in absolute amounts but remains relatively unchanged at ∼10% when expressed as a percentage of intake. However, the (epi)gallocatechin metabolites, which

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**Table 3** Quantification of flavan-3-ols in ileal fluid collected 0–24 h after ingestion of increasing doses of Polyphenon E

<table>
<thead>
<tr>
<th>Flavan-3-ols</th>
<th>200 mg</th>
<th>500 mg</th>
<th>1500 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>(−)-Epicatechin</td>
<td>15 ± 6a (21)</td>
<td>66 ± 3b (37)</td>
<td>293 ± 26b (55)</td>
</tr>
<tr>
<td>(−)-Epigallocatechin</td>
<td>160 ± 42b (52)</td>
<td>456 ± 17b (59)</td>
<td>2027 ± 10b (87)</td>
</tr>
<tr>
<td>(−)-Gallocatechin</td>
<td>7.8 ± 0.6b (71)</td>
<td>7.2 ± 0.2a (26)</td>
<td>30 ± 4b (36)</td>
</tr>
<tr>
<td>(−)-Epicatechin</td>
<td>11 ± 4a (34)</td>
<td>31 ± 1b (39)</td>
<td>118 ± 10b (49)</td>
</tr>
<tr>
<td>Total flavan-3-ols</td>
<td>194 ± 15b (43 ± 11b)</td>
<td>560 ± 83b (50 ± 7b)</td>
<td>2488 ± 41b (73 ± 12b)</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, \( n = 6 \), and percentage of the ingested parent compounds. Values in row with different superscripts are significantly different \((P < 0.05)\). For HPLC peak numbers see Figure 2.

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**Table 4** Quantification of flavan-3-ol metabolites in urine of volunteers with an ileostomy collected 0–24 h after the ingestion of 200 mg, 500 mg, and 1500 mg of Polyphenon E

<table>
<thead>
<tr>
<th>Flavan-3-ol (HPLC peak)</th>
<th>200 mg</th>
<th>500 mg</th>
<th>1500 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Epi)gallocatechin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Epi)gallocatechin-glucuronide (1)</td>
<td>0.9 ± 0.4</td>
<td>0.4 ± 0.2</td>
<td>0.6 ± 0.3</td>
</tr>
<tr>
<td>4′-Methyl-(Epi)gallocatechin-glucuronide (2)</td>
<td>0.8 ± 0.2</td>
<td>0.6 ± 0.1</td>
<td>1.1 ± 0.3</td>
</tr>
<tr>
<td>Methyl-(Epi)gallocatechin-sulfates (6,7)</td>
<td>3.9 ± 1.4</td>
<td>2.0 ± 0.5</td>
<td>3.6 ± 0.6</td>
</tr>
<tr>
<td>Total (Epi)gallocatechin metabolites</td>
<td>5.7 ± 1.9 (26 ± 9)</td>
<td>3.0 ± 0.8 (5.5 ± 1.4)</td>
<td>5.3 ± 1.2 (3.3 ± 0.6)</td>
</tr>
<tr>
<td>(Epi)catechin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Epi)catechin-glucuronide (3)</td>
<td>3.4 ± 0.8a</td>
<td>14 ± 4a</td>
<td>38 ± 6a</td>
</tr>
<tr>
<td>(Epi)catechin-sulfates (4,5,9)</td>
<td>18 ± 5a</td>
<td>40 ± 10a</td>
<td>104 ± 8a</td>
</tr>
<tr>
<td>Methyl-(Epi)catechin-sulfates (8,10–14)</td>
<td>15 ± 3a</td>
<td>53 ± 12b</td>
<td>120 ± 10b</td>
</tr>
<tr>
<td>Total (Epi)catechin metabolites</td>
<td>36 ± 6a (47 ± 12)</td>
<td>107 ± 26a (56 ± 14)</td>
<td>262 ± 26a (45 ± 4)</td>
</tr>
<tr>
<td>Total flavan-3-ol excretion</td>
<td>42 ± 11a (9.3 ± 2.4)</td>
<td>110 ± 26a (3.7 ± 2.7)</td>
<td>267 ± 26a (7.9 ± 0.8)</td>
</tr>
</tbody>
</table>

1 Values are means excreted over a 24-h period ± SEM, \( n = 5 \), and percentage of the ingested parent compounds. Values in row with different superscripts are significantly different \((P < 0.05)\). For HPLC peak numbers see Figure 2.
were the minor components, behaved differently from the
(–)-epicatechin metabolites. With the increasing dose, the
(7)-catechin metabolites were excreted in greater quantities,
but when expressed as a percentage of (–)-epicatechin and
(+)-catechin intake, figures of 47 ± 12%, 56 ± 14%, and 45 ± 4% were obtained, which are not significantly different. At the
same time, (7)-gallocatechin metabolites are not excreted in
higher quantities with the increasing dose with the excretion of
same time, (epi)gallocatechin metabolites are not excreted in
4% were obtained, which are not significantly different. At the
other articles in this supplement include references (63–72).

Finally, it should be noted that although there were person-
to-person variations in the flavan-3-ol content of ileal fluid and
the quantities of urinary metabolites that typify such feeding
studies, this was not related to either the gender of the subjects or
to whether or not they drank tea on a regular basis.

Other articles in this supplement include references (63–72).

**Literature Cited**


effects of drinking green tea on cancer and cardiovascular disease:

3. Arts IC, Hollman PC, Feskens Ej, Bueno de Mesquita HB, Kromhout D. Catechin intake might explain the inverse relation between

4. Arts IC, Jacobs DR Jr, Harnack LJ, Gross M, Folsom AR. Dietary
effectiveness of drinking green tea on cancer prevention: epidemiological evidence for multiple targeting prevention. Int J
Obes. 2006;30:561–8.


Mediates beneficial effects of flavanol-rich cocoa on vascular function in

8. Vankemmelbeke MN, Jones GC, Fowles C, Ilic MZ, Handley CJ,
Day AJ, Knight CG, Mort JS, Buttle DJ. Selective inhibition of
ADAMTS-1, -4 and -5 by catechin gallate esters. Eur J Biochem. 2003;
270:2394–403.

of prostate cancer cell growth and apoptosis induced by oral administration of green tea catechins in volunteers with high-grade prostate

10. Wolfram S, Raederstorff D, Preller M, Wang Y, Teixeira SR, Rieger C,

effects of drinking green tea on cancer and cardiovascular disease:


13. Arts IC, Hollman PC, Feskens Ej, Bueno de Mesquita HB, Kromhout D. Catechin intake might explain the inverse relation between

effectiveness of drinking green tea on cancer prevention: epidemiological evidence for multiple targeting prevention. Int J
Obes. 2006;30:561–8.


Mediates beneficial effects of flavanol-rich cocoa on vascular function in

18. Vankemmelbeke MN, Jones GC, Fowles C, Ilic MZ, Handley CJ,
Day AJ, Knight CG, Mort JS, Buttle DJ. Selective inhibition of
ADAMTS-1, -4 and -5 by catechin gallate esters. Eur J Biochem. 2003;
270:2394–403.

of prostate cancer cell growth and apoptosis induced by oral administration of green tea catechins in volunteers with high-grade prostate

20. Wolfram S, Raederstorff D, Preller M, Wang Y, Teixeira SR, Rieger C,


22. Diepvens K, Westerterp KR, Westerterp-Plantenga MS. Obesity and
thermogenesis related to the consumption of caffeine, epinephrine, cap-
casin, and green tea. Am J Physiol Regul Integr Comp Physiol. 2007;
292:R77–85.

23. Murase T, Hara Y. Antimutagenic and anticarcinogenic activity of tea


Mediates beneficial effects of flavanol-rich cocoa on vascular function in

27. Vankemmelbeke MN, Jones GC, Fowles C, Ilic MZ, Handley CJ,
Day AJ, Knight CG, Mort JS, Buttle DJ. Selective inhibition of
ADAMTS-1, -4 and -5 by catechin gallate esters. Eur J Biochem. 2003;
270:2394–403.


29. Kuroda Y, Hara Y. Antimutagenic and anticarcinogenic activity of tea


32. Sartippour MR, Pieters R, Marquez-Garban DC, Chen HW, Heber D,
Hemmings SM, Sartippour G, Zhang L, Lu M, et al. The combination of green tea and tamoxifen is effective against breast cancer. Carcinogen-
esis. 2006;27:2424–33.


34. Friedman M. Overview of antibacterial, anti-oxidant, antiviral, and antifungal activities of tea flavonoids and teas. Mol Nutr Food Res.

Absorption of flavan-3-ols


54. Hong YL, Choo HS, Harris RR. Green tea consumption is associated with decreased DNA damage among GSTM1 positive smokers regardless of their hOGG1 genotype. J Nutr. 2008;138:15438–78.


58. Hakim IA, Choo HHS, Harris RR. Green tea consumption is associated with decreased DNA damage among GSTM1 positive smokers regardless of their hOGG1 genotype. J Nutr. 2008;138:15675–715.

