Human studies on the absorption, distribution, metabolism, and excretion of tea polyphenols1–3

Michael N Clifford, Justin JJ van der Hooft, and Alan Crozier

ABSTRACT
Recent research on the bioavailability of flavan-3-ols after ingestion of green tea by humans is reviewed. Glucuronide, sulfate, and methyl metabolites of (epi)catechin and (epi)gallocatechin glucuronide reach peak nanomolar per liter plasma concentrations 1.6–2.3 h after intake, indicating absorption in the small intestine. The concentrations then decline, and only trace amounts remain 8 h after ingestion. Urinary excretion of metabolites over a 24-h period after green tea consumption corresponded to 28.5% of the ingested (epi) catechin and 11.4% of (epi)gallocatechin, suggesting higher absorption than that of most other flavonoids. The fate of (–)-epicatechin-3-O-gallate, the main flavan-3-ol in green tea, is unclear because it appears unmetabolized in low concentrations in plasma but is not excreted in urine. Possible enterohpetic recirculation of flavan-3-ols is discussed along with the impact of dose and other food components on flavan-3-ol bioavailability. Approximately two-thirds of the ingested flavan-3-ols pass from the small to the large intestine where the action of the microbiota results in their conversion to C-6–C-5 phenylvalerolactones and phenylvaleric acids, which undergo side-chain shortening to produce C-6–C-1 phenolic and aromatic acids that enter the bloodstream and are excreted in urine in amounts equivalent to 36% of flavan-3-ol intake. Some of these colon-derived catabolates may have a role in vivo in the potential protective effects of tea consumption. Although black tea, which contains theaflavins and thearubigins, is widely consumed in the Western world, there is surprisingly little research on the absorption and metabolism of these compounds after ingestion and their potential impact on health. Am J Clin Nutr 2013;98(suppl):1619S–30S.

PHYTOCHEMICAL CONSTITUENTS OF Camellia TEAS
Camellia-based teas, containing flavan-3-ols and derived compounds, are one of the most widely consumed beverages in the world (1). Their popularity is in part due to the fact that they also contain a substantial amount of the purine alkaloid, caffeine, and trace amounts of theobromine (2). In 2010, ~3.2 million metric tons of dried tea were produced, 61% of which was black tea and 31% green tea, with the balance made up of minor teas such as oolong and pu-erh. In all cases, the raw material is young leaves, the tea flush.

When harvested, the fresh tea leaf is unusually rich in (poly)phenols (~30% dry weight) with flavan-3-ols being the principal form. Usually (–)-epigallocatechin-3-O-gallate (EGCG)4 dominates, occasionally taking second place to (–)-epicatechin-3-O-gallate (ECG), together with smaller but still substantial amounts of (–)-epigallocatechin, (+)-gallocatechin, (–)-epicatechin, and (–)-epiafzelechin (2). There are at least 15 flavonol glycosides comprising mono-, di-, tri-, and tetra-O-glycosides based on kaempferol, quercetin, and myricetin and various permutations of glucose, galactose, rhamnose, arabinose and rutinose (3–6), flavone C-glycosides (7–10), several caffeoyl- and p-coumaroylquinic acids (chlorogenic acids) and galloyl-quinic acids (theogallins), and at least 27 proanthocyanidins including some containing epiafzelchin units (11, 12). In addition, some forms have a significant content of hydrolysable tannins, such as strictinin (13), whereas others contain chalconeflavon dimers known as assamaicins (14). Relevant structures are shown in Figure 1, and further information on the diversity of phenolic compounds in green tea can be found in earlier reviews (15, 16).

The production of black tea uses either the so-called orthodox method or the more recently introduced, cut-tear-curl process (17). In both processes, the objective is to achieve efficient disruption of cellular compartmentalization, thus bringing phenolic compounds into contact with polyphenol oxidase. The primary substrates for polyphenol oxidase are the flavan-3-ols monomers, which decline during fermentation, whereas there is a concomitant accumulation of theaflavins (Figure 2) and thearubigins (18). The nature of these transformations has been the subject of a detailed reviewed by Drynan

1From the Department of Nutritional Sciences, University of Surrey, Guildford, Surrey, United Kingdom (MNC); and the School of Medicine, College of Medical, Veterinary, and Life Sciences, University of Glasgow, Glasgow, United Kingdom (JJJvdH and AC).
2Presented at the conference “Fifth International Scientific Symposium on Tea and Human Health,” held at the US Department of Agriculture, Washington, DC, 19 September 2012. The conference was organized by Jeffrey Blumberg, Tufts University, Boston, MA, and a Steering Committee including representatives from each of the symposium cosponsors: the American Cancer Society, the American College of Nutrition, the American Institute for Cancer Research, the American Medical Women’s Association, the American Society for Nutrition, and the Linus Pauling Institute. The symposium was underwritten by the Tea Council of the USA. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the Tea Council of the USA or the cosponsoring organizations.
3Address correspondence to A Crozier, Plant Products and Human Nutrition Group, North Laboratory, Joseph Black Building, School of Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow G12 9QQ, United Kingdom. E-mail: alan.crozier@glasgow.ac.uk.
4Abbreviations used: \( C_{\text{max}} \), peak plasma concentration; ECG, (–)-epicatechin-3-O-gallate; EGCG, (–)-epigallocatechin-3-O-gallate; GIT, gastrointestinal tract; \( T_{\text{max}} \), time to reach peak plasma concentration.

First published online October 30, 2013; doi: 10.3945/ajcn.113.058958.
et al (19). Recent investigations of 15 commercial black teas, selected to represent the major types of black teas produced worldwide, detected, on average, 5000 caffeine-precipitable thearubigins in the mass range between $m/z$ 1000 and 2100 and a maximum of 9428 related compounds in the mass range of $m/z$ 300–1000. Those so far characterized are polyhydroxy derivatives of theaflavins, theacitrins, theanaphthoquinones, and theasinensins and their gallates and associated quinones.

**FIGURE 1.** Some of the phenolic compounds, including proanthocyanidins and hydrolysable tannins, present in green tea (*Camellia sinensis*) infusions. The predominant purine alkaloid is caffeine with trace amounts of theobromine.
(20–22). There are undoubtedly unresolved isomers present, further raising the total. A typical cup of black tea contains ~100 mg thearubigins (23, 24), suggesting that very few, if any, individual thearubigins exceed 100 mg/cup.

Beverages from green and black teas also have significant contents of flavonol glycosides and smaller amounts of chlorogenic acids, flavone-C-glycosides, and 5-O-galloylquinic acid (Figure 1), which are less affected by processing than the flavan-3-ols (18) but may vary more markedly with the origin of the fresh leaf (25–28).

During domestic brewing and production of instant teas, changes in the phytochemical constituents can occur, with flavan-3-ols, for instance, undergoing epimerization, producing (+)-epicatechin and (−)-catechin, (−)-gallocatechin, etc (Figure 3) (29, 30). Furthermore, the gut microbiota can transform (+)-catechin to (+)-epicatechin (31). The relative content of flavan-3-ol enantiomers consumed by study volunteers is generally not known, and variations therein might explain some of the reported variations in pharmacokinetic data.

ANALYSIS OF CONJUGATED FLAVAN-3-OL METABOLITES

Each flavan-3-ol and each associated phase II conjugate can, in theory, occur as 4 enantiomers, with the (+) and (−) forms resolvable only by chiral chromatography. Full chiral analysis of the flavan-3-ols consumed by volunteers is difficult and has been rarely performed (31–33). The corresponding analysis of the conjugated metabolites in urine or plasma is even more difficult, and so far as we are aware there are no reports of this having been achieved.

Because conjugated flavan-3-ol metabolites are rarely available commercially, most studies of flavan-3-ol metabolism have analyzed plasma and urine samples after treatment with mollusc glucuronidase/sulfatase preparations, providing data only for the aglycones. Some sulfate conjugates are resistant to enzyme hydrolysis (34, 35), and as a consequence this methodology generates inaccurate data. Reversed-phase HPLC–mass spectrometry without enzyme treatment can define the phase II conjugation but cannot determine the chirality of the aglycone, and this complication has sometimes been overlooked. In this review, metabolites will be described only as (epi)catechin or (epi)gallocatechin, or associated conjugates, unless they have been fully characterized.

CHIRALITY AND METABOLISM

Flavan-3-ols are nonplanar molecules, and thus in any biological phenomenon to which the “lock and key” concept might apply, it is plausible that the enantiomers will differ in potency. Donovan et al (32) reported that (−)-catechin is absorbed less readily than (+)-catechin. Ottaviani et al (33) investigated the

FIGURE 2. Theaflavins that occur in black tea.

FIGURE 3. During brewing and production of instant tea beverages, flavan-3-ols such as (+)-catechin, (−)-epicatechin, (+)-gallocatechin, and (−)-epigallocatechin may epimerize.
metabolism of flavan-3-ols in an acute study in which adult human males consumed equal quantities of (–)-epicatechin, (–)-catechin, (+)-epicatechin, and (+)-catechin (see Figure 3), albeit in a cocoa drink rather than in green tea. On the basis of plasma concentrations and urinary excretion of the aglycones after enzymic deconjugation, the bioavailability of the stereoisomers was ranked as (–)-epicatechin > (+)-epicatechin = (+)-catechin > (–)-catechin. The catechin and epicatechin epimers differed in their relative susceptibility to 3’- and 4’-O-methylation. The concentrations of nonmethylated conjugates of (–)- and (+)-epicatechin in plasma and urine also differed, indicating that flavan-3-ol stereochemistry affects other phase II pathways, but because the samples were analyzed after glucuronidase/sulfatase treatment the precise variations are not known. It is important that future feeding studies involve chiral analyses of the flavan-3-ols, as well as the conjugates appearing in plasma, urine, and, where appropriate, ileal fluid, preferably without any glucuronidase/sulfatase treatment.

GREEN TEA FEEDING STUDIES IN HEALTHY SUBJECTS

An acute supplement of a bottled green tea (500 mL) was given to 10 volunteers, and plasma and urine were collected over a 24-h period (36). The tea contained 648 μmol of flavan-3-ols, principally 257 μmol of (–)-epigallocatechin, 230 μmol of EGCg, 58 μmol of (–)-epicatechin, 49 μmol of ECG, and 36 μmol of (+)-gallocatechin (33). ECG and EGCg were identified unchanged in plasma along with glucuronide, methyl-glucuronide, and methyl-sulfate conjugates of (epi)gallocatechin and glucuronide, sulfate, and methyl-sulfate conjugates of (epi)catechin (36). Methylation would therefore appear to occur only after prior glucuronidation or sulfation. The plasma profiles of these flavan-3-ols and their conjugated metabolites are shown in Figure 4. Peak plasma concentrations ($C_{\text{max}}$) ranged from 25 to 126 nmol/L and the time to reach $C_{\text{max}}$ ($T_{\text{max}}$) varied from 1.6 to 2.3 h (Table 1), indicating absorption in the small intestine. Because of a short apparent half-life ($T_{1/2}$), which is an overestimate of the true absorption time, the plasma concentrations of flavan-3-ols and their conjugates were monitored for 8 h after tea consumption. The main components were epicatechin-3’-O-glucuronide, epicatechin-3’-O-sulfate, and 3’-O-methyl-epicatechin-5/7-O-sulfates (see Figure 5). It is likely that these are also the principal epicatechin conjugates to appear in plasma after green tea consumption. Reproduced from reference 36 with permission.

ECG, (–)-epicatechin-3-O-gallate; EC-3’-GlcUA, (–)-epicatechin-3’-O-glucuronide; EC-S, (epi)catechin-O-sulfates; EGCg, (–)-epigallocatechin-3-O-gallate; EGCG-K, (epi)gallocatechin-O-gluconide; Me-EC-S, 3’ and 4’-O-methyl-(epi)catechin-O-sulfates; 4’-Me-EGC-GlcUA, 4’-O-methyl-(epi)gallocatechin-O-glucuronide; 4’-Me-ECG-S, 4’-O-methyl-(epi)gallocatechin-O-sulfates.

**FIGURE 4.** Mean ($\pm$SE) concentrations of EGC-GlcUA, 4’-Me-EGC-GlcUA, EC-S, 4’-Me-ECG-S, EC-3’-GlcUA, Me-EC-S, EGCg, and ECg in the plasma of human subjects 0–8 h after the ingestion of 500 mL green tea; $n = 10$. Note that no flavan-3-ols or their conjugates were detected in plasma collected 24 h after ingestion of the green tea. In recent human bioavailability studies with chocolate and cocoa, a number of epicatechin conjugates were identified in plasma. The main components were epicatechin-3’-O-glucuronide, epicatechin-3’-O-sulfate, and 3’-O-methyl-epicatechin-5/7-O-sulfates (see Figure 5). It is likely that these are also the principal epicatechin conjugates to appear in plasma after green tea consumption. Reproduced from reference 36 with permission.
half-life5, the plasma concentration rapidly declines and marked increases in plasma accumulation are unlikely even with frequent consumption.

Few dietary flavonoids and related phenolic compounds appear unchanged in plasma. The exceptional behavior of ECG and EGCG in this regard might be a consequence of the galloyl moiety inhibiting phase II metabolism or enhancing absorption. Gallic acid per se is readily absorbed with a reported urinary excretion of 37% of intake (37, 38).

Urine excreted 0–24 h after green tea consumption contained a profile of flavan-3-ol conjugates similar to plasma except that 3 minor (epi)gallocatechin-O-sulfates were present (36). The aglycones, ECG and EGCG, were absent (Table 2). Even if phase II conjugates of these compounds were present below the limit of detection (~4 nmol/L), these observations indicate that the intact flavan-3-ols do not undergo extensive metabolism. In total, 52.4 μmol of flavan-3-ol metabolites were excreted, equivalent to 8.1% of the quantified green tea flavan-3-ols ingested (36). (Epi)afzelchin and its associated metabolites have not been reported in plasma or urine.

The recovery in 24-h urine of (epi)catechin metabolites accounted for 28.5% of intake, whereas the recovery of (epi)gallocatechin metabolites accounted for only 11.4% of the ingested (–)epigallocatechin and (+)-gallocatechin (Table 2). These recoveries are consistent with earlier studies with green tea and cocoa products (39, 40). In the absence of data for biliary excretion, and hence judged solely by 24-h urinary excretion of aglycones and associated phase II metabolites, these data confirm that flavan-3-ols are better absorbed than other flavonoids, with the possible exception of isoflavones (39, 41).

The inability to detect EGCG in urine despite its presence in plasma has been observed by several investigators (42–44) and is difficult to explain. It is possible that the kidneys are unable to clear EGCG from the bloodstream, but if this is the case, there must be other mechanisms that result in its rapid decline after reaching $C_{\text{max}}$. Auger et al (40) provided pure EGCG to ileostomists and failed to find (epi)gallocatechin or its metabolites in urine, establishing that degalloylation did not occur endogenously.

ECG has been detected immunocytochemically in human atherosclerotic lesions but was apparently absent from healthy human vascular tissue (45). The ability of macrophage-derived foam cells to take up the comparatively hydrophobic ECG [partition coefficient between n-octanol and water to simulate lipid membranes, etc, and aqueous plasma ($D = 62 \pm 7$)] (46) was confirmed in vitro (45), and the somewhat unexpected lower concentration in healthy tissue can be rationalized by assuming that healthy tissue is less hydrophobic and thus less able to take up the gallated flavan-3-ol. However, this rationalization cannot explain the presence of the hydrophilic quercetin-3-O-glucuronide ($D = 0.008 \pm 0.002$) (47) in lesions, whereas it is undetectable in healthy tissue (48). In this case, it is plausible that quercetin glucuronides in plasma have been deconjugated by β-glucuronidase released at the site of inflammation (49, 50), the hydrophilic quercetin absorbed ($D = 65.7 \pm 3.1$) (51) and re-metabolized after entry to the endothelial tissue. Alternatively,

### TABLE 1

<table>
<thead>
<tr>
<th>Flavan-3-ol conjugates</th>
<th>$C_{\text{max}}$ (mmol/L)</th>
<th>$T_{\text{max}}$ (h)</th>
<th>$T_{1/2}$ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Epi)gallocatechin-O-glucuronide</td>
<td>126 ± 19</td>
<td>2.2 ± 0.2</td>
<td>1.6</td>
</tr>
<tr>
<td>4′-O-Methyl-(epi)gallocatechin-O-glucuronide</td>
<td>46 ± 6.3</td>
<td>2.3 ± 0.3</td>
<td>3.1</td>
</tr>
<tr>
<td>(Epi)catechin-O-glucuronide</td>
<td>29 ± 4.7</td>
<td>1.7 ± 0.2</td>
<td>1.6</td>
</tr>
<tr>
<td>(Epi)catechin-O-sulfates</td>
<td>89 ± 15</td>
<td>1.6 ± 0.2</td>
<td>1.9</td>
</tr>
<tr>
<td>4′-O-Methyl-(epi)gallocatechin-O-sulfates</td>
<td>79 ± 12</td>
<td>2.2 ± 0.2</td>
<td>2.2</td>
</tr>
<tr>
<td>O-Methyl-(epi)catechin-O-sulfates</td>
<td>90 ± 15</td>
<td>1.7 ± 0.2</td>
<td>1.5</td>
</tr>
<tr>
<td>(–)Epigallocatechin-3-O-gallate</td>
<td>55 ± 12</td>
<td>1.9 ± 0.1</td>
<td>1.0</td>
</tr>
<tr>
<td>(–)Epicatechin-3-O-gallate</td>
<td>25 ± 3.0</td>
<td>1.6 ± 0.2</td>
<td>1.5</td>
</tr>
</tbody>
</table>

1Values are means ± SEs unless otherwise indicated; $n = 10$. Adapted from reference 36 with permission. $C_{\text{max}}$, peak plasma concentration; $T_{\text{max}}$, time to reach peak plasma concentration.

2$T_{1/2}$ is the apparent half-life because the flavan-3-ol dose was given orally rather than intravenously.

---

5A true $T_{1/2}$ value can be determined only by intravenous dosing of a metabolite. Assessments based on elimination after oral dosing overestimate $T_{1/2}$ because the metabolite is still entering the circulatory system when the elimination is being estimated.
TABLE 2

Quantification of the major groups of flavan-3-ol conjugates excreted in urine 0–24 h after the ingestion of 500 mL green tea by 10 volunteers

<table>
<thead>
<tr>
<th>Flavan-3-ol conjugates (no. of isomers)</th>
<th>Amount excreted after 0–24 h</th>
<th>μmol</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Epi)gallocatechin-O-glucuronide (1)</td>
<td>6.5 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>4′-O-Methyl-(epi)gallocatechin-O-glucuronide (1)</td>
<td>4.4 ± 1.5</td>
<td></td>
</tr>
<tr>
<td>4′-O-Methyl-(epi)gallocatechin-O-sulfates (2)</td>
<td>19.8 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>(Epi)gallocatechin-O-sulfates (3)</td>
<td>2.6 ± 3.0</td>
<td></td>
</tr>
<tr>
<td>Total (epi)gallocatechin conjugates</td>
<td>33.3 (11.4)</td>
<td></td>
</tr>
<tr>
<td>(Epi)catechin-O-glucuronide (1)</td>
<td>1.5 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>(Epi)catechin-O-sulfates (2)</td>
<td>6.7 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>O-Methyl-(epi)catechin-O-sulfates (5)</td>
<td>10.9 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>Total (epi)catechin conjugates</td>
<td>19.1 (28.5)</td>
<td></td>
</tr>
<tr>
<td>Total flavan-3-ol conjugates</td>
<td>52.4 (8.1)</td>
<td></td>
</tr>
</tbody>
</table>

1 Values are means ± SEs; n = 10. Adapted from reference 36 with permission.

2 Value in parentheses indicates amount excreted as a percentage of the amount ingested.

3 Total flavan-3-ol conjugates do recycle (56), but there are no human data for the extent to which flavan-3-ols are recycled in vivo or in vitro (53).

Studies in rats have led to speculation that EGCG may be excreted directly from the enterocyte rather than as a conjugate (52). It may occur at least for EGCG.

The varying plasma concentrations of (epi)catechin metabolites in Table 1 results in an approximate, hypothetical, overall maximum plasma concentration of 538 nmol/L being attained after the ingestion of green tea (36), the approximation arising because of variation in the T\textsubscript{max} of individual conjugates. This is lower than the analogous 1313 nmol/L C\textsubscript{max} of quercetin conjugates obtained after the ingestion of onions containing 250 μmol of quercetin-4′-O-glucoside and quercetin-3,4′-diglucoside (58) and is also less than the analogous 922 nmol/L C\textsubscript{max} of hesperetin-O-glucuronides that appear in plasma after the ingestion of orange juice containing 168 μmol of hesperetin-7-O-rutinoside (59).

**FOOD MATRIX EFFECTS**

The varying plasma T\textsubscript{max} values for (epi)catechin metabolites obtained in several studies are summarized in Table 3. In the study by Stalmach et al (36) in which volunteers ingested 500 mL of a bottled green tea containing vitamin C and 100 kcal, T\textsubscript{max} ranged from 1.7 to 2.2 h. When an infusion prepared from green tea leaves was consumed by ileostomists, the T\textsubscript{max} values were shorter, ranging from 0.8 to 1.3 h. Initially, this was thought to reflect more rapid transport through the small intestine of the ileostomists because of the absence of an ileal brake (57). However, this is clearly not the only factor affecting absorption in the small intestine because short T\textsubscript{max} times, 0.6–1.1 h, were also obtained when a drink containing a diversity of flavonoids was ingested by healthy subjects with intact colons (60). The rapid absorption of the flavan-3-ols in this drink is of interest because it occurred in the presence of substantial amounts of other (poly)phenolic compounds, including gallic acid, 5-O-cafeoylquinic acid, anthocyanins, flavonanes, and dihydrochalcones. This suggests that there is no major competition for transport of these components across the mucosa of the gastrointestinal tract (GIT) into the circulatory system. The varying (epi)catechin metabolite T\textsubscript{max} values observed in these studies indicate that other components in the drinks can affect flavan-3-ol absorption.

**TABLE 3**

Details of human feeding studies and times of peak plasma concentrations (T\textsubscript{max}) of (epi)catechin conjugates after the consumption of green teas, a polyphenol-rich juice, cocoas, and chocolate

<table>
<thead>
<tr>
<th>Beverage or food type (reference)</th>
<th>Volunteers, with or without colon</th>
<th>Amount consumed (mL)</th>
<th>Green tea (36)</th>
<th>Green tea (57)</th>
<th>Polyphenol-rich juice (60)</th>
<th>Cocoa (62)</th>
<th>Cocoa (63)</th>
<th>Chocolate (35)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount consumed (mL)</td>
<td>500</td>
<td>300</td>
<td>350</td>
<td>250</td>
<td>375</td>
<td>100(^2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy intake (kcal)</td>
<td>144</td>
<td>0</td>
<td>51</td>
<td>NS</td>
<td>224</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Vitamin C content (mg)</td>
<td>100</td>
<td>—</td>
<td>168</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Flavan-3-ol intake (μmol)</td>
<td>648</td>
<td>634</td>
<td>448</td>
<td>45</td>
<td>541</td>
<td>362</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma conjugate T\textsubscript{max} (h)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Epi)catechin-O-glucuronide</td>
<td>1.7 ± 0.2(^3)</td>
<td>0.8 ± 0.1</td>
<td>1.1 ± 0.2</td>
<td>ND</td>
<td>1.9 ± 0.1(^3)</td>
<td>3.2 ± 0.2(^3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Epi)catechin-O-sulfate(s)</td>
<td>1.6 ± 0.2</td>
<td>1.3 ± 0.3</td>
<td>0.9 ± 0.1</td>
<td>1.4 ± 0.2</td>
<td>2.0 ± 0.0(^3)</td>
<td>3.2 ± 0.2(^3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O-Methyl-(epi)catechin-O-sulfate(s)</td>
<td>1.7 ± 0.2</td>
<td>1.3 ± 0.3</td>
<td>1.0 ± 0.0</td>
<td>1.0 ± 0.2</td>
<td>2.0 ± 0.0(^3)</td>
<td>3.8 ± 0.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 ND, not detected; NS, not stated; —, not analyzed, probably absent.

2 Value for chocolate is expressed in grams.

3 Mean ± SE (all such values).

Clifford ET AL.
delay gastric emptying (61) and along with increases in the dose of flavan-3-ols may contribute to increases in (epi)catechin metabolite $T_{\text{max}}$ values after consumption of cocoa and chocolate. Two studies with cocoa drinks, in which there was a 10-fold difference in epicatechin/catechin intake, showed peak plasma concentration times ranging from 1.0 to 2.0 h (62, 63) (Table 3). When the flavan-3-ols were consumed as a 100-g chocolate supplement, $T_{\text{max}}$ values were further delayed to between 3.2 and 3.8 h. Although no data were presented on the topic, this could be a consequence of the high fat content of the chocolate (35) compared with the 19-g intake with one of the cocoa drinks (62, 63) (Table 3). Phillips et al (64) reported that sugar delays gastric emptying and that the different caloric intakes (Table 3) may be a further factor contributing to the delay.

No matrix effects were observed when polyphenon E, a flavan-3-ol–rich green tea extract, was provided to ileostomists either alone or with bread, cheese, or glucose (40). The influence of milk added to black tea on polyphenol absorption has been investigated by several groups, with some investigators reporting no major effects (65–67), whereas others observed that milk seeming reduced absorption (68, 69). The limited data that are available indicate that food matrix effects could be considerable, but more research is required to define its magnitude and the factors responsible.

FLAVAN-3-OL DOSE EFFECTS

Auger et al (40) provided ileostomists increasing doses of polyphenon E, and urinary excretion of flavan-3-ol metabolites was used as a measure of absorption in the small intestine. The data obtained with (epi)gallocatechin and (epi)catechin metabolites are summarized in Table 4. At a dose of 22 μmol, the 0- to 24-h excretion of (epi)gallocatechin metabolites was 5.7 μmol, and this figure did not increase significantly with intakes of 55 and 165 μmol. There appears to be a limit either of the extent to which (epi)gallocatechins can enter the blood or the degree to which the associated metabolites can be cleared by the kidneys, possibly suggesting significant biliary excretion. After the ingestion of 77 μmol (epi)catechins, 36 μmol were excreted; and with doses of 192 and 577 μmol, urinary excretion increased significantly to 107 and 262 μmol. Thus, even at the highest dose, (epi)catechins, unlike (epi)gallocatechin, are still readily absorbed and cleared by renal excretion. The addition of a 5′-hydroxyl group to (epi)catechin therefore either markedly reduces the extent to which the molecule can enter the circulatory system from the small intestine or predisposes to biliary excretion.

At the 3 doses administered, the ratio of the urinary glucuronide, sulfate, and methylated (epi)catechin metabolites changed little (Table 4). This indicates that, even at the highest intake, the uridine-5′-diphosphate glucuronotransferase, sulfotransferase, and catechol-O-methyltransferase enzymes involved in the formation of the (epi)catechin metabolites seemingly do not become saturated and limit conversions. It should be noted that in vitro EGCG inhibits human liver cytosolic catechol-O-methyltransferase with a half-maximal inhibition concentration (IC50) of 0.07 mmol/L (70), but it would appear that such inhibition does not occur in vivo.

GREEN TEA FEEDING STUDIES IN ILEOSTOMISTS AND COLONIC CATABOLISM

Dietary (poly)phenols that reach the colon, including metabolites secreted in bile and/or exported from the epithelia of the GIT, may be catabolized by the gut microbiota. A portion of at least some of these catabolites may be absorbed, a portion of some escaping subsequent phase II metabolism. It is generally accepted that such transformations will not be seen in ileostomists who have had their colon removed for medical reasons, commonly Crohn’s disease or ulcerative colitis. In reality, such transformations might occur to a limited extent in ileostomists if the upper GIT has been colonized by the microbiota. Nevertheless, valuable information can be obtained by comparing (poly)phenol absorption, metabolism, and excretion in ileostomists and volunteers with an intact colon.

Stalmach et al (57) gave ileostomists green tea containing 634 μmol flavan-3-ols—a very similar intake to that used in the study in healthy subjects (36) discussed in the section “Green tea feeding studies in healthy subjects.” The plasma profiles of (epi) catechins and their metabolites were similar to those shown in Figure 4 obtained in healthy subjects with an intact colon. Urinary excretion by the ileostomists was 8.0% of intake for (epi)gallocatechin conjugates and 27.4% of intake for (epi)catechin conjugates, values similar to those observed in healthy subjects (see Table 2). This shows unequivocally that the flavan-3-ol monomers are absorbed in the upper part of the GIT.

Despite substantial absorption of green tea flavan-3-ols in the upper GIT, 69% of intake was recovered in 0- to 24-h ileal fluid as a mixture of native flavan-3-ols and metabolites (57). Thus, in volunteers with a functioning colon, most of the ingested flavan-3-ols

### Table 4

<table>
<thead>
<tr>
<th>Urinary excretion of flavan-3-ol conjugates after the ingestion of increasing doses of (–)-epigallocatechin and (–)-epicatechin in polyphenon E by volunteers with an ileostomy&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Epi)gallocatechin</td>
</tr>
<tr>
<td>Conjugates [μmol (%)]</td>
<td>22 μmol</td>
</tr>
<tr>
<td>Glucuronides [μmol (%)]</td>
<td>1.8 (17)</td>
</tr>
<tr>
<td>Sulfates [μmol (%)]</td>
<td>3.9 (37)</td>
</tr>
<tr>
<td>Methylated [μmol (%)]</td>
<td>4.8 (46)</td>
</tr>
<tr>
<td>Total conjugates [μmol]</td>
<td>5.7 ± 1.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup>Data were from reference 40. Values for total (epi)gallocatechin and (epi)catechin conjugates with different superscript letters are significantly different (P < 0.05).

<sup>2</sup>Values are means ± SEs.
will pass from the small to the large intestine where they are subject to the action of the microbiota. To address this, data from in vitro anaerobic fecal incubations with (−)-epicatechin, (−)-epigallocatechin, and EGCG (50 μmol) were integrated with data for the 24-h urinary excretion of aromatic and phenolic acids after the ingestion of green tea and water by healthy subjects in a crossover study, as well as the analogous data after consumption of green tea by ileostomists (71). The results obtained provided the basis for the proposed catabolic pathways shown elsewhere in this issue by van Duynhoven et al (72).

The distinctive catabolites are several 5-(hydroxyphenyl)-γ-valerolactones and 5-(hydroxyphenyl)-4-hydroxyvaleric acids (Figure 6), which are unique colonic breakdown products of flavan-3-ols and associated proanthocyanidin oligomers (73, 74). It has been proposed that the hydroxyl at C-4 on the valeric acid side chain, used in lactonisation, corresponds to the C-3 hydroxyl of the parent flavan-3-ol (75). In vivo, these lactones are absorbed and subject to phase II metabolism. The 3′-O-glucuronide conjugate of 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone (Figure 6) is the most abundant valerolactone species in urine after green tea intake and is excreted in amounts 5–20 times its 4′-O-glucuronide conjugate. The analogous valerolactone-3′-O-sulfates, pyrogallol-2-O-sulfate and a pyrogallol-glucuronide, and vanilloyl-glycine are also excreted after ingestion of green tea (76).

There was an elevated excretion of hippuric acid (N-benzyol-glycine) by volunteers with an intact colon after green tea consumption compared with water, which is consistent with earlier studies in which volunteers consumed both green and black tea (77, 78). In association with in vitro fecal incubation studies, this suggests that the C-6–C-5 phenylvalerolactones and phenylvaleric acids are further degraded by side-chain shortening, either before or after absorption, to produce C-6–C-3 and C-6–C-1 phenolic and aromatic acids, and possibly also the C-6–C-2 analogs. Pyrogallol-2-O-sulfate, previously associated with catecholamine metabolism (79), and the pyrogallol-glucuronide might also arise by side-chain catabolism, although gallic acid and ester gallates are other known precursors in vitro (80). Other dietary sources of some phenolic and aromatic acids are known: for example, increased hippuric acid excretion occurs after consumption of benzoic acid, quinic acid, tryptophan, tyrosine, and phenylalanine (81–83).

Because of the multiple sources of some of these acids, it is difficult to accurately assess the extent to which the flavan-3-ol skeleton undergoes ring fission, although it does appear to be substantial. The excretion of 4-hydroxybenzoic acid, 3′-methoxy-4′-hydroxyphenylacetic acid, 3-(3′-hydroxyphenyl)hydracrylic acid, and 5-(3′,4′,5′-trihydroxyphenyl)-γ-valerolactone after ingestion of green tea was 172 μmol greater than after consumption of water, equivalent to 27% of the flavan-3-ols originally consumed (71); and in another volunteer study, the phenylvalerolactone derivatives excreted in urine accounted for 36% of the flavan-3-ols consumed (84). Judged solely by 24-h urinary excretion, considerably more of the flavan-3-ol dose is derived from gut flora–associated catabolites absorbed from the colon than from conjugates absorbed from the upper GIT.

**BLACK TEA FEEDING STUDIES**

Although consumed far more extensively in Europe and the United States than green tea, the absorption from black tea beverages of (poly)phenols, their human metabolism, and gut
flora catabolism have been studied much less extensively. Such studies as have been reported have focused on the absorption of flavan-3-ols (65) and flavonol glycosides (85, 86) from black tea with or without added milk. The appearance in urine of the gallic acid metabolites 3-O-methylgallic acid, 4-O-methylgallic acid, and 3,4-di-O-methylgallic acid has also been reported and used as an index of black tea consumption (87, 88). However, these metabolites are also to be expected in urine after the consumption of green tea (76) and after the ingestion of certain fruit, such as grapes, and associated wines. The absorption and metabolism of flavan-3-ols and flavonol glycosides from black tea are not obviously different from that observed after green tea consumption, although, pro rata, the dose of flavan-3-ols is much reduced. The dose of flavonol glycosides is similar because they are not so extensively transformed during fermentation (18).

To date, there is only one report to our knowledge on the absorption of mixed theaflavins [theaflavin (17.7%), theaflavin-3-gallate (31.8%), theaflavin-3′-gallate (16.7%), and theaflavin-3,3′-digallate (31.4%)] (Figure 2) (89). An extremely high 700-mg dose, equivalent to ~30 cups of black tea, was given to 2 healthy volunteers, one man and one woman. Plasma and urine concentrations were analyzed by HPLC–tandem mass spectrometry after enzymatic deconjugation with β-glucuronidase and sulfatase and extraction into ethyl acetate. Only theaflavin was detected because the enzyme treatment also removed the ester gallate. Maximum theaflavin concentrations detected in the plasma of the female and male volunteers were 1.0 and 0.5 μg/L, respectively (1.8 and 0.9 fmol/L), and maximum urine concentrations were 0.6 and 4.2 μg/L, respectively (1.1 and 7.4 fmol/L), all at 2 h. These values should be doubled to correct for the relatively poor recovery observed with standard theaflavin, but even so, the total amount of theaflavin excreted was considerably less than 0.001% of the very large dose consumed (89).

Attempts to investigate gut flora catabolism of mixed thearubigins in vitro using conditions that were suitable for flavonols, flavan-3-ols, and proanthocyanidin B2 (73, 90), failed to show the production of phenolic or aromatic acids, even when using a low-protein medium to minimize effects from protein binding (91, 92). In contrast, human studies have shown that the consumption of a black tea beverage results in a substantially increased excretion of hippuric acid relative to baseline, suggesting that a combination of gut flora catabolism and postabsorption metabolism results in a significant production of benzoic acid (77, 78, 93). The yield of benzoic acid excreted as hippuric acid is such that it points to both thearubigins and theaflavins serving as substrates in vivo and being degraded to phenolic acids. Urolithin A-3-O-glucuronide and its 8-O-isomer and a urolithin B-glucuronide have been detected in human urine after black tea consumption (76) (Figure 6). These gut microbiota-derived catabolites have previously been shown to originate from ellagitannins present in pomegranates, nuts, strawberries, and raspberries (94). Their appearance in urine after drinking black tea is most probably the result of colonic degradation of hydrolysable tannins such as straightin (Figure 1).

A number of compounds derived from (poly)phenols in the colon have been shown at low μmol/L concentrations to have potential protective effects in vitro, including antiinflammatory, anti-proliferative, antilygycative, neuroprotective, and photoprotective activity (95–103). As a consequence, there is a growing realization that colonic catabolites of dietary (poly)phenols as well as having an impact on colonic health may, once they enter the circulatory system, play a role in maintaining health in other parts of the body.

Because black tea is consumed with milk in many parts of the world, this is a topic that needs to be revisited, along with investigations into bioavailability and potential protective effects of theaflavins and thearubigins, in appropriately designed feeding studies and in vitro investigations that make use of state-of-the-art analytic methodology.

**FUTURE RESEARCH**

The identity and chirality of green tea (−)-epicatechin and (−)-epigallocatechin glucuronide, sulfate, and methylated conjugates that appear in the circulatory system after absorption in the small intestine need to be established, and their bioactivity investigated at low and submicromolar concentrations. Further information is required on the colon-derived catabolites of green tea flavan-3-ols, such as valerolactones and valeric acid conjugates, to determine whether or not they have a favorable impact on the colonic microflora and gastrointestinal health and their effects elsewhere in the body once they enter the circulatory system.

Matrix effects and their impact on the absorption, metabolism, and excretion of tea polyphenols require further study. To date, feeding studies have involved acute supplementation; studies should also involve repeat dosing at ~3-h intervals, which reflects the way in which teas are consumed by the general public. This will almost certainly extend the plasma concentration–time profiles of the various flavan-3-ol–derived products in the bloodstream.

Research on black tea should be a priority in view of the fact that black tea is widely consumed, with and without milk, in many parts of the Western world. Surprisingly little is known about the potential protective effects of black tea theaflavins and thearubigins in the large intestine and the impact on health of their low-molecular-weight microbiota-derived catabolites both within the lower bowel and when they enter the bloodstream and are transported to other sites within the body. This situation needs to be rectified.

The authors’ responsibilities were as follows—All 3 authors contributed equally to the literature searches and to the writing of this review. AC received an honorarium and travel support from the Tea Council of the USA for speaking at the Fifth International Scientific Symposium on Tea and Human Health and for preparing this manuscript for publication. The authors declared no competing financial interests.

**REFERENCES**

6. van der Hooft JJJ, Akermi M, Unlu FY, Mihaleva V, Bino RJ, de Vos RC, Vervoort J. Structural annotation and elucidation of conjugated...


68. Mulder TP, Rietveld AG, van Amelsvoort JM. Consumption of both black tea and green tea results in an increase in the excretion of hippuric acid into urine. Am J Clin Nutr 2005;81(suppl):2560–6S.


86. Daykin CA, van Duynhoven JP, Groenewegen A, Dachtler M, van Amelsvoort JM, Mulder TP. Nuclear magnetic resonance spectroscopic


