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Interaction between Phenolics and Gut Microbiota: Role in Human Health

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Dietary phenolic compounds are often transformed before absorption. This transformation modulates their biological activity. Different studies have been carried out to understand gut microbiota transformations of particular polyphenol types and identify the responsible microorganisms. Although there are potentially thousands of different phenolic compounds in the diet, they are typically transformed to a much smaller number of metabolites. The aim of this review was to discuss the current information about the microbial degradation metabolites obtained from different phenolics and their formation pathways, identifying their differences and similarities. The modulation of gut microbial population by phenolics was also reviewed in order to understand the two-way phenolic−microbiota interaction. *Clostridium* and *Eubacterium* genera, which are phylogenetically associated, are other common elements involved in the metabolism of many phenolics. The health benefits from phenolic consumption should be attributed to their bioactive metabolites and also to the modulation of the intestinal bacterial population.

KEYWORDS: *Eubacterium*; *Clostridium*; polyphenols; phenolics; flavonoids; tannins; human metabolism; gut microflora; microbial metabolism; bioavailability

INTRODUCTION

Phenolic compounds are currently receiving much attention because of their beneficial health effects related to their antioxidant, anti-inflammatory, antiestrogenic, cardioprotective, cancer chemopreventive, and neuroprotective properties (*1*−*6*). Most dietary polyphenols are transformed in the colon by the intestinal microbiota before absorption. This conversion is often essential for absorption and modulates the biological activity of these dietary compounds (*7*, *8*). Furthermore, dietary polyphenols are substrates for several enzymes located in the small intestine and colon and in the liver (hydrolyzing and conjugating enzymes) (*9*−*16*). Therefore, the colon has to be considered as an active site for metabolism rather than a simple excretion route and deserves further attention from the scientific community (*17*). Recent studies have investigated the relevance of the intestinal microbial activation of polyphenols in human health.

Gut bacteria can hydrolyze glycosides, glucuronides, sulfates, amides, esters, and lactones. They also carry out ring-cleavage, reduction, decarboxylation, demethylation, and dehydroxylation reactions (*17*−*19*). The hydrolysis of glycosides results in metabolites that are potentially more biologically active than the parent compounds. Further bacterial transformation of aglycones can lead to production of more or less active compounds, depending on the substrate being metabolized and the products formed (*18*, *19*). Several studies have been carried out to understand the transformations of particular polyphenol types and to identify the microorganisms involved during their colonic fermentation, which varies depending on the chemical structure.

On the other hand, phenolic compounds are also antimicrobial and can interact with the gut microbiota, producing a modulation of the microbial population of the gastrointestinal (GI) tract. This has effects on GI health and also in the metabolism of dietary phenolics (*20*, *21*).

The aim of this review was to summarize the current information about the microbial degradation metabolites and their formation pathways obtained from the different groups of dietary phenolic compounds, identifying their differences and similarities. The modulation of gut microbial population by phenolics has also been reviewed in order to understand the two-way phenolic−microbiota interaction. Recent progress in the identification of colonic microbial species responsible for phenolic metabolism and novel tools used to identify them and the effects of polyphenol microbial metabolism on their bioavailability and bioactivity were also reviewed.

MICROBIAL METABOLIC PATHWAYS AND METABOLITES OF THE DIETARY PHENOLIC COMPOUNDS

In nature, phenolics are usually found conjugated to sugars and organic acids and can be classified into two major types: flavonoid and nonflavonoid phenolics. All flavonoid phenolics share a basic structure consisting of two benzene rings (A and B) linked through a heterocyclic pyrone C ring (**Figure 1**). In contrast, nonflavonoid phenolics include a more heterogeneous group of compounds (**Figure 2**) including from the simplest of the class such as C_6-C_1 benzoic acids and C_6-C_3 hydroxycinnamates to

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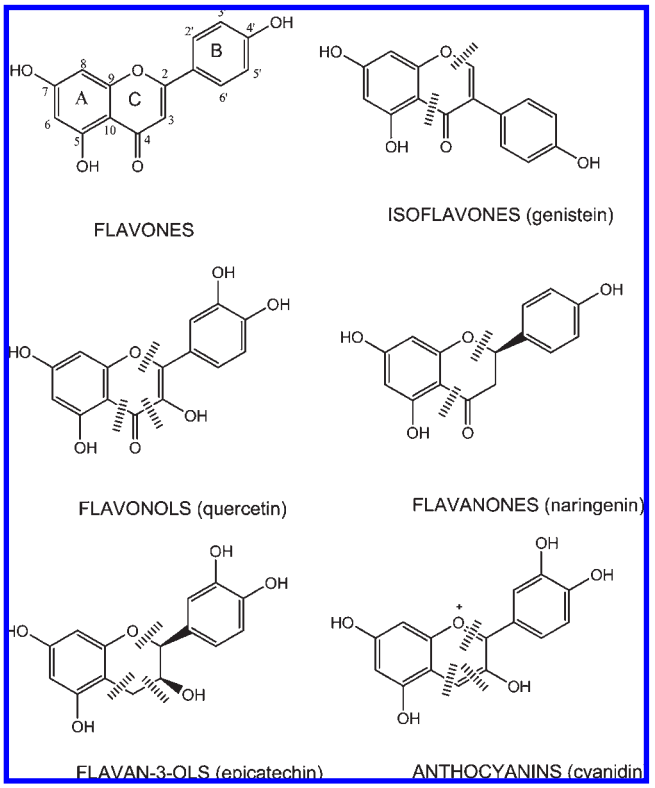

Figure 1. Microbiota heterocyclic C-ring cleavage of flavonoids. (III) Positions of the potential C-ring cleavages.

more complex compounds such as $C_6-C_2-C_6$ stilbenes, $C_6-C_3-C_3-C_6$ lignans and hydrolyzable tannins, gallotannins, and ellagitannins, with the principal component being gallic acid and hexahydroxydiphenic acid that upon hydrolysis releases ellagic acid.

Deconjugation of Phenolics. Deconjugation is the cleavage of either the glycosyl or glucuronosyl moiety from the phenolic backbone, resulting in an aglycone formation (*17*, *19*). Macdonald and Mader (*22*) showed that cell extracts of feces and saliva (fecalase and salivase) hydrolyze flavonoid glycosides to their corresponding aglycones. Some phenolics can also be transported through the epithelium as glycosides by sugar transporters. In the epithelial cells, cytosolic β-glucosidase hydrolyzes these glycosides, and aglycones are formed after absorption. Aglycones can also be formed in the lumen by the action of membrane-bound lactasephlorhizin-hydrolase (LPH), and they are absorbed passively through the epithelium (*23*, *24*). Deconjugation is catalyzed by fecal microbial enzymes (α-rhamnosidase, β-glucosidase, and β-glucuronidase), the specific activities of which reflect the in vitro deconjugation rates of phenolic compounds, when a fecal suspension is used (*17*). This is the case of three obligate anaerobic bacteria isolated from the human intestinal flora that were capable of hydrolyzing glycosides to aglycones (*25*) (**Table 1**). *Bacteroides distasonis* hydrolyzed robinin to kaempferol, and *Bacteroides uniformis* and *Bacteroides ovatus* converted rutin to quercetin. Microbial metabolites are absorbed from the colon after deconjugation and transformated by the human cell enzymes into phase II conjugates including methyl ether glucuronides and sulfates metabolized in the liver, resulting in their glucuronidated and sulfated derivatives (*9*−*16*). Through enterohepatic recirculation, conjugated compounds are excreted by the liver as components of bile into the intestine, and the deconjugated compounds are regenerated by microbial enzymes before being reabsorbed.

Flavonoid Phenolics. *Flavonols*. Flavonols are a class of flavonoids that use the 3-hydroxyflavone backbone (3-hydroxy-2-phenylchromen-4-one) (**Figure 1**). The basic ring system for flavonols is planar. Their diversity stems from the different positions of the phenolic −OH groups. Onions, apples, broccoli, tea, and red wine are rich in flavonols and are the main dietary sources of these compounds in Western populations (*26*). Flavonol intakes have been reported to vary widely across countries (*27*), with some of the lowest intakes being reported for northern European populations (*28*), whereas populations from the United States (*29*) and other European countries (*30*) have among the highest reported intakes. In Western populations, estimated daily intake is in the range of 20−50 mg/day for flavonols. These flavonoids are extensively degraded by colonic microbiota to produce simpler phenolic compounds derived from A and B rings after the flavonoid C ring has been broken (*31*, *32*). In this case the C-ring breakdown takes place at different positions, giving rise to a higher number of simple phenolics, and the equivalents to equol and *O*-DMA from daidzein are not present. Thus, quercetin gives 2-(3,4-dihydroxyphenyl)acetic acid, 2-(3-hydroxyphenyl)acetic acid, and 3,4-dihydroxybenzoic acid from the B ring (**Figure 3**), whereas phloroglucinol, 3-(3,4-dihydroxyphenyl)propionic acid, and 3-(3-hydroxyphenyl)propionic acid are produced from the A ring (**Figure 3**). Other metabolites such as 3,4-dihydroxybenzaldehyde, 2,4,6-trihydroxybenzoic acid, 2-(3,4-dihydroxyphenyl)ethanol, 3-(3,4-dihydroxyphenyl)benzoic acid methyl ester, 3-methoxy-4-hydroxybenzoic acid (vanillic acid), and 3-(*m* or *p*-hydroxyphenyl) propionic acid have also been reported. The hydroxylation pattern of the B ring affects the type of phenolic compounds produced. Flavonols with trihydroxylation on ring B, as is the case of myricetin, which has a 3′,4′,5′-trihydroxy-ring B, release 2-(3,5-dihydroxyphenyl)acetic acid, 2-(3-hydroxyphenyl)acetic acid, and 2-(3,4,5-trihydroxyphenyl)acetic acid. In the case of kaempferol (which has a 4′-hydroxy-ring B), only the metabolite 2-(4-hydroxyphenyl)acetic acid has been found (*19*, *29*) (**Table 2**). Therefore, in the intestine, flavonols seem to be transformed first by C-ring fission and then by dehydroxylation reactions (**Figure 3**). However, the corresponding aglycone is released from flavonol glucosides and rutinosides before specific enzymes of C-ring cleavage, demethylation, and dehydroxylation reactions transform these compounds (**Table 1**).

The fission of the flavonol C ring appears to be produced by breaking the bond between the 1- and 2-positions as occurs with isoflavones. In addition, in these flavonoids the C-ring cleavage can also be produced in the bonds between the 3- and 4-positions or between the 4- and 10-positions (**Figures 1** and **2**). These differences in the C-ring cleavage pattern of isoflavones and flavonols could be the consequence of the linkage of the B-ring in C-3 or C-2 of the C ring, respectively. The degradation rates of the quercetin monoglycosides showed a clear dependency on the hydroxyl pattern of the sugar moiety. The degradation of quercetin-3-*O*-β-D-glucopyranoside with all hydroxyl groups of the glucose in the equatorial position was the fastest. On the other hand, the intestinal metabolism of di- and trisaccharides (quercetin-3-*O*-β-D-rutinoside and quercetin-3-*O*-[α-L-dirhamnopyranosyl (1→2)-(1→6)-β-D-glucopyranoside, respectively) was much slower compared to that of the monoglycosides (quercetin-3-*O*-β-D-glucopyranoside) (*34*). The type of glycosylation can also affect the stability of the flavonoids in the intestine. However, the structure of the aglycone does not have much influence on the intestinal metabolism, whereas the type of glycosidic bond (*C*- or *O*-glycoside) has substantial influence on the degradation rate (*34*, *35*). In fact, the metabolism of a *C*-glycosidic bond seems to be much slower than the hydrolysis of an *O*-glycosidic bond according to a previous

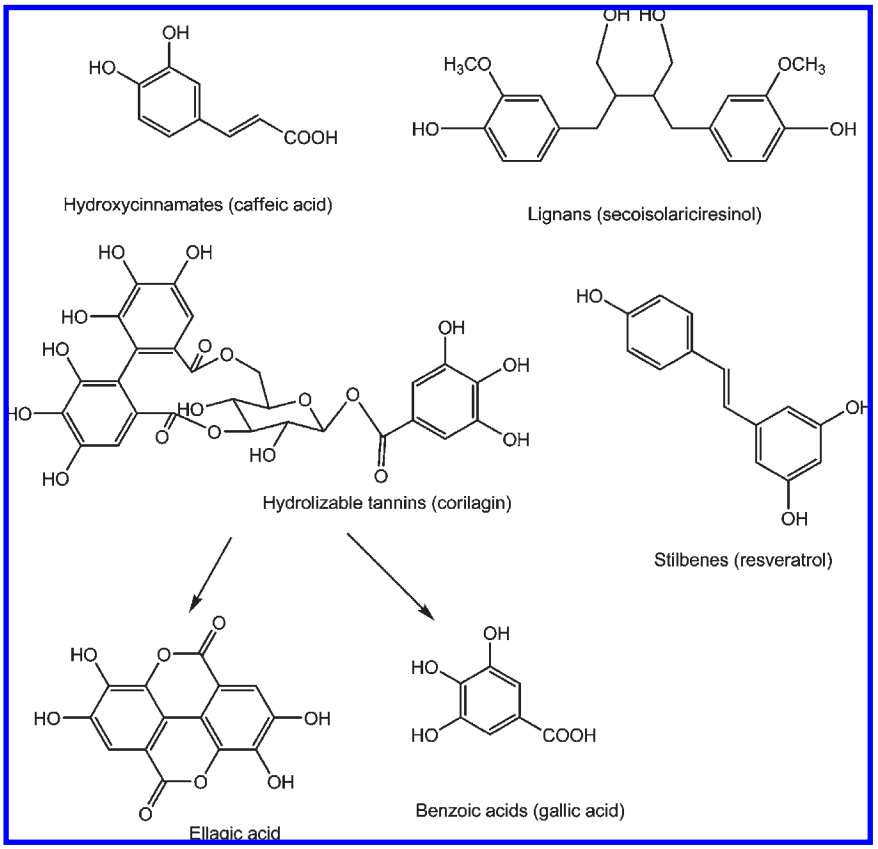

Figure 2. Nonflavonoid phenolics that are metabolized by the gut microbiota.

Table 1. Phenolic Enzymatic Reactions Achieved by the Human Intestinal Microbiota

reaction		compound	enzyme	microbiota containing enzymes	refs
hydrolysis	glucuronides	ellagitannins	β-glucuronidase	*Escherichia coli*	*182*
	glycosides	isoflavones, flavonols, flavanones, anthocyanins, ellagitannins, lignans	β-glucosidase	*Streptococcus faecalis*, *Eubacterium rectale*, *Clostridium sphenoides*, *Clostridium saccharogumia*, *Clostridium cocleatum*, *Bacteroides ovatus*, *Bacteroides fragilis*, *Bacteroides distasonis*	*138*, *182*
	ester	hydroxycinnamates	esterases	*Escherichia coli*, *Bifidobacterium lactis*, *Lactobacillus gasseri*	*107*, *139*
	carbonyl	isoflavones		*Bacteroide ovatus* spp., *Streptococcus intermedius* spp., *Ruminococcus productus*, SNU-Julong 732, *Enterococcus faecium*, *Lactobacillus mucosae*, *Finegoldia magna*, and *Veillonella* spp.	*16*, *119*−*123*
reductions		isoflavones, hydroxycinnamates, stilbenes	hydrogenases		*109*
dehydroxylation		flavonols, flavanones, hydroxycinnamates, ellagitannins, lignans	dehydroxylase	*Clostridium scindens*, *Eggerthella lenta*	*137*, *138*
demethylation		flavonols, flavan-3-ols, anthocyanins, lignans	demethylase	*Eubacterium limosum*, *Eubacterium callanderi*, *Streptococcus*, *Lactobacillus*, *Clostridium*, *Butyribacterium methylotrophicum*, *Peptostreptococcus productus*	*55*, *133*−*135*, *137*, *138*
decarboxylation		benzoic acids, hydroxycinnamates, ellagitannins	decarboxylase		*33*, *116*
isomerization		flavan-3-ols	isomerase		*46*
fission	ring	isoflavones, flavonols, flavanones, flavan-3-ols, anthocyanins		*Clostridium* spp. HGHA136, *Eubacterium ramulus*, *Clostridium orbiscindens*, *Eubacterium oxidoreducens*, *Butyrivibrio* spp.	*31*, *46*, *125*, *126*, *129*−*132*
	lactone	ellagitannins		*Butirivibrio* spp.	*136*

study (*34*). The metabolites are absorbed, and they can be detected in plasma and urine as phase II conjugates produced by the human cell enzymes, including methyl ethers as a product of catechol *O*-methyl transferase, and glucuronides and sulfates as products of

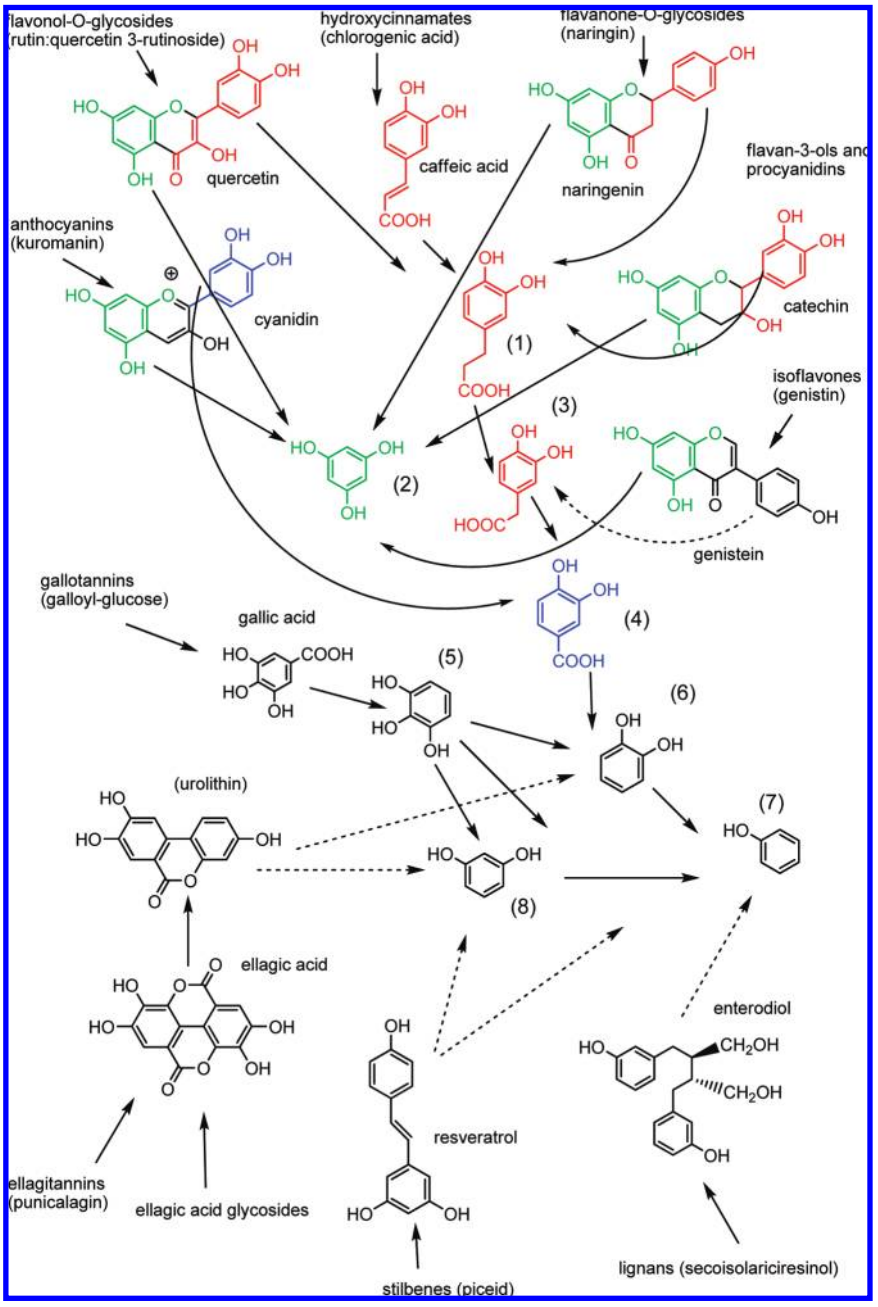

Figure 3. Confluence metabolic pathways and common metabolites of dietary phenolic compounds. (→) Demonstrated pathways; (- - ->) hypothetical pathways.

glucuronyl transferase and sulfate transferase. Conjugates with amino acids have also been described in humans and animals (glycinated derivatives). Glucuronidation and methylation occurs mainly in the intestinal cells and in the liver, whereas sulfation takes place mainly in the liver and kidney (*9*−*16*).

Flavanones. This class of flavonoids has a 2,3-dihydro-2-phenylchromen-4-one structure (**Figure 1**). The pyran ring of flavanones is nonplanar because of the saturation of the C2−C3 bond. These flavonoids can bind to estrogen receptors as occurs with the isoflavones, but the B ring is linked to the 2-position instead. Moreover, they do not have a double bond in the 2−3-position, in contrast to isoflavones (**Figure 1**). The major dietary sources of flavanones are citrus fruits. These compounds may make a greater contribution to the total daily intake of flavonoids than the more extensively studied flavonols (*36*). Also, flavanones seem to be more bioavailable than other related flavonoids such as flavonols or flavan-3-ols. This can be associated with the fact that these compounds are found to be less degraded than other flavonoids by colonic microbiota and therefore are more available for absorption, even in the distal part of the intestine. Flavanones occur as glycosides, usually rutinosides (6-*O*-α-D-rhamnosyl-D-glucosides) and neohesperidosides (2-*O*-α-L-rhamnosyl-D-glucosides) attached at position 7. They are characteristic compounds of citrus fruit and associated products. Grapefruit and sour (bitter) orange are dominated by neohesperidosides, mainly naringin in the former but similar amounts of naringin, neoeriodictyol, and neohesperidin in the latter. Flavanone glucosides are comparatively rare but are found in some species of *Mentha* used as culinary herbs (0.3 ± 3.3 g/kg of dry weight) (where they occur as 7-glucosides) (*37*) and as minor components of grapefruit, lemon, and sweet orange (where they occur as 4′-glucosides) (*38*). Flavanone glycosides, such as naringin (4′,5,7-trihydroxyflavanone-7-rhamnoglucoside), suffer first a deglycosylation to render the aglycone naringenin (**Figure 3**), and the aglycone, through C-ring

Table 2. Intestinal Microbiota Involved in the Metabolism of Flavonoids

phenolic compound		metabolite	responsible intestinal microbiota	refs
isoflavone	daidzein	equol	*Bacteroide ovatus* spp., *Streptococcus intermedius* spp., *Ruminococcus productus*, SNU-Julong 732 (AY310748), *Enterococcus faecium* EPI1, *Lactobacillus mucosae* EPI2, *Finegoldia magna* EPI3, and *Veillonella* spp. EP	*8*, *120*−*123*, *125*, *126*
	daidzein	*O*-demethylangolensin	*Clostridium* spp. HGHA136, *Eubacterium ramulus*	*125*, *126*
flavonol	quercetin	2-(3,4-dihydroxyphenyl)acetic acid	*Clostridium orbiscindens*, *Eubacterium oxidoreducens*, *Butyrivibrio* spp.	*31*, *83*
		2-(3-hydroxyphenyl)acetic acid 3,4-dihydroxybenzoic acid phloroglucinol 3-(3,4-dihydroxyphenyl)propionic acid 3-(3-hydroxyphenyl)propionic acid		
	kaempferol	2-(4-hydroxyphenyl)acetic acid	*Clostridium* strains	*31*
flavanone	naringenin	3-(4-hydroxyphenyl)propionic acid phloroglucinol	*Clostridium* strains	*31*
	isoxanthohumol	8-prenylnaringenin	*Eubacterium limosum*	*133*−*135*
flavan-3-ol	catechin and epicatechin	3-(3-hydroxyphenyl)propionic acid 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone 5-(3′-hydroxyphenyl)-γ-valerolactone 3-hydroxyhippuric acid pyrogallol 5-(3,4-dihydroxyphenyl)valeric acid 5-(3-hydroxyphenyl)valeric acid 3-(3,4-dihydroxyphenyl)propionic acid 5-(3-methoxyphenyl)valeric acid 2,3-dihydroxyphenoxyl 3-(3′,4′-dihydroxyphenyl) propionic acid	*Clostridium cocoides*−*Eubacterium rectale* group	*46*

cleavage, gives phloroglucinol and 3-(3,4-dihydroxyphenyl)propionic acid. This can be further dehydroxylated to produce 3-(*m*-hydroxyphenyl)propionic acid (**Figure 3**). Therefore, the flavanone degradation pathway is similar to that observed in flavonols and other flavonoids, including procyanidins (*19*). However, when the described metabolites are taken into account, C-ring cleavage seems to be produced by breaking the bonds between the 1- and 2-positions or the 4- and 10-positions but not between the 3- and 4-positions (**Figure 1**).

Flavan-3-ols. In contrast to other flavonoids, flavan-3-ols and procyanidins form a very complex group of polyphenols from simple flavan-3-ols (catechin and epicatechin; gallocatechin; and epigallocatechin and the corresponding gallate esters) to polymeric procyanidins known as condensed tannins, including the whole range of oligomeric intermediates from dimers to undecamers and dodecamers and then polymers. These polyphenols are some of the main constituents of the phenolic intake in the diet and are mainly provided by fruits, tea, and wine. The benzenoid B-ring position of the flavan-3-ols, such as catechin, epigallocatechin and epicatechin, is in the 2-position as occurs with flavanones and flavonols. However, the C ring of the flavan-3-ols does not have a carbonyl group in the 4-position or double bond in the 2−3-position, in contrast to flavonols and isoflavones (**Figure 1**). Flavan-3-ols are not planar, as is the case of flavanones.

During digestion and transfer across the small intestine, and in the liver, flavan-3-ols are rapidly metabolized by phase II enzymes to various O-sulfated, O-glucuronidated, and O-methylated forms (*39*−*41*). In fact, the methylated metabolites of catechin, epicatechin, and epicatechin gallate predominate over the original unmethylated forms in plasma (*42*, *43*). The main metabolites detected in urine after the intake of catechin and epicatechin are 3-(3-hydroxyphenyl)propionic acid, 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone, 5-(3′-hydroxyphenyl)-γ-valerolactone, and 3-hydroxyhippuric acid (produced by a combination of bacterial and human metabolism) (*19*, *44*, *45*) (**Figures 4** and **5**). Similar results were found in a recent in vitro study in which incubation of (−)-epicatechin or (+)-catechin with fecal bacteria led to the generation of 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone, 5-phenyl-γ-valerolactone, and 3-phenylpropionic acid (*46*) (**Table 2**). However, the formation of these metabolites from (+)-catechin required its initial conversion to (+)-epicatechin. After in vitro incubation of epicatechin with human intestinal bacteria, the metabolites pyrogallol, 5-(3,4-dihydroxyphenyl)valeric acid, 5-(3-hydroxyphenyl)valeric acid, 3-(3,4-dihydroxyphenyl)propionic acid, 3-(3-hydroxyphenyl)propionic acid, 3-(3-methoxyphenyl) valeric acid, and 2,3-dihydroxyphenoxyl 3-(3′,4′-dihydroxyphenyl)propionic acid were detected (*47*) (**Figure 5**). In contrast, epicatechin gallate and epigallocatechin gallate were not degraded by the microbiota (*47*).

The formation of phenylpropionic acids or *p*-hydroxyphenylacetic acids from flavan-3-ols and procyanidins seems to be the result of C-ring cleavages at the 1−2- and 4−10-bonds or at the 1−2- and 3−4-bonds, respectively, as occurs with flavonols (**Figures 1** and **3**). This can be related to the common feature with flavonols and anthocyanins of having a hydroxyl at the 3-position. Furthermore, the rupture at the 3−4 bond in these flavan-3-ol compounds shows that the absence of a carbonyl group in the 4-position or of a double bond in the 2−3-position did not prevent cleavage at this position (**Figure 1**). This is in agreement with our hypothesis about the absence of the OH group on the C ring of flavanones being the only cause of the nonrupture of the 3−4-bond. During the metabolism of epigallocatechin and epicatechin to give 5-(3′,4′,5′-trihydroxyphenyl)-γ-valerolactone and 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone, the rupture of the C ring at the 1−2-bond also seems to be necessary but, in this case, they should suffer a complete excision of the A-ring by means of A-ring cleavage at either the 8−9- or 5−10-bond (**Figure 4**).

Studies of the microbial metabolism of dietary condensed tannins (oligomeric and polymeric procyanidins) showed that benzoic, phenylacetic, phenylpropionic, and phenyllactic acid derivatives,

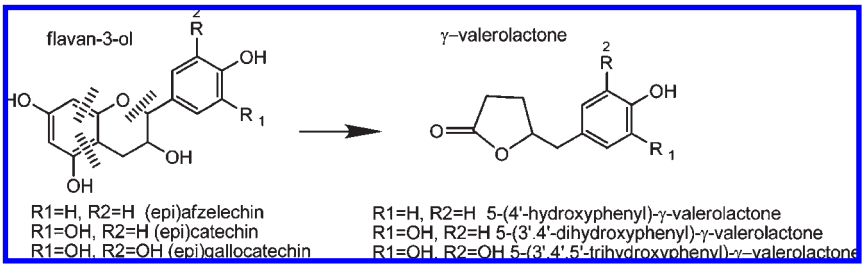

Figure 4. Valerolactone production by intestinal microbiota from dietary flavan-3-ols. (III) Positions of the potential C-ring cleavages.

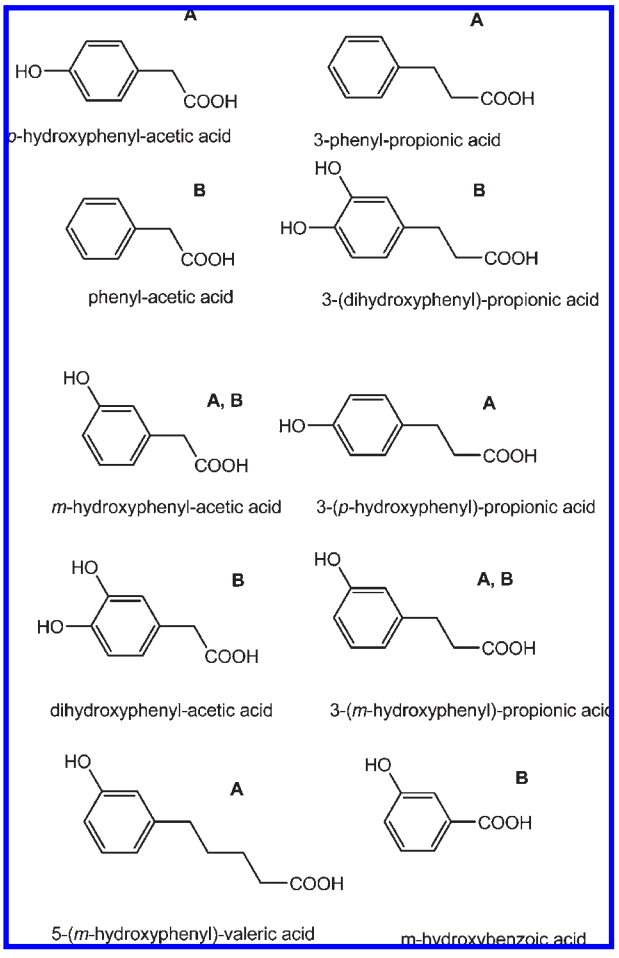

Figure 5. Intestinal microbiota metabolites of dietary procyanidins produced in vitro (**A**) (*116*) and in vivo (**B**) (*20*).

as well as phloroglucinol, 5-(3′-hydroxyphenyl)-γ-valerolactone, and 1-(3-hydroxyphenyl)-3-(2,4,6-trihydroxyphenyl)propan-2-ol, were produced (*48*). In another study, condensed tannins produced 2-(4-hydroxyphenyl)acetic acid, 3-phenylpropionic acid, 2-(3-hydroxyphenyl)acetic acid, 3-(4-hydroxyphenyl)propionic acid, 5-(3-hydroxyphenyl)valeric acid, and 3-(3-hydroxyphenyl)propionic acid, whereas valerolactones were not detected (*44*).

Anthocyanins. Anthocyanins are representative plant pigments widely distributed in colored fruits and flowers. These natural pigments, which are responsible for the blue, purple, violet, and red colors of fruit, are one of the major flavonoid classes (*49*). More than 300 different anthocyanin compounds have been identified in plants. They are planar molecules with a $C_6-C_3-C_6$ carbon structure typical of flavonoids. The daily intake of anthocyanins has been estimated to be 12.5 mg/day per person in the United States, but intakes could easily be over 200 mg/day if a regular diet of fruit and berries was consumed (*50*). The major sources of anthocyanins in edible plants are the families Vitaceae (grape) and Rosaceae (cherry, plum, raspberry, strawberry, blackberry, apple, peach, etc.). Other plant families that contain anthocyanin pigments are Solanaceae (tamarillo and eggplant), Saxifragaceae (red and black currants), Cruciferae (red cabbage), and Ericaceae (blueberry and cranberry) (*51*). Anthocyanins are present in nature mainly in the form of heterosides. The aglycone form of anthocyanins, also called anthocyanidins, is structurally based on the flavilium ion or 2-phenylbenzopyrilium and has hydroxyl and methoxyl groups in different positions (**Figure 1**). Depending on the number and position of the hydroxyl and methoxyl substituents, some dozen different anthocyanidins have been described in publications, of which six are commonly found in fruits and vegetables (*52*).

Studies looking at the colonic microbiota metabolism of anthocyanins are scarce. According to a previous study, overall in the gut, anthocyanin glucosides are the least stable, probably due to the β-glucosidase present in the small intestine (*54*). In feces, anthocyanin galactosides are also very unstable, probably influenced by large intestine microflora. However, anthocyanin arabinosides or xylosides seem to be quite stable in the gut. Acylation with *p*-coumaric acid or a second sugar moiety protected anthocyanins in the gut. Anthocyanidin diglycosides in the form of sambubioside or rutinoside impart increased stability to the anthocyanin molecule, and the quantities excreted in urine are $<0.1\%$ of intake (*50*). Only a small part of the dietary anthocyanins are absorbed as such or as hydrolysis products in which the sugar moiety is removed (*53*). Thus, large amounts of the ingested compounds are likely to enter the colon (*50*). Cyanidin 3-rutinoside is first transformed into the corresponding glucoside and then to the aglycone. It has been reported that these transformations are achieved by gut microbiota (*55*). The aglycones, which are chemically unstable, are also converted to phenolic acids, which can then suffer additional metabolism by the gut microbiota. The anthocyanin nucleus is broken down, and protocatechuic acid (3,4-dihydroxybenzoic acid) is detected as a product of human colonic microbiota (*17*, *33*, *56*) (**Figure 3**). Other metabolites produced in vitro by the pig gut microbiota include syringic acid (3,5-dimethoxy-4-hydroxybenzoic acid), vanillic acid (3-methoxy-4-hydroxybenzoic acid), phloroglucinol aldehyde (2,4,6-trihydroxybenzaldehyde), phloroglucinol acid (2,4,6-trihydroxybenzoic acid), gallic acid (3,4,5-trihydroxybenzoic acid), and 3-*O*-methylgallic acid depending on the chemical structure of the anthocyanins (*55*, *57*). Although anthocyanins are partly fragmented to phenolic acids, still a large part of metabolites remained unknown (*58*). In the case of methylated anthocyanins, the ring degradation products can be demethylated by the pig gut microbiota. This shows that anthocyanins, being flavonoid compounds, also suffer C-ring cleavage to release the B-ring- and A-ring-derived products (**Figures 1** and **3**).

Isoflavones. In contrast to the rest of the flavonoids, the benzenoid B-ring position of the isoflavones is in the 3-position

(**Figure 1**) (*59*). The basic ring system for isoflavones is planar. In soy, the health-promoting isoflavones are particularly abundant (*2*). The highest amounts of soy isoflavones can be found in soy nuts and tempeh. Another natural source of isoflavones is red clover. Isoflavones are a class of nonsteroidal estrogens that are similar in chemical structure to estrogens. These show conformational binding to the estrogen receptors (ERα and ERβ), which classifies them as natural selective estrogen receptor modulators (SERMs) rather than estrogens (*59*−*63*). Almost all soy isoflavones exist as glucosides, which are not absorbed intact across the enterocyte of healthy adults because of their higher hydrophilicity and molecular weights. There are 12 known isoflavone compounds in soybeans (3 aglycones of genistein, daidzein, and glycitein; 3 glucosides; 3 acetyl ester glucosides; and 3 malonyl ester glucosides). Among them, 6″-malonylgenistin, genistin (genistein glucoside), 6″-malonyldaidzin, and genistin are the major constituents. The isoflavone glucosides are very water-soluble. Their bioavailability requires the conversion of glucosides into the principal bioactive aglycones (daidzein, genistein, and glycitein) via the action of intestinal β-glucosidase from bacteria that colonize the small intestine for uptake to the peripheral circulation (*8*, *62*). The aglycones are either absorbed intact or further metabolized by intestinal microbiota (*64*). Genistein is converted to *p*-ethylphenol and 4-hydroxyphenyl-2-propionic acid, whereas daidzein is reduced to *O*-demethylangolensin (*O*-DMA) and equol (*2*). The higher antioxidant activity of equol with respect to soy isoflavones may be a result of its nonplanar structure, which gives equol a greater flexibility for conformational changes, enabling it to penetrate more easily into the interior of the membrane to prevent oxidative damage in situ than some of the other isoflavonoids that are more rigid in structure. However, humans produce relatively low levels of equol, in contrast to animal species assayed such as mouse, rat, and monkey (*2*, *8*). Furthermore, not all individuals consuming daidzein produce equol. Only approximately one-third to a half of the population is able to metabolize daidzein to equol. The inability of some subjects to produce equol is a consequence of the lack of specific components of the intestinal microflora (*2*). In addition to *O*-DMA and equol, dihydrodaidzein, tetrahydrodaidzein, 3′-hydroxydaidzein, 6-hydroxydaidzein, 8-hydroxydaidzein, 3-(4-hydroxyphenyl)benzopyran-4,7-diol, and 2-dehydro-*O*-DMA have also been reported to be microbial metabolites of daidzein (*2*). Therefore, these isoflavones are transformed by deglucosylation, reduction, C-ring cleavage, and hydroxylation reactions in the intestine (**Table 1**). C-ring cleavage occurs to break the bond between the oxygen in the pyrone ring and the C-2, only if a hydroxyl at the 5-position is missing, as is the case of daidzein, and both the 1- and 2-positions and the 4- and 10-positions, only if a hydroxyl at the 5-position is present, as is the case of genistein, and this breakdown is necessary to produce *O*-DMA (**Figure 1**). Glycitein comprises $<10\%$ of the total isoflavone amount in soybeans and soybean foods but comprises about 50% of the isoflavone mass in soy germ. Glycitein is demethoxylated in vitro by *Eubacterium limosum* to 6,7,4′-trihydroxyisoflavone (*65*). Glycitein metabolites, dihydroglycitein, dihydro-6,7,4′-trihydroxyisoflavone, 5′ -*O*-methyl-*O*-desmethylangolensin, and 6-*O*-methylequol have been isolated and characterized (*66*, *67*).

Nonflavonoid Phenolics. *Hydrolyzable Tannins*. These include gallotannins and ellagitannins. Upon hydrolysis gallotannins yield glucose and gallic acid, whereas ellagitannins undergo lactonization to produce ellagic acid (**Figure 3**). Gallotannins are typically found in berries (combined with condensed tannins) and particularly in pomegranates and persimmons, to which the astringent taste is attributable. The occurrence of ellagitannins in common foods is limited to a few berry, fruit, and nut species. They have been detected in berries of the genus *Rubus* (raspberry, blackberry, cloudberry, arctic bramble) and the genus *Fragaria* (strawberry), pomegranate, walnuts and some other nuts, and oak-aged wines (*68*). In addition to polymeric ellagitannins, minor amounts of ellagic acid are usually present as free and glycosylated forms (*69*). Some ellagitannins are not hydrolyzable, as they are linked by a *C*-glycosidic linkage to a polyol unit, but are nevertheless, for historical reasons, classified as hydrolyzable tannins (*70*). Both ellagitannins and ellagic acid are largely metabolized by the colon microbiota of different mammals including rats (*71*), pigs (*72*), and humans (*73*−*76*). In all of these cases, both ellagic acid and ellagitannins produce dibenzopyranones known as urolithin A (3,8-dihydroxy-6*H*-dibenzopyran-6-one) and its monohydroxylated analogue known as urolithin B (*73*) (**Figure 3**). The main ones reported in vivo are urolithins A and B. Therefore, in the intestine ellagic acid seems to be transformed by lactone-ring cleavage, decarboxylation, and dehydroxylation reactions (**Table 1**; **Figure 3**). Nothing is known about the microbial degradation of ellagitannin-*C*-glycosides such as castalagin and vescalagin. Ellagitannins are a very interesting group of phenolic metabolites; their bioavailability and metabolism by colonic microbiota deserve more study.

Lignans. These include a number of diphenolic compounds with a 1,4-diarylbutane structure (*77*) such as secoisolariciresinol, matairesinol, pinoresinol, lariciresinol, isolariciresinol, and syringaresinol (**Figures 2** and **3**). In a recent study, fruits and vegetables were the main sources of lignans (66% of total intake, with 35% of lignans from fruits, 30% from vegetables, 0.6% from potatoes, and 0.2% from legumes), followed by tea (11%), cereal products (7% of total intake, with 4% of lignans from bread, 2% from cold breakfast cereals, and 1% from rice and pasta), coffee (5%), and alcoholic drinks (5%) (*78*). The metabolism of plant lignans involves both mammalian (glucuronidation and to a lesser degree sulphation) and gut microbial processes (*79*−*82*). The biological activity of these lignans is related to the bioactivation of these compounds to enterolactone and enterodiol, which are mammalian phytoestrogens (*83*−*85*). Lignans are recognized as phytoestrogens due to their estrogen agonist and antagonist properties (*82*). These enterolignans are produced by the GI microbiota, and the biological activity of lignans depends on the occurrence of the microorganisms able to produce the necessary chemical conversions (*80*, *86*−*91*). Transformation of lignans into mammalian phytoestrogens is carried out after demethylation and dehydroxylation reactions. Demethylation takes place first from the naturally occurring lignans, and dehydroxylation happens as a second step (*92*). This is the case of secoisolariciresinol, which is demethylated before the conversion to enterodiol and enterolactone (*93*), and additional hydroxylation and conjugation patterns diversify enterolignan derivatives in the circulation (*94*, *95*). Thus, enterolactone conversion is a complex phenomenon, involving several precursors, different intermediary metabolites, and diverse conjugation patterns (*17*).

Hydroxycinnamates. In contrast to tannins and lignans, hydroxycinnamates are a simpler group of nonflavonoid compounds (**Figure 2**). The most common dietary C_6-C_3 hydroxycinnamates present in a large range of plant-derived food products are *p*-coumaric acid (4-hydroxycinnamic acid), caffeic acid (3,4-dihydroxycinnamic acid), ferulic acid (4-hydroxy-3-methoxycinnamic acid), and sinapic acid (4-hydroxy-3,5-dimethoxycinnamic acid) and their esters with quinic and tartaric acids (chlorogenic, caftaric, etc.) (**Figure 3**). Ferulic and *p*-coumaric acids occur, ester-linked, to pectic side chains in spinach and to the arabinoxylans of cereal brans (*96*, *97*). Coffee is a particularly rich source of chlorogenic acid, a water-soluble conjugate also widespread in fruits and vegetables (*98*). Potential health

effects of ferulic and cafeic acids have been demonstrated in many animal models and in vitro assays. Indeed, hydroxycinnamic acids exhibit in vitro chemoprotective, antioxidant, and anti-inflammatory properties (*99*−*101*), and it is suspected that they may contribute to the beneficial effects of a bran-rich diet (*102*). Caffeic acid was also able to inhibit chemically induced carcinogenesis in rats (*103*). However, it should be stressed that the antioxidant activity of these compounds does not necessarily reflect human health effects, because dietary components must be absorbed in the human gut to become bioavailable (*104*). Studies on the bioavailability of hydroxycinnamates in humans are scarce, but preliminary results from several groups suggest that hydroxycinnamates are absorbed in humans. In vitro studies with human microbiota indicated that the free acids were not detected after only 2 h of incubation. The main microbial metabolites of caffeic acid are 3-hydroxyphenylpropionic acid and benzoic acid. Both metabolites are also obtained from chlorogenic and caftaric acid, suggesting that the esterification has no influence on the metabolism of caffeic acid by the gut microbiota (*105*). The most frequent metabolites produced by colonic microbiota from ferulic acid are vanillin (3-methoxy-4-hydroxybenzoic acid) and 3-(4-hydroxyphenyl)propionic acid (**Figure 3**). Some conjugated derivatives such as vanilloylglycine and feruloylglycine were also detected in plasma and urine. In a similar way, in vivo phase II metabolism of the microbiota metabolites produced from caffeic acid produces the glycynated metabolites, including hippuric acid and 3-hydroxyhippuric acid (*19*, *106*). A general trend is that from esters, the free acid is released by bacterial esterases (*107*), and the free acid is then metabolized to reduce the double bond to give rise to phenylpropionic acid and, then, decarboxylated to produce phenylacetic acids (**Table 1**). Dehydroxylation occurs before reduction and decarboxylation to remove the hydroxyl at the C4-position of the hydroxycinnamic acid residue. Demethylation also occurs at different levels of the degradation pathway.

Stilbenes. Among stilbenes, the phytoalexin *trans*-resveratrol is the main compound studied due to its acknowledged health benefits (**Figure 2**). Stilbenes can be constitutive compounds in the woody part of the plant or induced metabolites in soft tissues such as fruits and leaves. These phytochemical compounds, which act as part of the plant's defensive arsenal (phytoalexins), can be induced by biotic and abiotic elicitors such as fungi and ultraviolet C, respectively (*108*). No conclusive data regarding the microbial transformation of stilbenes have been reported so far. The only previously reported tentative gut microbiota derived was dihydroresveratrol (*109*). This metabolite can be formed by the catalytic hydrogenation of *trans*-resveratrol (*110*), and it was found in the human urine as glucuronide and sulfate conjugates upon oral ingestion of resveratrol (*109*). On the contrary, this metabolite was not found in rat urine following oral administration (*111*), which could indicate differences in the metabolic pathway responsible for the formation of such a metabolite in humans. The presence of resveratrol in the diet is scarce, and its bioavailability is very low (*109*, *112*). It is rapidly absorbed and conjugated to yield mainly glucuronide and sulfate derivatives through an active enterohepatic circulation (*113*). This means that resveratrol could not reach colon distal portions in which the microbial metabolism is higher. In this context, the metabolism of stilbenes by gut microbiota, especially that of resveratrol, deserves further research as it is largely unexplored. In relation to resveratrol glucosides such as *trans*-piceid (*trans*-resveratrol-3-*O*-β-D-glucopyranoside), it is not confirmed whether or not the gut microbiota is able to hydrolyze the glucose to release the resveratrol aglycone. Kineman et al. (*114*) suggested in a mouse experiment that this hydrolysis requires an exogenous β-glucosidase. However, a previous study (*115*) showed that the transepithelial transport of *trans*-piceid occurs at a high rate and that the compound is deglycosylated in *trans*-resveratrol. They suggested two possible pathways by which *trans*-piceid is hydrolyzed in the intestine. The first was a cleavage of *trans*-piceid by the cytosolic-β-glucosidase, after passage through the brush-border membrane by the sodium-dependent glucose transporter 1. The second was deglycosylation on the luminal side of the epithelium by the membrane-bound enzyme, lactase phlorizin hydrolase, followed by passive diffusion of the released aglycone, which is further metabolized inside the cells into two glucuronoconjugates. However, the role of the gut microbiota in this transformation cannot be discarded.

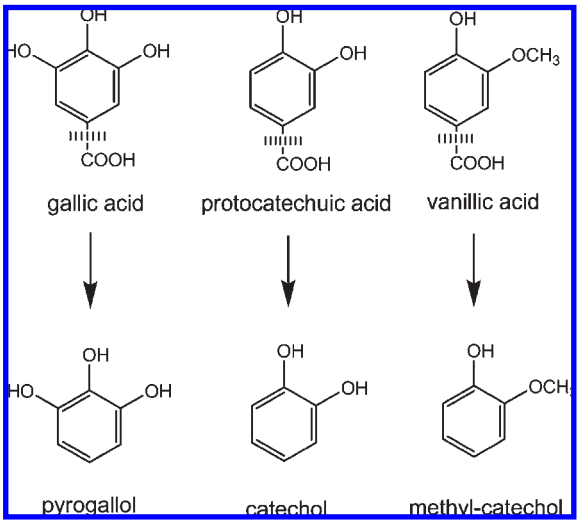

Figure 6. Microbial metabolites produced from benzoic acid derivatives.

Benzoic Acids. Benzoic acid, benzoates, and benzoic acid esters are commonly found in most fruits, especially berries. Cranberries are a very rich source of benzoic acid. Also, the common microbial degradation metabolites obtained in the body from flavonoid and nonflavonoid phenolics are the benzoic acids. These compounds are common metabolites produced from different phenolic groups (**Figures 3**). The main components of C_6-C_1 benzoic acids are gallic acid, *p*-hydroxybenzoic acid, vanillic acid, protocatechuic acid, and syringic acid (**Figure 6**). These phenolics are further transformed by colonic microbiota when a free hydroxyl group is present in the 4-position (**Figure 6**; **Table 1**). Thus, gallic acid (3,4,5-trihydroxybenzoic acid) produces pyrogallol (1,2,3-trihydroxyphenol) and protocatechuic acid (3,4-dihydroxybenzoic) produces catechol (1,2-dihydroxyphenol). In the same way, vanillic acid (3-methoxy-4-hydroxybenzoic acid) produces *O*-methylcatechol (**Figure 6**) (*33*). These metabolites are readily absorbed in the GI tract. In addition, a study in pigs showed that benzoic acid supplementation increased urinary hippuric acid concentration as a result of the conjugation of benzoic acid with glycine (*116*).

ROLE OF GUT MICROBIOTA IN PHENOLIC METABOLISM

Substantial levels of unabsorbed dietary phenolics remain in the gut. They and their metabolites may play a key role in the maintenance of intestinal health (*20*). The importance of intestinal bacteria for polyphenol metabolism was highlighted by the fact that germfree or antibiotic-treated animals no longer form the phenolic acid metabolites (ring-fission products) of (+)-catechin, apigenin, myricetin, hesperidin, naringin, rutin, 3′,4′,7-tri-*O*-β-hydroxyethylrutoside, and 3′,4′,5,7-tetra-*O*-β-hydroxyethylrutoside (*117*). Indeed, the majority of identified components derived from dietary phenolics are believed to be

generated by the action of intestinal bacteria (*20*). However, only a few species of intestinal bacteria responsible for phenolic metabolism have been identified, and there is scarce knowledge of the mechanisms involved. In addition, the transformation of the native phenolics into their metabolites depends on the individuals, and both metabolite "producers" and "nonproducers" have been reported (*75*, *118*). For example, the ability to produce equol has been related to the presence in the GI tract of specific microbiota (*2*), and therefore, the biological activity of equol depends on the colonization of the GI tract by specific bacteria that modulate the estrogenic activity through metabolism of some isoflavone to produce equol (**Table 2**). For this reason, current efforts are aimed at identifying the specific bacterial strains responsible for this metabolism. Strains of *Bacteroide ovatus* spp., *Streptococcus intermedius* spp., and *Ruminococcus productus* SNU-Julong 732 (AY310748) as well as a stable mixed culture consisting of *Enterococcus faecium* EPI1, *Lactobacillus mucosae* EPI2, *Finegoldia magna* EPI3, and *Veillonella* sp. EP have been isolated from human feces and reported to be able to convert daidzein into equol (*16*, *119*−*122*) (**Table 2**). The bacterial strain named Julong 732 is also able to metabolize dihydrodaidzein to *S*-equol (*123*). Thus, other bacteria have to be involved in the previous steps of the conversion of daidzein to equol (*124*). On the other hand, *Clostridium* sp. HGHA136 and *Eubacterium ramulus* have been reported to be able to cleave the C ring of daidzein to *O*-DMA (*125*, *126*). Therefore, the conversion of daidzein to equol or *O*-DMA will depend on the presence in the intestine of the microorganisms responsible for these transformations, which determine the interindividual differences between producer and nonproducer persons.

In the case of ellagic acid-related phenolics, such as ellagitannins, the production of the microbial-derived metabolites, urolithins, is also subjected to high interindividual variability (*75*). This process is independent of the chemical structure of the ellagitannin and its degree of polymerization. The same final metabolite, urolithin, is produced in some persons. However, nothing is known regarding the microbiota responsible for urolithin production. As urolithins have been reported to exert biological activity (*76*, *127*, *128*), the identification of the microorganisms involved in ellagic acid metabolism and their use as probiotics could be of great interest to enhance the production of urolithins in low-producing subjects.

Butyrivibrio spp. from ruminal fluid has also been reported as being responsible for cleaving the C ring of rutin and quercitrin but not the C ring of the aglycone quercetin (*129*−*131*) (**Table 1**). In contrast, quercetin is cleaved by another bacterial species, *Eubacterium oxidoreducens*, which was recovered from the bovine rumen as reported by Krumholz and Bryant (*132*). *Clostridium orbiscindens* strains capable of cleaving the C ring of quercetin and kaempferol at the bond between the 3- and 4-positions were also isolated from the fecal microbiota of humans (*31*) (**Table 1**). Therefore, C-ring cleavage is achieved by bacteria from *Clostridium* genera as occurred with isoflavones. Furthermore, flavonol C-ring fission could be carried out by different bacterial communities in humans compared to ruminants, as the *Clostridium* genus has been reported to be the responsible microorganisms in the human colon but *Butyrivibrio* spp. in rumen fluid. However, *Eubacterium* seems to be a common bacterial genus for the flavonoid metabolism in both humans and ruminants. *Clostridium* strains capable of cleaving the C ring of quercetin and kaempferol at the 3−4-bond were also able to act on the aglycone naringenin (*31*). The potential inability of C-ring cleavage in the 3−4-bond seems to be linked to the absence of a hydroxyl group in the 3- position, which marks the difference of flavanones from other flavonoid groups that suffer this cleavage such as flavonols and flavan-3-ols.

Isoxanthohumol is a prenylated flavanone from hops and beer that has interesting biological activities. It can be metabolized in vivo by colonic microbiota to render 8-prenylnaringenin (*133*, *134*). This metabolic O-demethylation is carried out by *Eubacterium* species and particularly by *Eubacterium limosum* (**Table 2**). In fact, it has recently been demonstrated that this species activates isoxanthohumol from hops (*Humulus lupulus* L.) into the potent phytoestrogen 8-prenylnaringenin in vitro and in rat intestine (*135*).

Currently, attention is mainly focused on intestinal microbiota biodegradation of nonflavonoid phenolics, which can contribute to the definition of their bioavailability for both humans and ruminants. A previous study reported that tannin-resistant *Butyrivibrio* from the rumen was able to metabolize tannins completely into volatile fatty acids (*136*). The microbial metabolism of other nonflavonoid phenolics is being investigated, such as the microorganisms involved in hydroxycinnamate and lignan degradation. Two isolated fecal bacterial strains, *Peptostreptococcus productus* SECO-Mt75m3 and *Eggerthella lenta* SECO-Mt75m2, have recently been shown to be responsible for demethylation and dehydroxylation of the lignan secoisolariciresinol, respectively (*137*). Demethylation of secoisolariciresinol is also catalyzed by strains of *Butyribacterium methylotrophicum*, *Eubacterium callanderi*, and *Eubacterium limosum*, whereas dehydroxylation of secoisolariciresinol is catalyzed by strains of *Clostridium scindens* (*138*). An in vitro study reported six bacterial isolates from human feces able to release ferulic acid from its ethyl ester, and these were identified through genotypic characterization (16S rRNA sequencing) as *Escherichia coli* (three isolates), *Bifidobacterium lactis*, and *Lactobacillus gasseri* (two strains) (*139*). Therefore, certain gut bacteria including some already recognized as potentially health-promoting ones (i.e., some species belonging to the genera *Bifidobacterium* and *Lactobacillus*) are involved in the release of bioactive hydroxycinnamic acids in the human colon. The use of dietary supplements of beneficial bacteria, which modify the colonic microbiota by increasing the number of specific microbial strains able to transform some of the phenolics mentioned above, could have wide-ranging implications for the health of the host, resulting in beneficial effects. The application of *Eubacterium limosum* as a probiotic for the intestinal activation of isoxanthohumol into the phytoestrogen 8-prenylnaringenin has recently been assayed (*135*). This research (*135*) demonstrated that the administration of the probiotic increased 8-prenylnaringenin production in nonconverting rats. In the published studies, *Clostridium* and *Eubacterium* genera are a common element of the metabolism of several phenolic compounds. Given the phylogenetic association between these two genera, the association of them in the phenolic transformations is not surprising.

The degree of degradation of many phenolic compounds is significantly influenced by the substrate concentration in the diet as well as individual variations in the composition of the colonic microbiota. This will contribute to justifying the large interindividual variability in the health benefits observed, which has been attributed to dissimilarities in the populations of colonic microbiota.

EFFECT OF PHENOLICS ON GUT MICROBIOTA

Interactions between bacteria and their hosts can be viewed in terms of a continuum between symbiosis, commensalism, and pathogenicity, with symbiosis and commensalism grouped under the general heading of mutualism (*140*). Unabsorbed dietary phenolics and their metabolites, in addition to their direct beneficial effect on the human tissues, exert significant effects

on the intestinal environment by modulation of the microbiota (*20*). This is the case of tea phenolics, including epicatechin, catechin, 3-*O*-methylgallic acid, gallic acid, and caffeic acid, which have been identified as responsible for the repression of *Clostridium perfringens*, *Clostridium difficile*, and *Bacteroides* spp. growth, whereas commensal anaerobes such as *Clostridium* spp., *Bifidobacterium* spp., and probiotics such as *Lactobacillus* spp. were less severely affected (*20*). Similar results were obtained in another study in which (+)-catechin incubation affected the growth of selected microbiota, resulting in a significant increase in the growth of the *Clostridium coccoides*−*Eubacterium rectale* group, *Bifidobacterium* spp., and *Escherichia coli*, as well as a significant inhibitory effect on the growth of the *Clostridium histolyticum* group (*46*). The increase of the *C. coccoides*−*Eubacterium rectale* group could be related to its capacity for metabolizing these flavonoid compounds. The stilbenoid resveratrol, the ellagitanins of pomegranate, and their main microbiota-derived metabolite urolithin A have also been identified as responsible for changes in intestinal microbiota in rats with an increase of *Bifidobacterium* and *Lactobacillus* levels (*141*, *142*).

Several phenolics have been recognized as potential antibacterial compounds able to repress pathogenic bacteria in the human gut (*20*) and also in other human tissues. The importance of dietary phenolics as antimicrobial compounds has increased due to the fact that the incidence of antimicrobial resistance by several pathogens has also become more frequent. Mechanisms of action of food phytochemicals and their metabolites are not well-known. They could have bacteriostatic or bactericidal actions or also act to inhibit the adhesion of infection-causing bacteria within cells of the intestinal and urinary tract. This is the case of anthocyanins of several purple and red fruits such as cherry, pomegranate, raspberry, and strawberry, which have in vitro bacteriostatic activity against *Staphylococcus epidermis* and *Klebsiella pneumoniae* (*143*). Among different berries, which are rich in anthocyanins, cloudberry and raspberry were shown as the best in vitro inhibitors of pathogenic bacteria such as *Staphylococcus* spp., *Salmonella* spp., *Helicobacter pylori*, and *Bacillus cereus* (*144*, *145*). Raspberry juice totally inhibited the growth of *Escherichia coli* in vitro (*146*). These findings suggest that these phenolic compounds have potential to be developed as antimicrobial agents against human infections.

Some stilbenoids also show antimicrobial activity. This is the case of resveratrol, which has growth inhibitory effects on some ochratoxigenic fungi inoculated on grapes (*108*) as well as on fungal and bacterial human pathogens as demonstrated during in vitro antimicrobial assays (*147*, *148*). Resveratrol also inhibits in vitro swarming and virulence factor expression in *Proteus mirabilis*, an important pathogen infecting the urinary tract (*149*). Furthermore, this compound inhibited the ability of *Proteus mirabilis* to invade human urothelial cells in a NTUB1 cell culture assay (*149*). Proanthocyanidins of cranberries are also shown to be effective in reducing the risk of *Escherichia coli* adhesion to bladder cells and the onset of urinary tract infection in patients with a previous history of urinary tract infections (*150*, *151*) as well as in inhibiting adhesion of *Helicobacter pylori* to the confluent monolayers of gastric cell line in microtiter plate (*152*). The addition of cranberry to triple therapy with omeprazole, amoxicillin, and clarithromycin also improves the rate of *Helicobacter pylori* eradication in human females as shown in a double-blind randomized clinical study (*153*). The antiadhesion agents may be considered as antimicrobial agents even though they do not act by killing or arresting the growth of the target bacteria, but rather impair their ability to gain a foothold in the host. As these agents are not bactericidal, their use would lessen the selective propagation and spread of strains resistant to them (*152*).

In addition to research aiming at the evaluation of dietary compounds that target bacterial virulence, there seems to be considerable potential to investigate phenolics from plants with activity against the multidrug resistance pumps (MDR) of microorganisms. There has indeed been a considerable effort to discover plant-derived antibacterials active against strains such as methicillin-resistant *Staphylococcus aureus*, which have developed resistance to most existing antibiotics (*154*, *155*). In other studies, it has been shown that the isoflavone biochanin A exhibits inhibiting activity against drug-resistant tuberculosis produced by *Mycobacterium smegmatis* (*156*). The flavonoids luteolin and genistein and the stilbene resveratrol were also active MDR inhibitors, but less active than biochanin A (*156*). The intake of phenolics with bacterial MDR inhibiting capacity should decrease the intrinsic resistance of bacteria to antibiotics, reverse acquired resistance, and reduce the frequency of emergence of resistant mutant strains (*157*).

NEW TOOLS FOR THE ANALYSIS OF INTERACTIONS BETWEEN PHENOLICS AND GUT MICROBIOTA

The gut microbiota consists of a diverse collection of microbial species that are mostly bacterial and are commonly detected in human feces. Nevertheless, a significant fraction of the microbiota that can be seen by direct microscopic examination of diluted fecal specimens cannot be grown in culture media. Indeed, 40−80% of the total microscopic counts are not recoverable by culture, although estimates vary between individuals and between studies (*158*). Molecular biological procedures can now also be used to investigate the gut microbial ecology and the microbial changes derived from the consumption of phenolics without the use of cultures. Metagenomic analyses enable us to study microorganisms by deciphering their genetic information from DNA that is extracted directly from communities of environmental microorganisms, thus sidestepping the need for culturing or isolation. These molecular methods involve the amplification by Polymerase Chain Reactions (PCR) of 16S rRNA genes (16S rDNA) from microbial DNA extracted from samples collected from particular habitats (*159*). The amplified 16S rDNA sequences are cloned and should contain copies of the gene from all of the species represented in the sample. Alignment of the 16S rDNA sequences with those stored in databanks permits the recognition of which species are represented in the habitat, including those that cannot be cultivated by conventional techniques. Also, species can be enumerated directly in samples by means of oligonucleotide probes based on the 16S rDNA sequences (in situ hybridization). These probe molecules are labeled with a fluorescent dye, and the procedure is termed fluorescent in situ hybridization (FISH) (*159*). Molecular analyses of gut microbiota by FISH analyses will enable the adequate evaluation of the effect of phenolic consumption on the intestinal microbiota.

Cultivation-independent molecular techniques based on the 16S rRNA gene have given us a broader and less biased view of the gut microbiota (*160*, *161*). Full-length 16S rRNA sequences offer the highest possible degree of taxonomic resolution using this gene, but the cost of dideoxy Sanger sequencing limits our ability to survey the less abundant members of this diverse community. These approaches can be focused more narrowly on particular taxonomic groups, which facilitates the investigation of less abundant taxa, but at the expense of a broader view of community composition that might reveal important microbial interactions. Pyrosequencing ameliorates some of these constraints by generating much larger numbers of 16S rDNA sequence data at a lower cost (*162*). Both with this approach

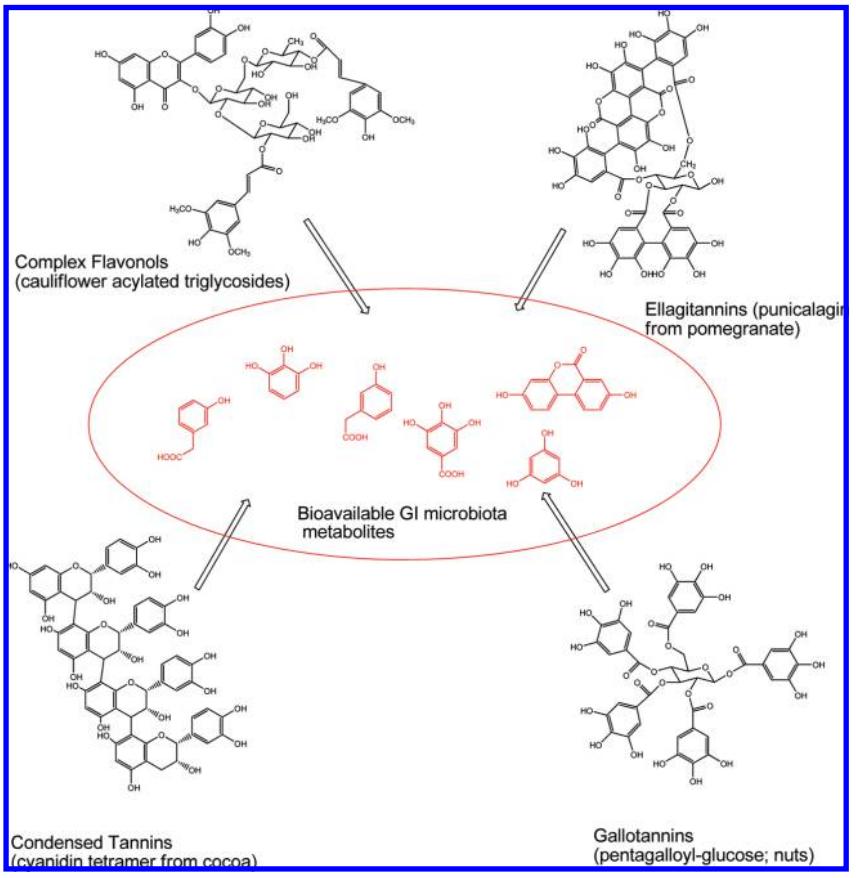

Figure 7. Gut microbiota affects dietary phenolics bioavailability. Large-size dietary phenolics (poorly absorbable) are converted to small-size microbial metabolites (highly bioavailable) in the colon.

and with the established approach of clone library sequencing, DNA is extracted from a sample, and PCR primers complementary to conserved regions of the 16S rRNA are used to amplify the intervening variable sequence. The diversity and relative abundance of 16S rRNA sequence variants in the pool of amplicons is analyzed as a proxy for the diversity and relative abundance of the microbial populations in the sample (*163*). Studies examining the effects of phenolics in humans and animal models might be improved by considering interindividual variability in phenolic compound absorption, as it seems to be a controllable factor. For instance, in a previous study (*164*), the cholesterol-lessening effect of soy protein in hamsters largely depended on the extent of apparent absorption of isoflavones, which varies according to distinct phenotypes. These isoflavone absorption phenotypes are probably determined by gut microbial ecology, but isoflavone degrading microbial species and other factors determining this relatively stable variability in isoflavone absorption remain to be identified.

In conjunction with the analyses and quantification of gut bacterial populations, the use of appropriate analytical tools that allow comprehensive and robust metabolic analyses for various chemical structures is also essential to understand the effect of the microbiota on the phenolic metabolism. Metabonomic/metabolomic analyses involve the combination of high-density spectral data generated from biofluids and tissues in combination with a computer-based pattern recognition strategy (*165*). Currently, several analytical techniques, including high-resolution NMR spectroscopy and various other hyphenated approaches such as gas chromatography−mass spectrometry (GC-MS) and high-performance liquid chromatography−mass spectrometry (HPLC-MS) and LC-NMR-MS are used to generate spectral profiles, from which information that pertains to physiology and latent disease can be extracted (*165*). GC-MS methods can be especially useful to detect the low molecular weight phenolics, which are common microbial degradation metabolites obtained from the different groups of dietary phenolic compounds. Combination of HPLC-MS-MS and GC-MS should be used for this purpose as these are complementary methods that will help to cover the whole range of potential phenolic metabolites present in the gut.

MICROBIOTA METABOLISM AND POLYPHENOL BIOAVAILABILITY AND BIOACTIVITY

The biological activity of phenolics has been extensively studied in cell cultures, animal models, and, to a lesser extent, clinical trials. The effects observed have been traditionally attributed to the phenolics ingested, as they occur in the foods. In the past few years, increasing attention has been paid to the phase II enzyme-conjugated metabolites, that is, glucuronide and methylated and sulfate derivatives (*166*, *167*), as these metabolites are found in the bloodstream and can reach different tissues (*13*). Gut microbiota metabolism can also modulate the health effects of dietary phenolics by altering their absorption, bioavailability, and biological activity. However, the biological properties of the microbiota metabolites have scarcely been explored.

It is generally accepted that the bioavailability of phenolics is rather low and the values of the relative urinary excretion of the intake range from 0.3% for anthocyanins to 43% for isoflavones such as daidzin (*168*). This demonstrates the great variability in the bioavailability of the different polyphenols. This bioavailability can be even lower when the food polyphenols have a large

molecular weight, as is the case of hydrolyzable and condensed tannins and complex flavonoid conjugates with several sugars and acylated with hydroxycinnamic acids (**Figure 7**). The content of these complex phenolics in food is generally higher than that of simpler phenolics, and these complex molecules have been underestimated in many papers mainly due to analytical problems. The microbiota metabolites of these complex polyphenols are smaller molecules in which some of the original phenolic hydroxyls have been removed, and these metabolites are generally better absorbed in the intestine. In fact, this increased absorption has been demonstrated in the case of urolithins, the microbiota metabolites derived from ellagic acid and ellagitannins (*72*−*75*), valerolactones, phenylpropionic, and phenylacetic derivatives produced from procyanidins (*169*). This indicates that the systemic effects of polyphenols can be modulated by the microbial metabolism.

In terms of biological activity, the microbial metabolism could also be relevant. This activity could be exerted at both local and systemic levels. The phenolic structure of some microbial metabolites could contribute to protection against oxidative stress (*170*, *171*). The ellagic acid microbiota metabolites (urolithins) have been reported to show anti-inflammatory (*142*), estrogen-modulating (*76*), and cancer chemopreventive effects (*128*, *172*). The soy isoflavone microbiota metabolite (equol) (*173*−*176*), the lignan-derived metabolites (enterolactone and enterodiol) (*177*, *178*), and the microbiota metabolite of the hop isoxanthohumol (8-prenylnaringenin) (*135*, *179*) exert more potent estrogenic effects than their precursors. The 3,4-dihydroxyphenylacetic and 4-hydroxyphenylacetic acid metabolites have been reported to exert higher inhibition of platelet aggregation than their precursors rutin or quercetin (*180*). In addition, the hydroxyphenylacetic and phenylpropionic derivatives, produced by microbiota degradation of many flavonoids, have structural characteristics similar to apocyinin, a relevant inhibitor of NADPH oxidase. This enzyme is associated with the endothelial function (*181*), and these metabolites could act as inhibitors of this enzyme, which is deserving of more intensive studies as a potential target of phenolics.

CONCLUSION

Metabolites of food polyphenols play an important role in the health benefits of fruits and vegetables. Many dietary phenolics suffer similar transformations in the gut by means of microbial enzymes and release the same compounds, which can be considered biomarkers of colonic metabolism if they are subsequently absorbed. This could probably explain some common physiological effects observed after the intake of foods with very diverse phenolic compositions. However, some intact phenolics of foods with well-known bioactivity are also absorbed. The varied health effects of each phenolic compound between different people are the result of the interindividual variability in gut microbial ecology, which determines phenolic compound absorption. Thus, studies examining the effects of phenolics in humans and animal models might be improved by considering compound absorption, as it seems to be a controllable factor. Availability of good methods for monitoring the total bacterial communities and their metabolic activities is a prerequisite for informative studies in the future. The combination of metagenomic and metabolomic studies will contribute to understanding better the interaction between microbial communities and dietary phenolics. Furthermore, a better understanding of the dietary phenolic and gut microbiota relationship should help in the prevention of intestinal diseases, such as inflammatory bowel diseases and colon cancer, as well as in improvement of human health avoiding diseases in other tissues.

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