Influence of Formulation and Processing on Absorption and Metabolism of Flavan-3-Ols from Tea and Cocoa

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Abstract

Flavan-3-ols are a major subclass of the class of plant phytochemicals known as flavonoids. Flavan-3-ols are commonly found in fruit, vegetable, and botanical products, including tea, cocoa, grapes, and apples. Both monomeric catechins and polymeric procyanidins are common in the diet, along with several derivatives produced by degradation of these species during processing. Both epidemiological and biological evidence suggests a health-protective role for dietary flavan-3-ols, leading to increased interest in the bioavailability of these compounds from foods. Flavan-3-ol bioavailability depends on numerous factors, including digestive release, absorption, metabolism, and elimination. In addition to these in vivo factors, the complexity of whole-food systems (physical form, flavan-3-ol form and dose, macronutrient and micronutrient profile, processing, etc.) influences the absorption efficiency and circulating profile of flavan-3-ols. An understanding of how food matrices may influence flavan-3-ol absorption will provide a framework to design and develop functional products that positively affect flavan-3-ol absorption and, by extension, potential bioactivity.

INTRODUCTION

Flavan-3-ols are polyphenols belonging to the broader class of plant phytochemicals known as flavonoids. Interest in flavonoids has intensified over the past decade due to the significant number of epidemiological associations linking flavonoid-rich diets with prevention of several chronic and degenerative diseases, including cancer (Neuhouser 2004), cardiovascular disorders (Ding et al. 2006), obesity, and diabetes (Nagao et al. 2009, Thielecke & Boschmann 2009), as well as neurodegenerative disorders (Mandel et al. 2005). Flavan-3-ols are a major subclass of flavonoids that includes monomeric catechins and polymeric procyanidins. This flavonoid subclass is believed to account for approximately 83.5% (~157 mg/d) of the total flavonoid consumption in the U.S. diet (estimated to be ~190 mg/day) (Chun et al. 2007), making flavan-3-ols a significant dietary flavonoid form. In addition to epidemiological associations and high dietary exposure, specific biological activities consistent with disease prevention have been reported for flavan-3-ols, including antioxidant activities (Fraga & Keen 2003), stimulation of endogenous antioxidant systems (Pietta & Simonetti 1998), stimulation of nitric oxide (NO) production and vasodilation (Grassi et al. 2006), regulation of xenobiotic-metabolizing enzymes (Moon et al. 2006), increased fatty acid oxidation and insulin sensitivity, and alteration of glucose absorption and utilization (Boschmann & Thielecke 2007).

With a growing body of epidemiological and biological evidence suggesting a protective role for dietary flavan-3-ols, interest in the bioavailability and metabolism of these compounds from foods and dietary supplements has expanded. Current knowledge of flavan-3-ol bioavailability from foods is variable (Manach et al. 2004, Williamson & Manach 2005) and dependent on numerous factors, including source and type of flavan-3-ol, interindividual variability in absorption, metabolism, and elimination (Feng 2006, Lambert et al. 2007). Although numerous studies have focused on flavan-3-ol absorption from pure compounds or refined extracts, knowledge of flavan-3-ol absorption from food remains limited. This is due, in part, to the complexity of whole-food systems and potential interactions between flavan-3-ols with specific macronutrients, micronutrients, or other food components that often complicate interpretations (Neilson et al. 2009, Peters et al. 2010, Roura et al. 2008, Schramm et al. 2003). A better understanding of flavan-3-ol absorption, metabolism, and tissue distribution from foods remains essential to understanding the role these flavonoids may play in prevention of chronic disease. Furthermore, understanding how the food matrix may influence flavan-3-ol absorption provides guidance in design and development of products to positively affect flavan-3-ol absorption.

In this context, the purpose of this review is to provide an overview of flavan-3-ol composition and bioavailability from tea and cocoa products, which are common dietary sources of these compounds. Key research describing the impact of processing on flavan-3-ol composition and bioavailability is described, including the impact of digestion, intestinal uptake, and metabolism on physiological flavan-3-ol profiles. Finally, the impact of specific food and beverage formulation factors on bioavailability of flavan-3-ols is discussed.

CLASSIFICATION OF FLAVAN-3-OLS

As a subclass of the flavonoid family, flavan-3-ols can be subdivided based upon degree of polymerization, oxidative state, and substitution pattern of the B- and C-rings (Beecher 2003, Heim et al. 2002). In this review, both monomeric and oligomeric flavan-3-ol forms are described.

Monomeric Flavan-3-ol (Catechins)

Five major monomeric flavan-3-ols, referred to as catechins, are found in the diet: (+)-catechin (C), (−)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG), and

(−)-epigallocatechin gallate (EGCG) (**Figure 1*a***) (Del Rio et al. 2004). Structurally, gallocatechins (EGC and EGCG) differ from catechins (C, EC, and ECG) by having a third B-ring hydroxyl group at C5′. Catechin gallates (EGCG and ECG) have a gallic acid residue esterified to the C3 hydroxyl. Due to the two chiral carbons in the C-ring (C2 and C3), multiple stereoisomers exist for each catechin. Oxidation of catechin monomers during processing results in formation of several products, including theaflavins (TFs) (**Figure 1*b***), theasinensins, and other polymers such as thearubigins and theabrownins (Menet et al. 2004, Tanaka et al. 2002).

Oligomeric Flavan-3-ols (Procyanidins and Proanthocyanidins)

In addition to monomers, more complex flavan-3-ols exist, including the procyanidins (PCs). The PCs are dimers (2 monomer residues), oligomers (3–7), and polymers (≥8) of flavan-3-ol monomers (Beecher 2003, Jeong & Kong 2004, Manach et al. 2004). Monomers are bonded by interflavan linkages between the C-ring of the first monomer and either the A- or C-ring of the next. B-type PCs have only one interflavan linkage (typically a C4→C8 or C4→C6 carbon-carbon bond) (**Figure 1*c***). A-type PCs have monomers joined by two interflavan linkages: the C4→C8 bond plus a C2→O→C7 ether bond (**Figure 1*d***) (Beecher 2003, Khanbabaee & van Ree 2001). C-type condensed tannins are trimeric B-type condensed tannins.

DIETARY SOURCES OF FLAVAN-3-OLS

Numerous reviews on flavonoid contents of foods have identified tea, cocoa, grapes, apples, and other fruits and vegetables as the predominant dietary sources of monomeric and complex flavan-3-ols (Manach et al. 2004, Scalbert & Williamson 2000).

Tea

Tea (brewed from leaves of *Camellia sinensis*) is one of the richest dietary sources of monomeric flavan-3-ols, accounting for up to 77% of flavonoid intake by adults in the United States (Chun et al. 2010). Various types of tea are consumed, which differ primarily in type and extent of leaf processing and, by extension, flavan-3-ol profiles. Green tea is a minimally processed (unfermented) tea product (Astill et al. 2001). In green tea leaf, catechins represent up to 85% of the total flavonoid content (Astill et al. 2001, Yao et al. 2005). On a wet-weight basis (wwb), total catechins levels in green tea have been reported between 4 and 140 mg g^{-1}. Extreme variability exists, arising from agroclimactic factors as well as between varieties, brands, and area of harvest (Friedman et al. 2005, Khokhar & Magnusdottir 2002). EGCG is most abundant (7–74 mg g^{-1} wwb), followed by EGC (0–55 mg g^{-1}), ECG (1–40.5 mg g^{-1}), EC (0.1–17 mg g^{-1}), and C (0–8 mg g^{-1}) (Friedman et al. 2005, Khokhar & Magnusdottir 2002, Lee et al. 2000). Traditional brewing of green tea with hot water generates infusions containing 50–540 mg per cup (approximately 8 oz or 236 mL) of total catechins (Bronner & Beecher 1998, Henning et al. 2003).

Black tea is produced from the same botanical material as green tea but differs in that the leaf is highly processed by natural oxidative enzymes present in the leaf, including polyphenol oxidase and peroxidase. Known as fermentation, this processing significantly alters the flavonoid profile (Astill et al. 2001). Specifically, oxidation of catechin monomers results in generation of complex products, including theaflavin (TF), TF-monogallate (TFMG), TF-digallate (TFDG), theasinensins, and thearubigins, which provide characteristic color and flavor to black tea (Bailey et al. 1992, Menet et al. 2004, Tanaka et al. 2002). Although the extent of fermentation varies significantly (between products and regions), generally, monomer oxidation (particularly EGCG

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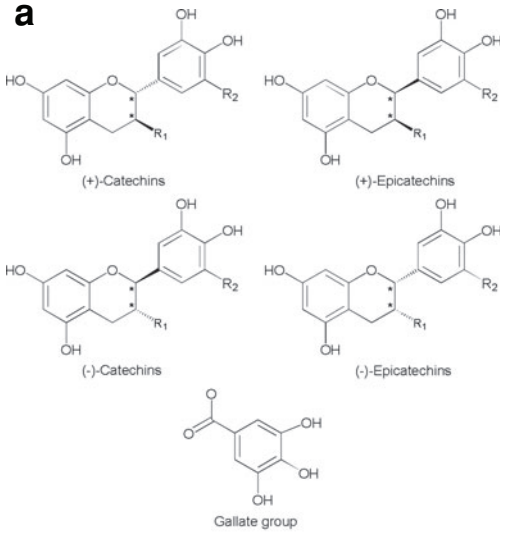
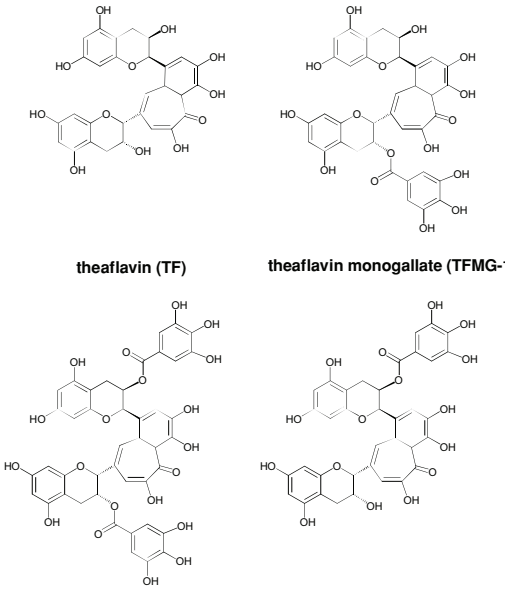


C3 Configuration	Compound	Abbreviation	R_1	R_2
(+) or (−)	Catechin	C	OH	H
(+) or (−)	Gallocatechin	GC	OH	OH
(+) or (−)	Catechin gallate	CG	Gallate	H
(+) or (−)	Gallocatechin gallate	GCG	Gallate	OH
(+) or (−)	Epicatechin	EC	OH	H
(+) or (−)	Epigallocatechin	EGC	OH	OH
(+) or (−)	Epicatechin gallate	ECG	Gallate	H
(+) or (−)	Epigalocatechin gallate	EGCG	Gallate	OH

b



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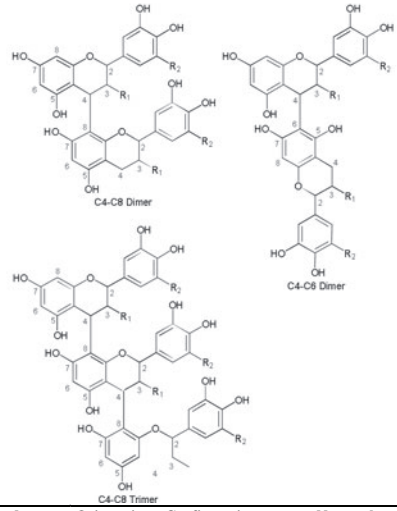
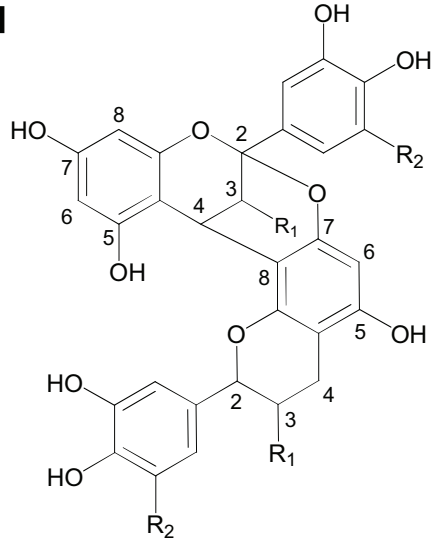


Bond	Orientation	Configuration	Nomenclature
C4-C8 (interflavan)	⟋	β	monomer$_1$-(4β→8)-monomer$_2$
	⟍	α	monomer$_1$-(4α→8)-monomer$_2$
C4-C6 (interflavan)	⟋	β	monomer$_1$-(4β→6)-monomer$_2$
	⟍	α	monomer$_1$-(4α→6)-monomer$_2$

d



C4-C8, C2-O-C7 Dimer

Bond	Orientation	Configuration	Nomenclature
C2-C7 (interflavan)	⟋	β	monomer$_1$-(2β→O→7)-monomer$_2$
	⟍	α	monomer$_1$-(2α→O→7)-monomer$_2$

and EGC) reduces the levels of catechins relative to green tea. Levels of total catechins in black tea leaf range widely from 5–110 mg g^{-1} wwb, with theaflavins present at 5–21 mg g^{-1} wwb (Friedman et al. 2005, Wright et al. 2002). EGCG (0.5–47 mg g^{-1} wwb) and ECG (2–67 mg g^{-1} wwb) are the most abundant catechins in black tea, followed by EGC (0–10 mg g^{-1} wwb) and EC (0.4–7 mg g^{-1} wwb). Individual theaflavin species (TF, TFMG, and TFDG) are typically present at similar levels in black tea (Friedman et al. 2005, 2006). Brewing of black tea generates beverages containing between 50 and 370 mg per cup total catechins and 4–18 mg per cup theaflavins (Bronner & Beecher 1998, Henning et al. 2003).

Cocoa and Chocolate

Cocoa and chocolate products, made from beans of *Theobroma cacao* fruit, are another major source of flavan-3-ols (Cooper et al. 2007, Gu et al. 2002, Natsume et al. 2000). Significant variation exists in the qualitative and quantitative profiles of flavan-3-ols and PCs in cocoa owing to differences in geographical region, season, processing, and formulation. The predominant flavan-3-ol monomers in chocolate are (−)-EC, as well as two forms of C: (+)-C and (−)-C (referred to collectively as C) (Cooper et al. 2007). Cocoa also contains PCs with varying of degrees of polymerization (Nelson & Sharpless 2003, Sanchez-Rabaneda et al. 2003).

On a fat-free basis (ffb), cocoa powder contains 0.7–2 mg g^{-1} C, 2–15 mg g^{-1} EC, and 25–55 mg total PCs (Gu et al. 2006, Miller et al. 2006, Natsume et al. 2000). Dutched cocoa has considerably lower contents of C (0.3–0.4 mg g^{-1}), EC (0.2–0.5 mg g^{-1}), and PC (8–13 mg g^{-1}) than standard cocoa (Gu et al. 2006). Dark chocolate has relatively high levels of flavan-3-ols, with C at 0.15–0.5 mg g^{-1}, EC at 0.7–2 mg g^{-1}, and PCs at 0.5–31 mg g^{-1}. Milk chocolate contains less cocoa powder by weight than dark chocolate and therefore has proportionally lower levels of C (0.7–0.2 mg g^{-1}), EC (0.3–0.4 mg g^{-1}), and PCs (0.6–3.2 mg g^{-1}) (Adamson et al. 1999, Gu et al. 2006, Miller et al. 2006, Natsume et al. 2000). Major cocoa PCs have a degree of polymerization (DP) of 2–10 (Adamson et al. 1999, Hammerstone et al. 2000). The predominant PCs in cocoa products are dimers B2 and B5, trimer C1, and cinnamtannin A2 (Cooper et al. 2008).

The source, processing method, and finished form of cocoa products greatly influence the profile of monomers and PCs present. Processing of cocoa involves physical and chemical alterations to the raw beans. This typically involves fermentation, air drying, cleaning of the bean, roasting and winnowing, grinding of the nibs, separating cocoa butter from cocoa powder via pressing, alkalization of cocoa powder (also called Dutching, an optional step), refining, formulating, conching, and repeated cooling/heating (Wollgast & Anklam 2000).

Fermentation of raw cocoa beans results in oxidative degradation of monomers (C, EC) and PCs to form large, insoluble tannins (Hansen et al. 1998, Kealey et al. 1998). Roasting of the fermented beans induces epimerization, along with other reactions that impact flavan-3-ol profile.

Figure 1

Primary dietary flavan-3-ol derivatives present in cocoa and tea. (*a*) Structures and stereochemistry of the major monomeric flavan-3-ols (catechins). Chiral carbons are identified with asterisks. (*b*) Structures of derived tannins (theaflavins). (*c*) Structures of B-type condensed tannins (dimers are shown) and C-type (trimeric) condensed tannins. The identities of the R1 group (-H or -OH) and R2 group (-OH or -gallate), the configuration of C2 (+/−), and the configuration of the C2/C3 substituents relative to the C-ring plane (*cis*/*trans*) depend upon the identity of each constituent flavan-3-ol monomer residue (see **Figure 3**). The procyanidins are composed of (−)-EC and/or (+)-C residues, whereas the prodelphinidins are composed of (−)-EGC and/or (−)-EGCG residues. (*d*) Structures of A-type condensed tannins (a dimer is shown). The identities of the R1 group (-H or -OH) and R2 group (-OH or -gallate), the configuration of C2 (+/−), and the configuration of the C2/C3 substituents relative to the C-ring plane (*cis*/*trans*) depend upon the identity of each constituent flavan-3-ol monomer residue (see **Figure 3**). See **Figure 4** for the stereochemistry of the C4-C8 interflavan linkage.

Specifically, (+)-C in fermented beans appears to be highly degraded/epimerized, with losses of 67%–97% during roasting (Kofink et al. 2007, Oliviero et al. 2009).

Cocoa and cocoa products are a rare and significant dietary source of (−)-C. Although the majority of plant foods contain mostly (+)-C, as do the native *Theobroma cacao* beans (Gotti et al. 2006), (+)-C is largely epimerized during processing to (−)-C (Andres-Lacueva et al. 2008, Kofink et al. 2007), resulting in cocoa products that contain mixtures of (±)-C, with up to 90% (−)-C and 10% (+)-C (Donovan et al. 2006, Gotti et al. 2006). Additionally, the levels of PCs as well as total polyphenols decrease during roasting, with greater losses at higher temperatures (Kealey et al. 1998).

CHEMICAL PROPERTIES OF FLAVAN-3-OLS

With growing interest in bioavailability and biological activities of flavan-3-ols, it is critical to consider their susceptibility to heat and oxidative conditions typically encountered in food processing. These properties determine stability and the extent of chemical changes that occur between harvest of the raw plant and consumption (i.e., during holding, processing, packaging, self-storage, and digestion), thereby affecting the qualitative profiles and concentrations of flavan-3-ols available to influence human health.

Thermal Stability

Numerous foods containing flavan-3-ols are subjected to thermal processes, including fermentation, retorting, pasteurization, and in-home cooking/preparation, that influence qualitative and quantitative profiles of flavan-3-ols in finished products and make them available for absorption and utilization. Several studies have investigated the thermal stabilities for flavan-3-ols in aqueous solutions (Chen et al. 2001, Wang & Helliwell 2000, Xu et al. 2003). The predominant reactions of catechins during exposure to heat appear to be isomerization and autooxidation (Komatsu et al. 1993, Wang et al. 2006). Isomerization of epicatechins to their nonepi isomers is thermodynamically favorable (Okumura et al. 2008). The heats of formation (ΔH_f) of the nonepi isomers are 1–2 kcal mol^{-1} lower than those of epi forms, and this difference is sufficient to drive epimerization during typical thermal processes (Okumura et al. 2008). The significance of thermally induced isomerization is reflected in studies demonstrating that retorted green tea beverages contain (−)-GCG as their predominant catechin species, whereas (−)-EGCG is the predominant species present in unprocessed green tea (Chen et al. 2001, Zhu et al. 2003). Dutching and roasting of cocoa also facilitate epimerization of (−)-EC to (−)-C (Kofink et al. 2007).

The theoretical thermal stabilities of catechins are believed to be ECG > EGC > EC > EGCG (Okumura et al. 2008). However, stabilities in food systems are confounded by autooxidation reactions, which preferentially degrade EGCG and EGC (Komatsu et al. 1993). This was illustrated by a study demonstrating that brewing tea (100°C for 5 min) in tap water (containing metal ions and dissolved O_2 at 1.1 mg L^{-1}) resulted in infusions with more nonepi species (GCG and GC) and less epi species (EGCG and EGC) than brewing in purified water (Wang & Helliwell 2000).

Oxidative Stability of Flavan-3-ols

The oxidative stability of catechins in aqueous systems is highly dependent on pH (Zhu et al. 1997). The relative stabilities of catechins to elevated pH conditions (pH > 5.5) have been reported to be EC > ECG > EGCG ≥ EGC (Chen et al. 2001, Sang et al. 2005, Su et al. 2003, Zhu et al. 1997). Although Dutching, or alkaline processing of cocoa, is typically a desired process to enhance cocoa

color, this treatment at high pH reduces levels of monomers C and EC and PCs by 3- to 8-fold in finished products (Andres-Lacueva et al. 2008, Gu et al. 2006). pH-driven degradation of EGCG and EGC is thought to proceed by oxidative mechanisms involving the donation of $H^{\bullet}$ to quench oxygen radicals (Hou et al. 2005, Miura et al. 1998, Sang et al. 2005). Catechins with the catechol B-ring structures (EC and ECG) are more stable, compared to those with the pyrogallol B-ring structures (EGCG and EGC) (Mochizuki et al. 2002). The half-life of EGCG at pH 7.2–7.4 is 30 min to 2 h (Chen et al. 1998, 2001; Hong et al. 2002; Sang et al. 2007). EGCG is highly unstable in cell culture media (pH 7.4), with a half-life of 30 min (Sang et al. 2005) and 95% loss in 4 h (Hou et al. 2005). Eighty-five percent of EGCG was degraded within 30 min in intestinal juice (pH 8.5), and 79% was degraded within 30 min in mouse plasma (Yoshino et al. 1999). Oxidation of EGCG by reactive oxygen species (ROS) at elevated pH results in the generation of several autooxidation products, including dimers. These dimers include the theasinensins (THSNs) A and D, and a more complex dimer referred to as P-2 (Hou et al. 2005; Sang et al. 2005,2007; Yoshino et al. 1999) (**Figure 2*a***).

Although the majority of research regarding pH-driven autooxidation has focused on EGCG, relatively little is known regarding the behavior of EGC, which is one of the most abundant flavan-3-ols in green tea. EGC appears to be highly labile to oxidative degradation and also generates oxidative dimers during tea processing (Matsuo et al. 2008) and in solution (Neilson et al. 2007, 2010b). Additionally, autooxidation of these flavan-3-ol mixtures (commonly present in foods, as opposed to individual compounds) results in heterodimerization between species. For example, autooxidation of EGCG and EGC mixtures in model systems forms EGC homodimers structurally analogous to the THSNs and P-2 as well as EGCG and EGC heterodimers (Neilson et al. 2007). Although the relevance of these species remains to be determined, conditions favoring autooxidation exist in select food and beverage systems, as well as in the small intestinal lumen (**Figure 2*b***). These conditions include elevated pH ($\geq$5.5), residual dissolved O_2, and presence of ROS (Parks 1989). More recently, the presence of several of these autooxidation dimers of EGC and EGCG have been identified in fermented black and oolong teas (Neilson et al. 2010b), indicating their presence in the diet and highlighting the need to better understand factors driving their formation and/or biological significance.

DIGESTION, ABSORPTION, AND METABOLISM OF FLAVAN-3-OLS FROM TEA AND COCOA

Absorption of flavan-3-ol is a multistep process, starting with the (*a*) digestive release of the flavan-3-ol from the food or beverage matrix, followed by (*b*) solubilization of stabile flavan-3-ols in the gut lumen, (*c*) uptake and transport by intestinal epithelial cells, and (*d*) metabolism (colonic, intestinal, and hepatic) of flavan-3-ols (**Figure 3**). Each step of this process can ultimately influence the circulating and/or tissue flavan-3-ol profiles. For the purpose of this review, bioavailability is defined as the fraction of flavan-3-ol compounds from a food absorbed and secreted into circulation (as native or metabolized forms) and made available for tissue uptake and metabolism. The term bioaccessibility is often utilized to describe the fraction of flavan-3-ols made available for absorption at the luminal surface of the intestinal epithelia during the initial stages of digestive release and solubilization/stability of flavan-3-ols in the intestine (Ferruzzi 2010).

Digestive Stability and Bioaccessibility

Several factors determine the bioaccessibility of flavan-3-ols. First, flavan-3-ols must be released from molecular interactions with other food components as well as bulk-phase interactions with

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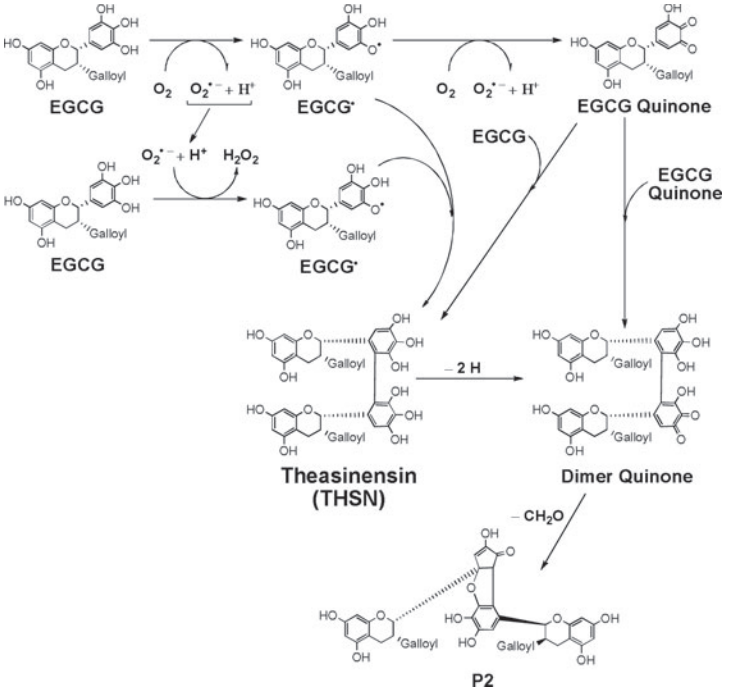

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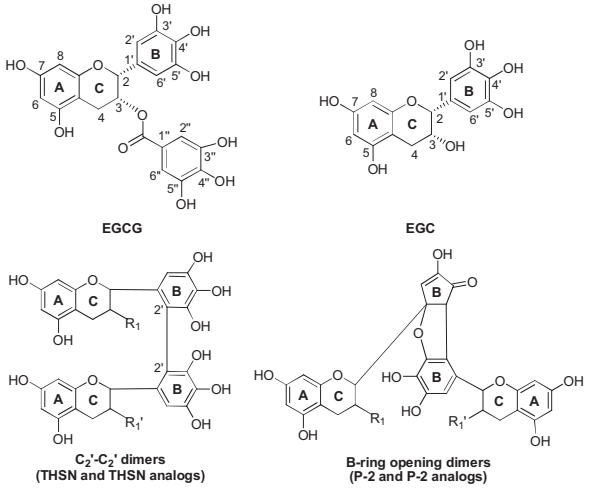

Linkage	Type	Compound	Precursors	MW[a] (g/mol)	Substitution[b] R_1	Substitution[b] $R_{1'}$
C2'-C2'	Homo	THSN A/D	2 EGCG	914	G	G
	Homo	THSN C/E	2 EGC	610	OH	OH
	Hetero	THSN B	EGCG + EGC	762	G	OH
B-ring opening	Homo	P-2	2 EGCG	884	G	G
	Homo	P-2 analog	2 EGC	580	OH	OH
	Hetero	P-2 analog	EGCG + EGC	732	G/OH	OH/G

[a]Nominal molecular weight
[b]G = galloyl residue

the physical food matrix. Second, flavan-3-ols must be soluble in the bulk aqueous phase in order to diffuse across the unstirred water layer that protects the enterocyte surface. Finally, flavan-3-ols must be stable to gastrointestinal conditions, including exposure to saliva, gastric juice, and intestinal secretions, as well as wide pH variations. Only the flavan-3-ol fraction that meets these criteria will be available for absorption (i.e., bioaccessible).

Both monomeric flavan-3-ols and PCs appear to be generally stable in both oral and gastric environments. Recovery of monomeric catechins and select PCs, following short incubations (10–60 min) with authentic or simulated saliva (pH 6.9, α-amylase), has been reported between 85% and 102% (Laurent et al. 2007, Tsuchiya et al. 1997). Simulated gastric recovery of C and EC, and PCs B2 and B3 is 97% to125% (some PCs were hydrolyzed to C and EC, resulting in >100% recovery for these compounds) (Laurent et al. 2007). This finding was similar to a report that found the gastric stability of flavan-3-ols from both green and black tea to be >80%, with the exception of EGCG, EGC, and GCG, which experienced gastric losses of roughly 50% for black tea only (Record & Lane 2001). GCG was found to increase by 30% for green tea, suggesting that acid-catalyzed epimerization of EGCG may occur under gastric conditions (Record & Lane 2001).

In contrast, individual flavan-3-ols appear to be less stable in intestinal conditions. Record & Lane (2001) reported that the recovery of flavan-3-ols during simulated intestinal digestion (pH 7.5, no enzymes) of green teas was 1% for EGCG, 8% for EGC, 38% for GCG, 59% for ECG, and 71% for EC. Separate studies confirmed the simulated digestive stability of catechol-containing EC, C, and ECG compared with pyrogallol-containing EGCG and EGC (Green et al. 2007, Neilson et al. 2007). These results suggest that flavan-3-ol degradation may be driven by autooxidation at near-neutral or greater pH common in the small intestine. In support of this hypothesis, autooxidation dimers of EGCG and EGC have been identified in simulated intestinal digesta containing monomeric flavan-3-ols (Neilson et al. 2007, 2010b).

In addition to instability, intestinal solubility of flavan-3-ols may be a factor that limits bioaccessibility. Laurent et al. (2007) reported that recoveries of EC and C and PCs B2 and B3 could be enhanced from <55% to >85% (with PCs improving from 0% to >85%) from simulated digesta by extraction with acetonitrile. This suggests that physical associations with food and intestinal secretions, as well as solubility, may be important factors limiting the bioaccessibility of flavan-3-ols, particularly the PCs.

In one of the few studies of actual intestinal stability and recovery in vivo, Auger et al. (2008) reported that recovery of flavan-3-ols in ileal fluid (i.e., unabsorbed and nondegraded) of humans consuming green tea extract (Polyphenon E) was 21%–36%, 47%–59%, 53%–74%, and 26%–34% for EC, EGCG, GCG, and ECG, respectively. Additionally, ileal recovery of total flavan-3-ols was 39% to 46%. Although these data should be taken in context due to the altered physiological state (lack of a colon) of the individuals included in the study, the higher ileal recoveries of flavan-3-ols observed compared to those predicated by in vitro experiments, particularly for EGCG and

Figure 2

Flavan-3-ol autooxidation leads to formation of complex products. (*a*) Proposed autooxidation reaction mechanism of (−)-epigallocatechin gallate (EGCG) at near-neutral or greater pH, leading to the formation of the homodimers theasinensin, and P-2 (Hou et al. 2005, Miura et al. 1998, Mochizuki et al. 2002, Sang et al. 2007). Two EGCG monomers form a C-C bond in the B-ring, resulting in the net loss of 2 H atoms, to generate the homodimers theasinensin (THSN A and THSN D). Two EGCG monomers also undergo B-ring opening and subsequent condensation, resulting in the net loss of 2 H atoms and formaldehyde (CH2O), to generate the homodimer P-2. (*b*) Structures of EGCG and (−)-epigallocatechin (EGC), as well as the known autooxidation dimers (THSNs and P-2 analogs) of EGCG and EGC formed though in vitro digestion, incubation in a variety of fluids at near-neutral pH (cell-culture media, authentic intestinal juices, plasma, etc.), and enzymatic oxidation both in vitro as well as in tea. Adapted from Neilson et al. 2010b with permission.

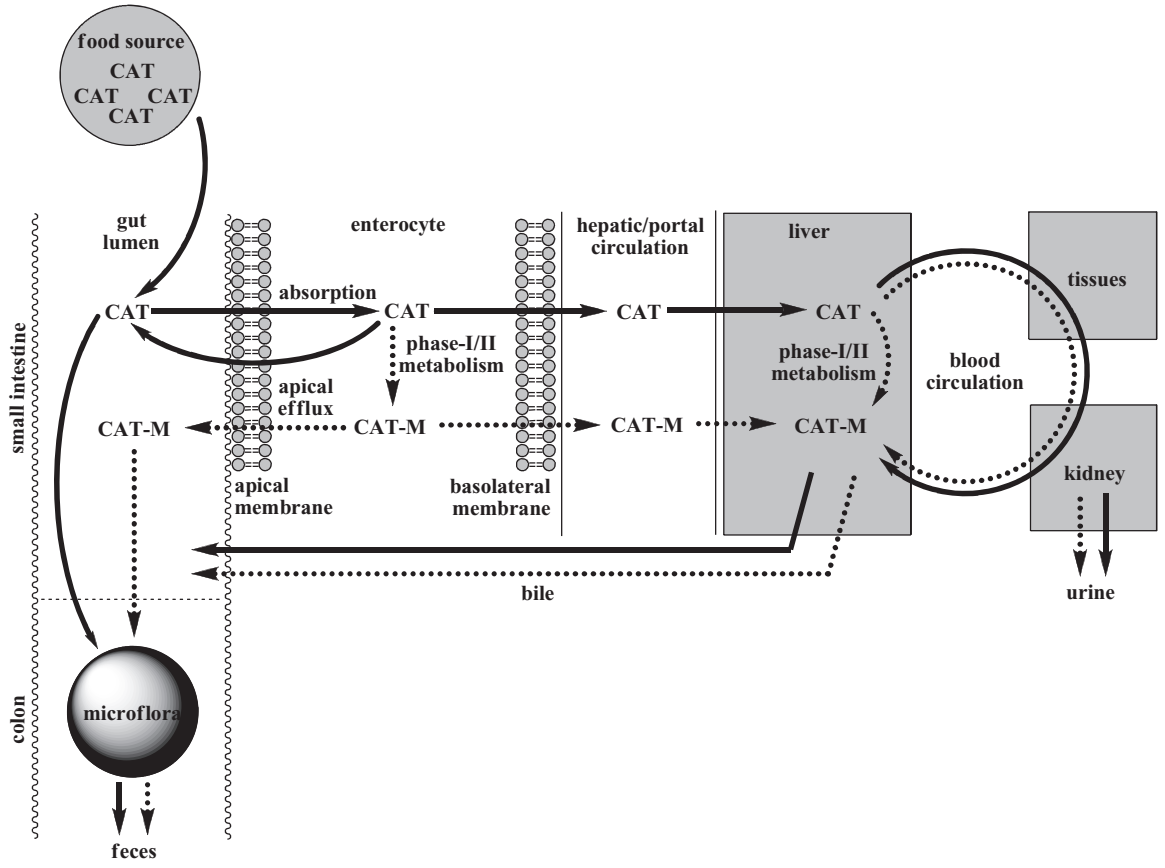

Figure 3

Schematic of the processes that affect systemic bioavailability and metabolism of flavan-3-ols. CAT, catechins; CAT-M, catechin phase-II metabolites; →, pathways of native catechins; ⋯→, pathways of catechin metabolites.

EGC, suggest that the extent to which intestinal degradation of flavan-3-ols occurs in vivo requires additional investigation.

Intestinal Absorption

Following digestive release and solubilization, flavan-3-ols are absorbed in the upper small intestine. Intestinal uptake of flavan-3-ols is believed to proceed principally through the monocarboxylic acid (MCT) transporter present in the brush border of intestinal epithelial cells. Additionally, but to a more limited extent, flavan-3-ol absorption may proceed by passive diffusion (Crespy et al. 2003, Lambert et al. 2007, Vaidyanathan & Walle 2003). Animal and Caco-2 cell models have been widely applied in the study of flavan-3-ol intestinal absorption. The relative apical → basolateral permeability of flavan-3-ols in Caco-2 monolayers has been reported to be EGC (1.5×10^{-7} cm s^{-1}) > EC (1.4) > ECG (1) > EGCG (0.8), suggesting that poor transepithelial transport efficiency for flavan-3-ol monomers limits overall bioavailability especially for gallated derivatives (EGCG and ECG) (Zhang et al. 2004).

A key factor limiting transepithelial intestinal transport is the affinity for flavan-3-ols of the ATP-binding cassette (ABC) trans-membrane transporters, specifically P-glycoprotein (Pgp) and multidrug resistant proteins (MRP) 1 and 2 (Feng 2006, Takano et al. 2006). These transporters actively remove xenobiotics from the cell interior to the lumen, interstitial space, or bloodstream surrounding the cells (Feng 2006). The affinity of flavan-3-ols for these transport systems significantly limits the ability of flavan-3-ol to cross into the bloodstream. Although 35% to 80% of a flavan-3-ol dose may be absorbed by the intestinal epithelia, 11% to 52% may be subsequently effluxed back into the lumen (Feng 2006, Vaidyanathan & Walle 2001). The effective efflux rate was found to be as high as 20% to 80% of the absorption rate for flavan-3-ols in a perfused rat intestine (Crespy et al. 2003), indicating that along with digestive instability, affinity for this transport system is a major barrier to the overall systemic bioavailability of flavan-3-ols from foods.

Flavan-3-ol Metabolism and Plasma Profiles

Following uptake by intestinal absorptive cells, flavan-3-ols are subject to xenobiotic metabolic transformation. Although flavan-3-ols are not typically substrates for phase-I metabolizing systems (Chan et al. 2004, Williamson et al. 2000), they serve as substrates for several phase-II conjugation systems, both in the intestine and the liver. Glucuronidation of C5, C7, and/or C3′ on a flavan-3-ol is carried out by uridine diphosphate glucuronyl-transferase (UDPGT). Sulfation of absorbed flavan-3-ols at various sites is carried out by sulfotransferase (SULT) or phenol-sulfotransferase (PST). O-methylation of flavan-3-ols may occur at C3′, C4′, C3″, and/or C4″ positions by catechol O-methyl transferase (COMT) (Feng 2006, Williamson et al. 2000).

The majority of flavan-3-ol metabolism is believed to occur in the small intestine. Flavan-3-ol phase-II conjugates formed in intestinal enterocytes are efficiently effluxed into the interstitial space and bloodstream by MRP1 and into the gut lumen by MRP2 (Feng 2006, Takano et al. 2006, Vaidyanathan & Walle 2001). Although reduced relative to intestinal metabolism (Cai et al. 2002, Lambert et al. 2003), first-pass hepatic metabolism does exert an effect on the profile of circulating phase-II metabolites in rats. COMT activity is highest in the liver, generating 3′ O-methyl, 4′ O-methyl, 4″ O-methyl, and 3′,4″ di-O-methyl flavan-3-ol metabolites (Piskula & Terao 1998, Zhu et al. 2001). Liver COMT appears to preferentially form 3′ O-methyl derivatives over 4′ O-methyl derivatives, with 3″ and 4″ O-methyl derivatives formed in small amounts (Feng 2006, Kohri et al. 2003, Silberberg et al. 2005, Zhu et al. 2001). Additionally, glucuronidation of the A-ring does not appear to prohibit methylation by liver COMT isoforms (Feng 2006). The liver also possesses strong UDPGT and SULT activity (Feng 2006). Liver microsomes glucuronidate EGC and EGCG (8% to 12%) more effectively than intestinal epithelial microsomal fractions (1% to 3%), suggesting that ECG and EGCG are predominantly glucuronidate in the liver (Crespy et al. 2003). EC and EGCG are sulfated in the liver, and the liver appears to be the predominant site of PST expression (Feng 2006).

Individual flavan-3-ols are metabolized differentially, generating a diverse plasma profile of metabolites and native forms. EGCG is metabolized to a lesser extent than other species. EGCG was predominantly in the native form in plasma, following consumption of EGCG-rich green tea or Polyphenon E by humans (Chow et al. 2004, Stalmach et al. 2009, Van Amelsvoort et al. 2001). Some studies have reported phase-II metabolites of EGCG, including sulfated forms (58% to 72% of circulating species) and glucuronide forms (8% to 19% of circulating species) (Feng 2006). ECG exhibits similar plasma profiles to EGCG and is found predominantly in the native form in plasma, following consumption by humans (Chow et al. 2004, Stalmach et al. 2009, Van Amelsvoort et al. 2001). Native ECG was eight times more abundant than its

phase-II metabolites in plasma of rats fed pure ECG (Kohri et al. 2003). EGC exists in several metabolized forms in plasma (glucuronides, sulfates, O-methyl forms, O-methyl sulfates, and O-methyl glucuronides) (Chow et al. 2004, Stalmach et al. 2009, Yang et al. 1998). Following consumption of green tea, 14% of EGC was in methylated form (O-methyl or O-methyl conjugates) in plasma, whereas 10% was found as free form (Van Amelsvoort et al. 2001). C and EC appear to be the most extensively metabolized flavan-3-ols. C and EC predominantly exist as glucuronides, with some sulfates and O-methyl forms, in the plasma of rats fed C and EC (Harada et al. 1999, Piskula & Terao 1998, Silberberg et al. 2005). EC was almost exclusively phase-II metabolites in plasma following consumption of green tea and Polyphenon E in humans (Chow et al. 2001, 2004).

Metabolism by Intestinal Microflora

Small intestinal absorption and systemic (plasma/urine) bioavailability of intact catechins and their phase-II metabolites are poor (<25%), with most figures suggesting 0.1% to 10% (Donovan et al. 2002, Kohri et al. 2001a, Lee et al. 2002, Scalbert & Williamson 2000). These data suggest that a large portion of the ingested dose is not absorbed in the small intestine but rather reaches the colon and its microflora as native compounds (or phase-II metabolites that have been effluxed by enterocytes) (Kohri et al. 2001a, Scalbert & Williamson 2000). Additionally, native catechins and their phase-II metabolites may be excreted into the bile and reintroduced into the intestinal lumen via enterohepatic recycling (Donovan et al. 2001, Harada et al. 1999, Kohri et al. 2001b).

The colon harbors a complex bacterial ecology composed of more that 500 species and a bacterial load of approximately 10^9–10^{12} cells g^{-1} of luminal contents (O'Hara & Shanahan 2006, 2007). The metabolic capacity of colonic bacteria results in extensive fermentation of unabsorbed material, and colonic bacteria metabolize polyphenols to simpler metabolites (Bravo et al. 1994, Kohri et al. 2001a). In vitro fermentation studies using fecal inocula have demonstrated that fecal bacteria metabolize 5% to 100% of polyphenols (Justesen et al. 2000, Lin et al. 2003, Tzounis et al. 2008, Winter et al. 1989). Native polyphenols are extensively degraded in the colon by a variety of reactions to generate a wide array of 1,3-diphenylpropanes, γ-valerolactones, phenylalkyl carboxylic acids, benzoic acids, and other aromatic compounds (**Figure 4**) (Kohri et al. 2003, Lin et al. 2003, Simons et al. 2005, Tzounis et al. 2008). Following formation, colonic bacterial metabolites are absorbed into the bloodstream, providing another source of potentially bioactive compounds (Gonthier et al. 2003, Kohri et al. 2001b, Rios et al. 2003).

Excretion and Elimination

Circulating flavan-3-ols and their metabolite forms are largely extracted from the bloodstream by the kidneys and subsequently excreted in the urine. Glucuronide and sulfate conjugates appear to be more readily excreted into the urine than the native forms (Lambert et al. 2003, Yang et al. 2000). C, EC, and EGC appear to be readily excreted in the urine as glucuronides, sulfates, and O-methylated forms of these conjugates (Auger et al. 2008, Chow et al. 2004, Li et al. 2001, Stalmach et al. 2009, Van Amelsvoort et al. 2001, Yang et al. 2000). In spite of the high urinary excretion of the other flavan-3-ols, human studies have reported that virtually no EGCG is excreted in the urine in conjugated, O-methylated, or native forms; similarly, little ECG is believed to be excreted in the urine in any form (Auger et al. 2008, Chow et al. 2004, Stalmach et al. 2009, Yang et al. 1998, 2000). Free EGCG, ECG, and O-methylated forms of these and other flavan-3-ols are believed to be secreted from the liver into bile, either by first-pass or subsequent metabolism (Harada et al. 1999, Kohri et al. 2003, Yang et al. 1998).

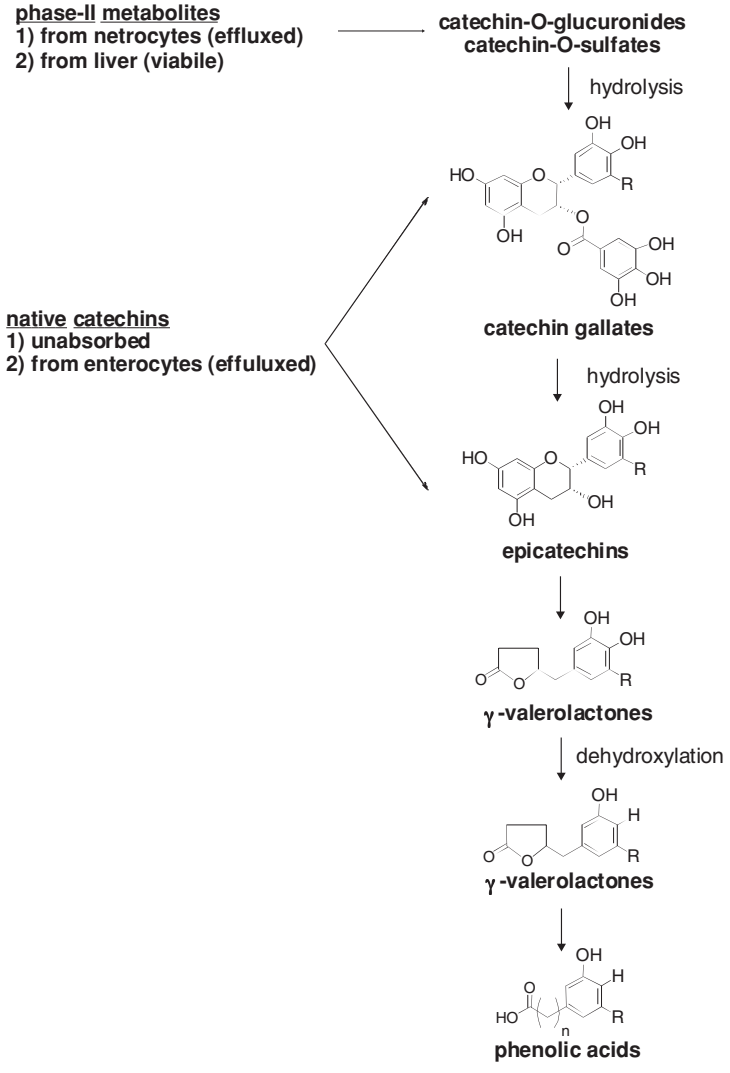

Figure 4

Colonic metabolism of dietary epicatechins.

FACTORS AFFECTING FLAVAN-3-OL BIOAVAILABILITY AND METABOLISM

The impact that food formulation and processing have on flavan-3-ol bioavailability is particularly critical for tea and cocoa, considering they are typically consumed as formulated products rather than purified extracts or supplements. Although cocoa is most commonly consumed as chocolate, tea may be formulated by consumers and food processors with specific adjuncts. In such complex food systems, both physical and chemical interactions between the flavan-3-ols and the food matrix may impact preabsorptive and absorptive events, ultimately influencing circulating flavan-3-ol profiles in humans.

Numerous pharmacokinetic investigations of flavan-3-ol absorption in humans are reported in the literature. Generally, these studies follow the appearance of individual flavan-3-ols and their metabolites in plasma and urine, following an acute dose of tea- or cocoa-containing foods/beverages. Several pharmacokinetic parameters are subsequently calculated and reported, including area under the plasma pharmacokinetic curve (AUC), maximum plasma flavan-3-ol concentration (C_{MAX}), and time of maximum plasmaflavan-3-ol concentration (T_{MAX}). The following discussion focuses on these parameters in describing the impact of food matrix and formulation on flavan-3-ol bioavailability.

Tea

As one of the most prominent dietary sources, the bioavailability of tea flavan-3-ols has been the subject of numerous clinical studies, some of which are summarized in **Table 1** (Chow et al. 2003; Henning et al. 2004; Kyle et al. 2007; Lee et al. 2002; Puch et al. 2008; Reddy et al. 2005; Stalmach et al. 2009, 2010; Van Amelsvoort et al. 2001; van het Hof et al. 1998; Warden et al. 2001; Yang et al. 1998). From plain green and black tea products, flavan-3-ols appear to be rapidly absorbed following consumption, with plasma C_{MAX} levels varying between 0.5 h and 2 h postadministration followed by rapid metabolism and clearance and a return to baseline levels within 8 h to 12 h postadministration. Interestingly, bioavailability of gallated catechins (EGCG and ECG) appears to be markedly lower than nongallated catechins (EGC and EC), making EGC and EC metabolites the most abundant circulating tea-derived flavan-3-ols in humans (Henning et al. 2004, Stalmach et al. 2009, 2010, Van Amelsvoort et al. 2001, Warden et al. 2001).

Impact of formulation to the bioavailability of flavan-3-ols from tea. Tea is commonly consumed with food and/or formulated with sweeteners (caloric and noncaloric) and creamers (dairy or nondairy). It appears that absorption of flavan-3-ols from tea may be influenced by consumption with or without a meal. Chow et al. (2005) reported overall bioavailability (measured as AUC) to be approximately fourfold higher in participants administered 400 mg EGCG as a green tea extract (Polyphenon E) in a fasted (127 ng*min ml^{-1}) compared to fed state (37 ng*min ml^{-1}). Additionally, average T_{MAX} values were lower in the fasted state (~1.5 h) relative to the fed state (~2 h), suggesting that coconsumption with food may slow the rate and extent of flavan-3-ol from tea.

In addition to consumption with food, tea is commonly prepared with milk. Several studies have assessed the influence of milk on the bioavailability of flavan-3-ols from black and more recently green tea. Van het Hof et al. (1998) reported that addition of skim milk did not impact any of the pharmacokinetic parameters (AUC, C_{MAX}, T_{MAX}, or $t_{1/2}$) of flavan-3-ols from black tea. However, Reddy et al. reported that the presence of milk with black tea did not negate increases in plasma antioxidant activity but did lower plasma AUC of total catechins over 3 h in subjects consuming milk with black tea compared to plain (0.95 versus 1.14 min*μM, respectively) (Reddy et al. 2005). These findings should be considered in the context of potential differences in kinetics of absorption and that plasma levels were only monitored for 3 h. Overall, these results suggest that formulation of tea with milk has a limited impact on absorption of flavan-3-ols from tea.

In addition to traditional in-home preparation, commercial ready-to-drink tea products have expanded in popularity in recent years. These products are often formulated with food additives such as ascorbic acid and EDTA (antioxidants and chelators), as well as citric acid or other acidulants and buffers to minimize loss of flavan-3-ols to autooxidative reactions in beverage systems (Chen et al. 1998). Additionally, tea beverages blended with other botanical extracts and fruit juices are increasingly common in the marketplace. Although these products are becoming a large portion

of the tea market in the United States (Del Rio et al. 2010), limited information is currently available on the potential impact of these added ingredients on bioavailability of flavan-3-ols from tea.

As described previously, the primary flavan-3-ols in tea (EGC and EGCG) are sensitive to autooxidation reactions, and conditions of the small intestinal lumen may facilitate such reactions, leading to a diminished bioaccessibility (Green et al. 2007, Neilson et al. 2007, Record & Lane 2001). Similar to ascorbic acid's stabilizing effect in beverage systems (Chen et al. 1998), formulation of green tea with ascorbic acid has been reported to markedly enhance digestive stability (bioaccessibility) of EGCG and EGC in in vitro models (Green et al. 2007, Peters et al. 2010). Furthermore, formulation of green tea with sucrose or ascorbic acid–rich citrus juices enhanced in vitro digestive recovery, suggesting that these formulation factors may enhance bioavailability in vivo (Green et al. 2007). These data are in line with the observation that EGCG absorption from green tea extracts was enhanced 14% in humans by coformulation of tea with nutrient-rich mixtures including ascorbic acid (Gawande et al. 2008). Similarly, bioavailability of EGC and EGCG was enhanced by 2.5- to 3-fold in rats treated with green tea [50 mg kg^{-1} body weight (BW)] formulated with 1.25 g kg^{-1} BW sucrose and 10 mg kg^{-1} BW ascorbic acid compared to unformulated green tea (GT) (Peters et al. 2010). Combined, these data suggest that formulation of tea products with common food additives may alter absorption of bioactive flavan-3-ols. However, more research is required to determine the clinical relevance of these modifications to flavan-3-ol bioavailability and the extent to which metabolism of tea derived flavan-3-ols may be impacted by formulation.

Cocoa and Chocolate

Although C and EC are the predominant monomeric flavan-3-ols in chocolate, C is typically present at extremely low concentrations in blood, relative to EC, in the majority of studies. Therefore, the majority of published data regarding the bioavailability of monomeric flavan-3-ols from chocolate has focused exclusively on EC. This phenomenon is likely due to three primary factors: (*a*) the lower C content of most cocoa powders relative to EC (EC is typically present at 2- to 5-fold higher concentrations) (Cooper et al. 2007, Gu et al. 2006, Natsume et al. 2000), (*b*) the fact that, unlike most foods, C in cocoa is predominantly (−)-C as opposed to (+)-C (Cooper et al. 2007, Donovan et al. 2006), and (*c*) the reported lower bioavailability of (−)-C compared to (+)-C and (−)-EC (Baba et al. 2001, Donovan et al. 2006). It should be noted that the reverse-phase high performance liquid chromatography (HPLC) methods typically used to assess C and EC levels do not resolve (+)-C and (−)-C, and therefore both elute as one peak and are quantified together collectively as (±)-C in biological samples.

Bioavailability. Owing to its typical consumption in beverages and confections, the food matrix composition of chocolate has great potential to modulate the absorption and pharmacokinetics of flavan-3-ols. The main factors affecting the pharmacokinetics of flavan-3-ols from cocoa are the macronutrient composition [carbohydrates (typically sucrose), lipids, and proteins (typically milk or milk solids)] and physical state (liquid versus solid) of the product. Numerous studies have been performed on the bioavailability of EC, and these are summarized in **Table 2** (Engler et al. 2004, Heiss et al. 2005, Holt et al. 2002, Keogh et al. 2007, Mullen et al. 2009, Muniyappa et al. 2008, Rein et al. 2000, Richelle et al. 1999, Roura et al. 2005, Schramm et al. 2001, Schroeter et al. 2006, Serafini et al. 2003, Taubert et al. 2007, Wan et al. 2001, Wang et al. 2000, Wiswedel et al. 2004).

Carbohydrates, and particularly sucrose, have generally been reported to increase C_{MAX} of EC relative to control and other macronutrients (lipid, milk protein) for confections as well as

Table 1 Plasma bioavailability of catechins from tea

Study	Formulation	Time (h)	Compound	Dose (mg)	AUC (nM*h)	C_{MAX} (nM)	T_{MAX} (h)	AUC/dose (nM*h mg^{-1})	C_{MAX}/dose (nM mg^{-1})
Stalmach 2010	GT	24	EC+C[a]	18	1120	369	0.8–1.3	61.6	20.3
			EGC+GC[a]	73	1720	487	0.5–2.2	23.4	6.6
			ECG	28	50	17	1.0	1.8	0.6
			EGCG+GCG	111	90	35	0.6	0.8	0.3
Stalmach 2009	GT	24	C+EC[a]	19.4	~1020	~208	~1.7	52.6	10.7
			GC+EGC[a]	89.7	~1320	~251	~2.2	14.7	2.8
			ECG	21.7	120	25	1.6	5.5	1.3
			EGCG+GCG	109	170	55	1.9	1.6	0.5
Puch 2008[b]	GT, milk, w/ meal	0-6	Total catechins	47	248	98	2	5.3	2.1
	GT w/ meal				310	88	4.5	6.6	1.9
Kyle 2007[c,d]	BT	0-3	Total catechins	395 μmol	?	~350	1.3	?	?
	BT, 25% milk				?	~300	1.3	?	?
Reddy 2005	BT, sugar	0-3	Total catechins	~200	1140	670	2	~5.7	~3.4
	BT, sugar, 20% milk				950	420	2	~4.8	~2.1
Henning 2004	GT	0-8	EC	76.5	1010	330	1.2	13.2	4.3
			EGC	269.6	2590	740	1.3	9.6	2.7
			ECG	119.3	320	82	1.4	2.7	0.7
			EGCG	213.6	270	80	1.3	1.3	0.4
	BT		EC	39.8	270	80	1.4	6.8	2.0
			EGC	103.4	970	220	1.5	9.4	2.1
			ECG	122.5	290	70	1.5	2.4	0.6
			EGCG	230.8	370	100	1.4	1.6	0.4
Chow 2003	EGCG	24	EGCG	400	1707	301.3	3.1	4.3	0.8
	EGCG (after 4-week exposure)				1598	351.5	2.3	4.0	0.9
	EGCG			800	3479	513.1	3.7	4.3	0.6
	EGCG (after 4-week exposure)				5298	851.5	3.5	6.6	1.1

Lee 2002	GT	0-24	EC	? (20 mg GT solids/kg body weight)	1826	428	1.2	?	?
			EGC		3089	730	1.3	?	?
			EGCG		1110	170	1.6	?	?
	GT, decaffeinated		EC		533	112	1.0	?	?
			EGC		964	262	1.1	?	?
			EGCG		198	53	1.2	?	?
Warden 2001[c]	BT, co-consumed w/ sugar cookie (4 servings over 6 h)	0-24	EC	67	?	135	7	?	2.0
			EGC	61.9	?	72	5	?	1.2
			ECG	124.6	?	22	24	?	0.18
			EGCG	146.2	?	16	5	?	0.1
Van Amelsvoort 2001	Pure compounds in hot water w/ syrup	0-24	EGC[a]	459	65,500	13,600	1.4-2	142.7	29.6
			ECG	663	12,100	1300	4	18.3	2.0
			EGCG	687	39,900	3100	2.9	58.1	4.5
Yang 1998	GT, decaffeinated, 45 g sugar, 8 g coffee creamer	25	EC	37.5	963	190	1.4	25.7	5.1
				75	3654	652	1.8	48.7	8.7
				112.5	4137	656	1.8	36.8	5.8
			EGC	68	2018	484	1.4	29.7	7.1
				136	8152	1661	1.8	59.9	12.2
				204	10,729	1799	1.3	52.6	8.8
			EGCG	73	1955	259	1.6	26.8	3.5
				146	4846	711	2.4	33.2	4.9
				219	5367	700	2.7	24.5	3.2
van het Hof 1998	GT	0-8	Total catechins	930	2220	550	2.3	2.4	0.6
	BT			300	530	170	2.2	1.8	0.6
	BT, 17% milk			300	600	180	2	2.0	0.3

Abbreviations: GT, green tea; BT, black tea.

[a]Sum of reported metabolites.

[b]Units are as follows: AUC in $\mu g^{*}h\ L^{-1}$, C_{MAX} in $\mu g\ L^{-1}$, AUC/dose in $\mu g^{*}h/L\ mg^{-1}$, and C_{MAX}/dose in $\mu g/L\ mg^{-1}$. Easier to read?

[c]Baseline values subtracted.

[d]C_{MAX} is reported as nmol.

Table 2 Plasma bioavailability of cocoa catechins

Study	State	Formulation	Time (h)	Compound	Dose (mg)	AUC (nM*h)	C_{MAX} (nM)	T_{MAX} (h)	AUC/dose (nM*h mg^{-1})	C_{MAX}/dose (nM mg^{-1})
Mullen 2009[a]	L	Water, 1 g paracetamol, 5 g lactulose	0–8	C+EC	13.1	296	143	1-1.4	22.6	10.9
		Milk, 1 g paracetamol, 5 g lactulose				260	127	1.3	19.8	9.7
Neilson 2009	L	Water, 6 g milk solids	0–6	EC	27	143	42	1.1	5.3	1.6
		Water, 15 g sugar, 6 g milk solids				132	43	0.9	4.9	1.6
	S	20 g fat, 7 g sugar	0–6	EC	27	121	32	2.3	4.5	1.2
		12 g fat, 15 g sugar				128	34	1.8	4.7	1.3
		14 g fat, 7 g sugar, 6 g milk solids				101	25	2.3	3.7	0.9
Muniyappa 2008[b]	L	Water, 1g fat, 17g CHO, 9g protein	0–3	C+EC	118	1754	765	1.4	14.9	6.5
Roura 2007[b]	L	Water, 58 g CHO, 2 g fat, 6 g protein	2	EC	28	?	330	?	?	11.8
		Milk, 31 g CHO, 11 g fat, 14 g protein				?	274	?		9.8
Taubert 2007	S	2 g fat, 3 g CHO, 0.3 g protein	0–8	C	1.7	13	3.9	1.3	7.6	2.3
				EC	5.1	44	12.5	1.3	8.6	2.5
Keough 2007	L	Water, 7 g fat, 7 g sugar	0–8	C	? (2 g	1100	210	~3.5	?	?
		3 g fat, 8 g (sugar+		EC	polyphenols	58,615	12,890	3		
		lactose), 3 g milk		C		1075	200	2		
		protein		EC		58,340	12,420	3		
Schroeter 2006[a,c]	L	Water, ?	0–6	C+EC	?	8875	150	2.5	?	?
Heiss 2005	L	Water, 0.5 g fat, 6 g sugar, 1.5 g protein	0–2	C	?	?	9	?	?	?
				EC	~10.5		188			~17.9
		Water, 1 g fat, 12 g sugar, 3 g protein		C	?		19			?
				C	~21		289			~13.8
		Water, 2 g fat, 25 g sugar, 6 g protein		EC	?		18			?
					~42		386			~9.2
Roura 2005	L	Milk	2	EC	54	?	626	?	?	11.6
Engler 2004	S	15 g fat, 21 g sugar, 2 g protein	2	EC	46	?	~200	?	?	~4.3

Serafini 2004	S	Dark chocolate Dark chocolate + 200 mL milk Milk chocolate	4	EC	?	225 120 69	?	?	?	?
Wiswedel 2004	L		2	EC	? (187 mg favanol)	?	144	?	?	?
Schraam 2003	P	Control, water	0–8	EC	1.53 mg C+EC kg^{-1} body weight (~107 mg for 70 kg subject)	4230	1022	~1.5	?	?
		Sugar, water (69 g CHO)				5172	1209	~1.5		
		High sugar, water (138 g CHO)				6072	1436	~2		
		Control, water				4398	1185	~1.5		
		Bread, water (3 g fat, 45 g CHO, 7 g protein)				5748	1517	~1.5		
		Butter, water (29 g fat, 0 g CHO, 0 g protein)				4171	1177	~1.5		
		Steak, water (9 g fat, 0 g CHO, 48 g protein)				4966	1221	~1.5		
		Control, water				4930	1109	~1.5		
		Bread, water (3 g fat, 45 g CHO, 7 g protein)				6954	1514	~1.5		
		Milk (14 g fat, 20 g CHO, 14 g protein)				5769	1163	~1.5		
		Grapefruit juice (1 g fat, 61 g CHO, 3 g protein)				5944	1273	~1.5		
Holt 2002	L	Water, co-consumed w/ bread	0–6	C EC	? (323 mg C+EC)	?	160 5920	2 2	?	?
Wan 2001	P/S	?	0–24	EC	? (111mg C+EC)	?	36	2	?	?
Schraam 2001	S	12.2 g fat, 18.8 g CHO, consumed w/ bagel	0–6	EC	40.7	?	21	2	?	0.5
Rein 2000	S	27 g fat	0–6	EC	137	?	257	~2	?	1.9
Wang 2000	S	?, consumed w/ bread: 0.8 g fat, 25 g CHO, 4.5 g protein)	0–6	EC	27	500	133	2	18.5	4.9
					53	1000	258	2	18.9	4.9
					80	1500	355	2	18.8	4.4
Richelle 1999	S	?, consumed w/ bread, water	0–8	EC	82	1534	355	2	18.7	4.3
					164	3686	676	2.6	22.5	4.1

[a] Sum of reported metabolites.
[b] EC, glucuronide.
[c] AUC estimated from the author's published data.
Abbreviations: S, solid; L, liquid; P, powder.

beverages (Neilson et al. 2009, Roura et al. 2007, Schramm et al. 2003). Although the mechanism by which sucrose enhances the absorption rate of catechins is unclear, similar studies with green tea have indicated that formulation with sucrose may improve catechin bioavailability by enhancing solubility and intestinal uptake (Peters et al. 2010).

The formulation factor that has been the most controversial for chocolate is the presence of milk and milk protein. Several studies have been performed regarding the influence of milk protein on the bioavailability of EC from cocoa beverages and chocolate. Serafini et al. (2003) reported that milk resulted in a reduced AUC for EC relative to control in chocolate confections, whereas other studies (Keogh et al. 2007, Roura et al. 2007, Schramm et al. 2003, Schroeter et al. 2003) reported no statistical difference between the AUC of EC from cocoa beverages consumed with water or milk. It is critical to note that Serafini examined confections, whereas the studies demonstrating no difference between milk and control were performed using cocoa beverages. Recently, we (Neilson et al. 2009) compared absorption of EC from beverages versus confections with differing macronutrient composition, finding that the AUC and C_{MAX} of EC from a milk chocolate confection were lower, though not significantly different, than control dark chocolate. However, the highest AUC and C_{MAX} values in this study were observed from milk-containing beverages of these chocolate formulations. Taken together, these studies suggest that milk protein may modulate the pharmacokinetics of flavan-3-ol absorption from confections, exerting a mild, but not always significant, suppressive effect on their bioavailability.

In addition to milk protein, the lipid content of cocoa and chocolate products has been associated with lower AUC and C_{MAX} of EC in confections (Neilson et al. 2009, 2010a, Roura et al. 2007, Schramm et al. 2003). However, this effect may be related to slower gastric emptying induced by lipid and digestive release of EC from the food matrix, as the lipid matrix must melt and be emulsified for EC to be solubilized in the intestine.

In addition to macronutrient composition of either beverages or confections, the physical form of the product may play a large role in determining the relative pharmacokinetic properties of cocoa-containing products, specifically the rate of absorption from the intestine and the subsequent plasma T_{MAX} and C_{MAX}. It is possible that the physical state of the food matrix may significantly modulate GI mobility (stomach-emptying time and transit through the intestine) and the rate of EC release and solubilization in the intestine, resulting in the observed distinct pharmacokinetic curve shapes and parameters between beverages and confections (Neilson et al. 2009). For example, milk does not appear to exert the same suppressive effects of EC bioavailability in beverages compared to confections. Milk-containing beverages produce generally higher serum AUC and C_{MAX} values than confections formulated with or without milk (Neilson et al. 2009). Rapid emptying of beverages from the stomach and rapid digestive release from beverages compared to confections may explain a more rapid appearance of EC (T_{MAX}) in the blood. Additionally, slower absorption from confections may result in pharmacokinetic curves that do not return to baseline as quickly as beverages. This may result in incomplete curves with apparently different AUC values that may in fact be similar if the entire curve were available (Neilson et al. 2009, 2010a; Serafini et al. 2003).Overall, these findings suggest that the absorption rate, but not the bioavailability of EC (AUC) from physiologically relevant doses of cocoa and chocolate, is more likely to be influenced by physical form rather than ingredient composition.

CONCLUSIONS

Interest in the bioavailability of flavan-3-ols from foods has grown because of the epidemiological and biological evidence of their health effects. Absorption of flavan-3-ols from food is a complex multistep process that appears to be influenced by several factors including (*a*) botanical source

and flavan-3-ol profile, (*b*) type and extent of food processing, and (*c*) formulation and product formulation/composition. Bioavailability of flavan-3-ols from tea appears to be differentially affected by formulation with carbohydrate and ascorbic acid positively influencing absorption, whereas milk is believed to have minimal impact on overall bioavailability of these compounds from tea. Interestingly, for cocoa products, bioavailability of flavan-3-ols (C and EC, specifically) do not appear to differ greatly based on formulation, but the physical state of the product may influence pharmacokinetic parameters, including T_{MAX} and C_{MAX}, suggesting that beverages may be employed for more rapid absorption and higher peak plasma levels, whereas confections may provide more sustained plasma levels of flavan-3-ols.

Future efforts should consider these factors when designing experiments to assess the efficacy or bioavailability of flavan-3-ol from food products. Also, specific information on how food formulation factors influence metabolism and tissue distribution of flavan-3-ols remains limited and requires additional exploration. Finally, definition of target tissue profiles and identification of biologically active flavan-3-ol metabolites are also required to better define food matrix factors that favor delivery of physiologically relevant flavan-3-ol forms.

DISCLOSURE STATEMENT

Mario G. Ferruzzi has received grants and honoraria from, and has consulted for, food, beverage, and ingredient companies with interests in flavan-3-ols, including but not limited to Kraft Foods, Mead Johnson, Sensient Flavors, and Heinz.

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Errata

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