Insights into the metabolism and microbial biotransformation of dietary flavan-3-ols and the bioactivity of their metabolites

Maria Monagas,*[a] Mireia Urpi-Sarda,[b] Fernando Sánchez-Patán,[a] Rafael Llorach,[b] Ignacio Garrido,[a] Carmen Gómez-Cordovés,[a] Cristina Andres-Lacueva[b] and Begoña Bartolomé[a]

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Flavan-3-ols, occurring in monomeric, as well as in oligomeric and polymeric forms (also known as condensed tannins or proanthocyanidins), are among the most abundant and bioactive dietary polyphenols, but their *in vivo* health effects in humans may be limited because of their recognition as xenobiotics. Bioavailability of flavan-3-ols is largely influenced by their degree of polymerization; while monomers are readily absorbed in the small intestine, oligomers and polymers need to be biotransformed by the colonic microbiota before absorption. Therefore, phenolic metabolites, rather than the original high molecular weight compounds found in foods, may be responsible for the health effects derived from flavan-3-ol consumption. Flavan-3-ol phenolic metabolites differ in structure, amount and excretion site. Phase II or tissular metabolites derived from the small intestine and hepatic metabolism are presented as conjugated derivatives (glucuronic acid or sulfate esters, methyl ether, or their combined forms) of monomeric flavan-3-ols and are preferentially eliminated in the bile, whereas microbial metabolites are rather simple conjugated lactones and phenolic acids that are largely excreted in urine. Although the colon is seen as an important organ for the metabolism of flavan-3-ols, the microbial catabolic pathways of these compounds are still under consideration, partly due to the lack of identification of bacteria with such capacity. Studies performed with synthesized or isolated phase II conjugated metabolites have revealed that they could have an effect beyond their antioxidant properties, by interacting with signalling pathways implicated in important processes involved in the development of diseases, among other bioactivities. However, the biological properties of microbe-derived metabolites in their actual conjugated forms remain largely unknown. Currently, there is an increasing interest in their effects on intestinal infections, inflammatory intestinal diseases and overall gut health. The present review will give an insight into the metabolism and microbial biotransformation of flavan-3-ols, including tentative catabolic pathways and aspects related to the identification of bacteria with the ability to catabolize these kinds of polyphenols. Also, the *in vitro* bioactivities of phase II and microbial phenolic metabolites will be covered in detail.

I. Introduction

Proanthocyanidins or condensed tannins are polymers of flavan-3-ols and are among the most abundant polyphenols in our diet. Proanthocyanidins exhibit a wide range of biological activities, including antioxidant, anti-carcinogenic, cardioprotective, antimicrobial and neuro-protective activities, as has been demonstrated in many *in vitro* and *ex vivo* studies.[1] In the last decade, a large body of epidemiological data has been accumulated supporting the assumption that the consumption of flavan-3-ol-rich food such as cocoa, red wine or tea may reduce the risk of coronary heart disease (CHD).[2–4] Proanthocyanidins exhibit a high structural diversity and a wide range of degree of polymerization (DP), and their content varies considerably between the different plant sources. Procyanidins, consisting of (epi)catechin units, are the most abundant type of proanthocyanidins in nature. Propelargonidins and prodelphinidins contain (epi)afzelechin and (epi)gallocatechin units, respectively, and are usually mixed with procyanidins. With regard to the interflavanic bond nature, B-type procyanidins [C-4 (upper unit) → C-6 or C-8 (lower unit)] are more abundant than A-type procyanidins, which contain an additional ether-type bond [C-2 (upper unit)–O–C-7 (lower unit)]. Fruits (grapes, apples and pears), legumes, cocoa and beverages such as wine, cider and beer are among the most important sources of B-type proanthocyanidins.[5] Polymeric proanthocyanidins with DP >10 represent the largest amount in 21 kinds of food.[5] The daily intake of flavan-3-ols in the United States has been estimated to be around 60 mg/day for proanthocyanidins with a DP <2.[5] In the Spanish population it has been estimated to be 18–31 mg/day when considering proanthocyanidins with a DP up to 3,[6] and 450 mg/day when considering highly polymerized proanthocyanidins.[7]

Polyphenols are recognized as xenobiotics (*i.e.* foreign or artificial substances, usually of synthetic origin) by the human organism, and therefore bioavailability is a factor that limits the health benefits derived from proanthocyanidin consumption. Bioavailability of proanthocyanidins is largely influenced by their DP. While monomeric flavan-3-ols are readily absorbed in

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[a] *Institute of Industrial Fermentations (CSIC), Juan de la Cierva 3, 28006 Madrid, Spain. E-mail: monagas@ifi.csic.es*
[b] *Department of Nutrition and Food Science, Xarta, INSA, Pharmacy School, University of Barcelona, Av/Joan XXIII s/n., 08028 Barcelona, Spain*

the small intestine, oligomeric and polymeric forms pass intact through the gastrointestinal tract, reaching the colon where they are transformed by the intestinal microbiota before absorption. The scientific evidence accumulated during the last decade indicates that the beneficial effect of these phytochemicals could be attributed to the conjugated metabolites (formed during the phase II metabolism of monomeric flavan-3-ols), and mainly to metabolites derived from the microbial catabolism of proanthocyanidins, rather than to the original forms found in food which have been widely used in most bioactivity studies.[8–10] Recent studies have estimated that the amount of non-absorbable polyphenols reaching the colon is very high and that microbe-derived phenolic metabolites excreted in urine represent the largest proportion of polyphenol intake. This recognition is leading to a reformulation of estimated bioavailability values and the potential bioactivity of polyphenols.[11] Currently, there is an increasing interest in the determination of the possible health implications derived from the interaction between phenolic compounds and human microbiota, in particular concerning the effect on microbiota composition and gut health. However, the biological properties of microbial-derived metabolites are still largely unknown.

The aim of the present review is to provide updated information on metabolites formed from dietary flavan-3-ols, as well as their bioactivity and potential health effects. After giving a general overview about the bioavailability of dietary flavan-3-ols in humans, structures of main phase II or tissular metabolites derived from small intestine and liver metabolism are presented. A special section is dedicated to the microbial catabolism of monomeric flavan-3-ols and proanthocyanidins, describing possible catabolic pathways, microbial reactions, and characteristic metabolites derived from the biotransformation process. Intrisic characteristics of candidate catabolic bacteria and structural flavan-3-ol features limiting bacteria degradation are also discussed. Finally, the main biological activities reported for both phase II or tissular and microbial metabolites derived from flavan-3-ols are reviewed, taking into consideration the results of studies performed with conjugated metabolites at *in vivo* concentrations.

II. Bioavailability of monomeric flavan-3-ols and proanthocyanidins

Bioavailability is a key issue linking polyphenols and health effects. In the case of flavan-3-ols, the degree of polymerization (DP) and galloylation are factors affecting their bioavailability (Fig. 1). Monomeric flavan-3-ols are absorbed in the small intestine and extensively metabolized into glucuronide conjugates by phase II enzymes.[12,13] These metabolites can reach the systemic circulation or be eliminated in the bile. Further metabolism into sulfate conjugates and methyl derivatives occurs in the liver. However, oligomers with DP >3 and polymers are not absorbed in the small intestine and reach the colon, where they are subjected to microbial catabolism. Microbial metabolites are further absorbed and metabolized by phase II enzymes, to finally enter the circulation or be eliminated in urine.

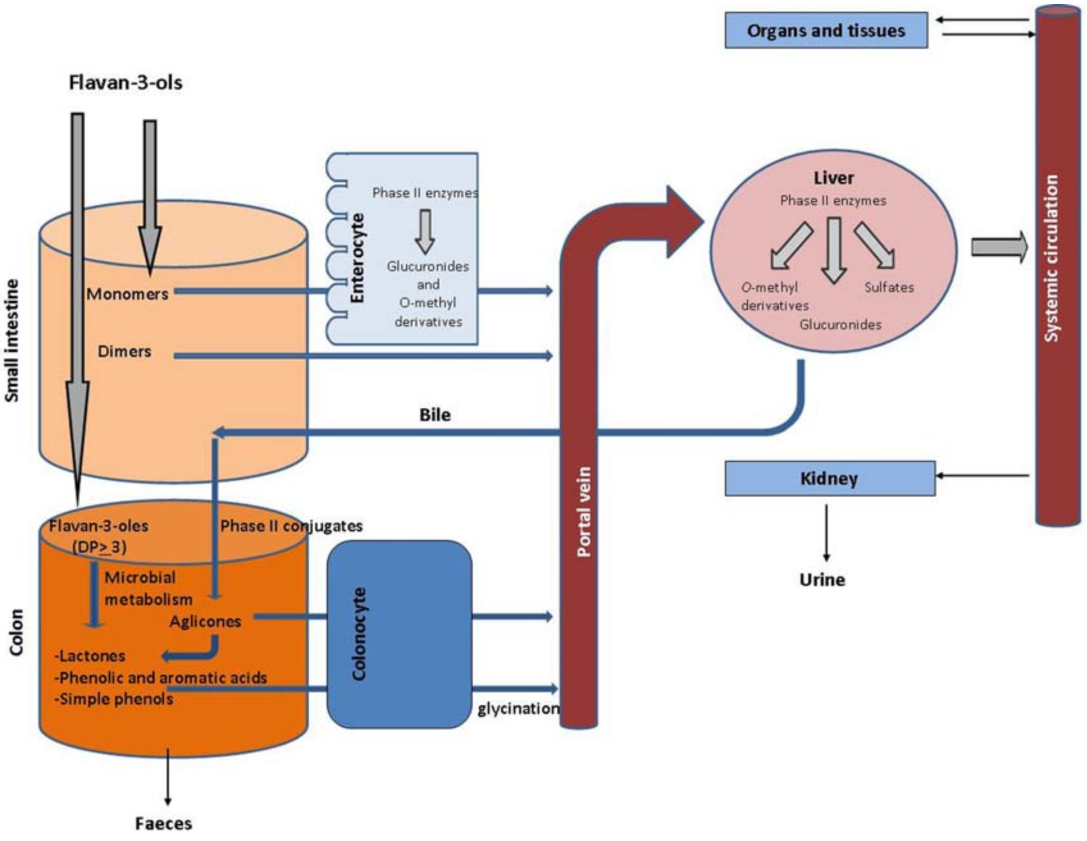


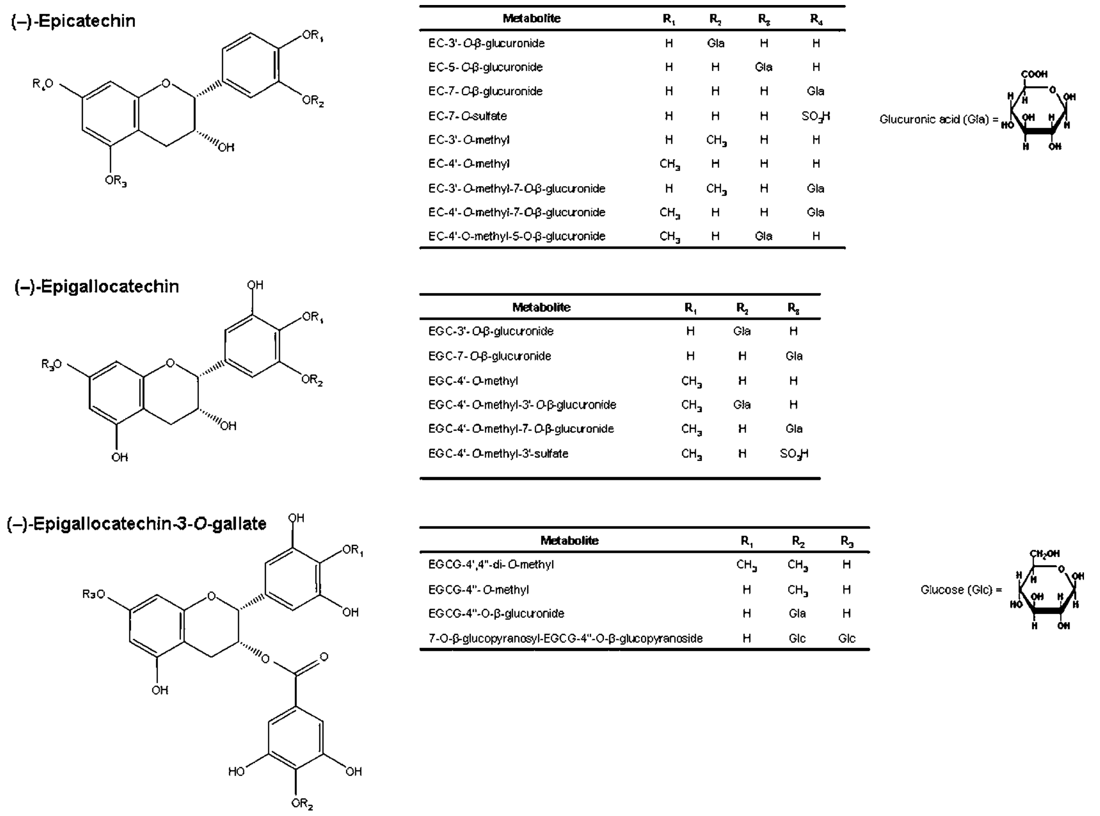
Fig. 1 Schematic diagram of organs, reactions and agents involved in the bioavailability of flavan-3-ols.

II.1 Absorption and metabolism of monomeric flavan-3-ols

With regard to small intestine and liver metabolism, the small intestine is the main site for glucuronidation, which occurs in the luminal part of the endoplasmic reticulum *via* the superfamily of uridine 5′-diphosphate glucuronosyltransferases (UGTs). In particular, UGT1 is considered to be responsible for the glucuronidation of flavonoids.[14] Sulfation and methylation mainly occur in the liver through cytosol sulfotransferases (SULT) and catechol-*O*-methyltransferase (COMT). Specifically, SULTA1 and SULTA3 are considered to be responsible for the sulfation of (−)-epicatechin.[15] The preferred positions for conjugation are the hydroxyl groups at C-3′ and C-4′ (B ring), and C-5 and C-7 (A ring) (Fig. 2). Generally, conjugated metabolites of (−)-epicatechin are presented in the form of monoglucuronides (5-, 7- and -3′-*O*-glucuronides), sulfates (7-*O*-sulfate), methyl ethers (3′- and 4′-*O*-methyl) or as combined derivatives (3′-*O*-methyl-7-*O*-glucuronide, 4′-*O*-methyl 5- or -7-*O*-glucuronide).[16–19] In the case of (−)-epigallocatechin (EGC), the 3′- and 7-*O*-glucuronides and the 4′-*O*-methyl and its derivatives (4′-*O*-methyl-3′-*O*-glucuronide, 4′-*O*-methyl-7-*O*-glucuronide, and 4′-*O*-methyl-3′-sulfate) have been identified following consumption of green tea.[20–24]

In general, *O*-sulfated metabolites of (−)-epicatechin are the predominant metabolites in urine samples after the intake of a single nutritional dose of cocoa powder in humans.[25] However, *O*-methyl-*O*-sulfate derivatives of (−)-epicatechin have been found as major urinary metabolites, followed by glucuronide, sulfate and methyl-glucuronide conjugates, after the intake by humans of a single dose of flavonol-rich cocoa powder.[26] Major urinary amounts of (epi)catechin-*O*-methyl-*O*-sulfates, followed by sulfates and glucuronide conjugates, have also been reported after the intake of a single dose of tea extracts in humans.[27–29] Among *O*-glucuronides, the 3′-*O*-glucuronide is the main glucuronide derivative of both (−)-epicatechin and EGC in humans.[17,22,24,30] EGC-4′-*O*-methyl is the major methylated metabolite of EGC after tea intake in humans.[23]

Among the different pairs of diastereomers, (−)-epicatechin presents higher absorption than (+)-catechin,[16] but the latter is more bioavailable than (−)-catechin.[31]With regard to galloylated monomers, (−)-epicatechin-3-*O*-gallate (ECG) seems to be better absorbed than (−)-epigallocatechin-3-*O*-gallate (EGCG),[27–29,32] but is considerably less bioavailable than the non-galloylated monomers. No conjugated metabolites of the 3-*O*-galloylated flavan-3-ols (ECG and EGCG) have been detected in biological fluids. The possible hydrolysis of ECG after absorption was suggested at first, but no esterases have been described in plasma or liver, being found only at the level of the oral cavity.[33] It has been suggested that the low $C_{\max}$ of EGCG is probably due to not all possible conjugated forms being identified, particularly those conjugated in the gallic acid ring such as EGCG-4″-*O*-methyl, EGCG-4′,4″-di-*O*-methyl, and EGCG-4″-*O*-glucuronide[24,34] (Fig. 2). Recently, a new 7-*O*-glucopyranosyl-EGCG-4″-*O*-glucopyranoside has also been identified.[21] Other studies have confirmed that both ECG and EGCG, but in particular the latter, also appear in unmetabolized form in plasma.[28,35–37] Conjugated forms of monomeric flavan-3-ols usually reach a $T_{\max}$ at 1.5 h after ingestion, which is characteristic of absorption in the small intestine.[38]



Metabolite	R_1	R_2	R_3	R_4
EC-3′-*O*-β-glucuronide	H	Gla	H	H
EC-5-*O*-β-glucuronide	H	H	Gla	H
EC-7-*O*-β-glucuronide	H	H	H	Gla
EC-7-*O*-sulfate	H	H	H	SO_3H
EC-3′-*O*-methyl	H	CH_3	H	H
EC-4′-*O*-methyl	CH_3	H	H	H
EC-3′-*O*-methyl-7-*O*-β-glucuronide	H	CH_3	H	Gla
EC-4′-*O*-methyl-7-*O*-β-glucuronide	CH_3	H	H	Gla
EC-4′-O-methyl-5-O-β-glucuronide	CH_3	H	Gla	H

Metabolite	R_1	R_2	R_3
EGC-3′-*O*-β-glucuronide	H	Gla	H
EGC-7-*O*-β-glucuronide	H	H	Gla
EGC-4′-*O*-methyl	CH_3	H	H
EGC-4′-*O*-methyl-3′-*O*-β-glucuronide	CH_3	Gla	H
EGC-4′-*O*-methyl-7-*O*-β-glucuronide	CH_3	H	Gla
EGC-4′-*O*-methyl-3′-sulfate	CH_3	H	SO_3H

Metabolite	R_1	R_2	R_3
EGCG-4′,4″-di-*O*-methyl	CH_3	CH_3	H
EGCG-4″-*O*-methyl	H	CH_3	H
EGCG-4″-O-β-glucuronide	H	Gla	H
7-O-β-glucopyranosyl-EGCG-4″-O-β-glucopyranoside	H	Glc	Glc

Fig. 2 Chemical structure of conjugated metabolites of (−)-epicatechin, (−)-epigallocatechin (EGC) and (−)-epigallocatechin-3-*O*-gallate (EGCG).

II.2 Absorption and metabolism of dimeric proanthocyanidins

In the last decade, the absorption and metabolism of dimeric proanthocyanidins have been a subject of speculation. It was first thought that procyanidins could be depolymerized into bioavailable monomers under the acidic conditions of the stomach,[39] but later studies failed to demonstrate this occurrence *in vivo*.[40–42] In contrast to monomers, glucuronidated or sulfated metabolites of dimeric procyanidins have not been detected in biological fluids,[43] although some methylated forms have been reported.[44] Procyanidins B1 [epicatechin-(4β → 8)-catechin] and B2 [epicatechin-(4β → 8)-epicatechin] have been detected in their intact form at very low levels in human plasma (nM range) after consumption of cocoa[45] or grape seeds,[46] and present the lowest $C_{\max}$ in plasma among flavonoid compounds.[13] Besides dimer B2, procyanidin B5 (epicatechin-(4β → 6)-epicatechin) has also been detected in the plasma of rats fed cocoa extracts,[47,48] but it was not detected in human plasma after cocoa consumption.[45] However, dimer B3 [catechin-(4α → 8)-catechin] and trimer C2 [catechin-(4α → 8)-catechin-(4α → 8)-catechin] were not detected in the plasma of rats fed the corresponding purified compounds.[49] Recently, oligomers with DP 2–5 have been detected in rat plasma after the administration of apple procyanidin fractions (1 g/kg weight) with the same DP.[44]

III. Microbial catabolism of monomeric flavan-3-ols and proanthocyanidins

It has been estimated that 90–95% of dietary polyphenols are not absorbed in the small intestine and therefore accumulate in the colon.[50] In the case of flavan-3-ols, in studies performed with ileostomy patients (*i.e.* patients whose colon has been removed surgically), it was calculated that approximately 70% of the ingested monomeric flavan-3-ols from green tea could pass from the small to the large intestine, with 33% corresponding to the intact parent compounds.[29] Recently, it has been reported that after oral administration of [^{14}C]procyanidin B2, 63% of the total radioactivity was excreted *via* urine, indicating that a large quantity of the parent compound is degraded by the gut microflora.[51] The recognition that the colon is a very active organ for the metabolism of flavan-3-ols, particularly proanthocyanidins, has led to a resurgence in the study of the biotransformation of these compounds and other polyphenols by the intestinal

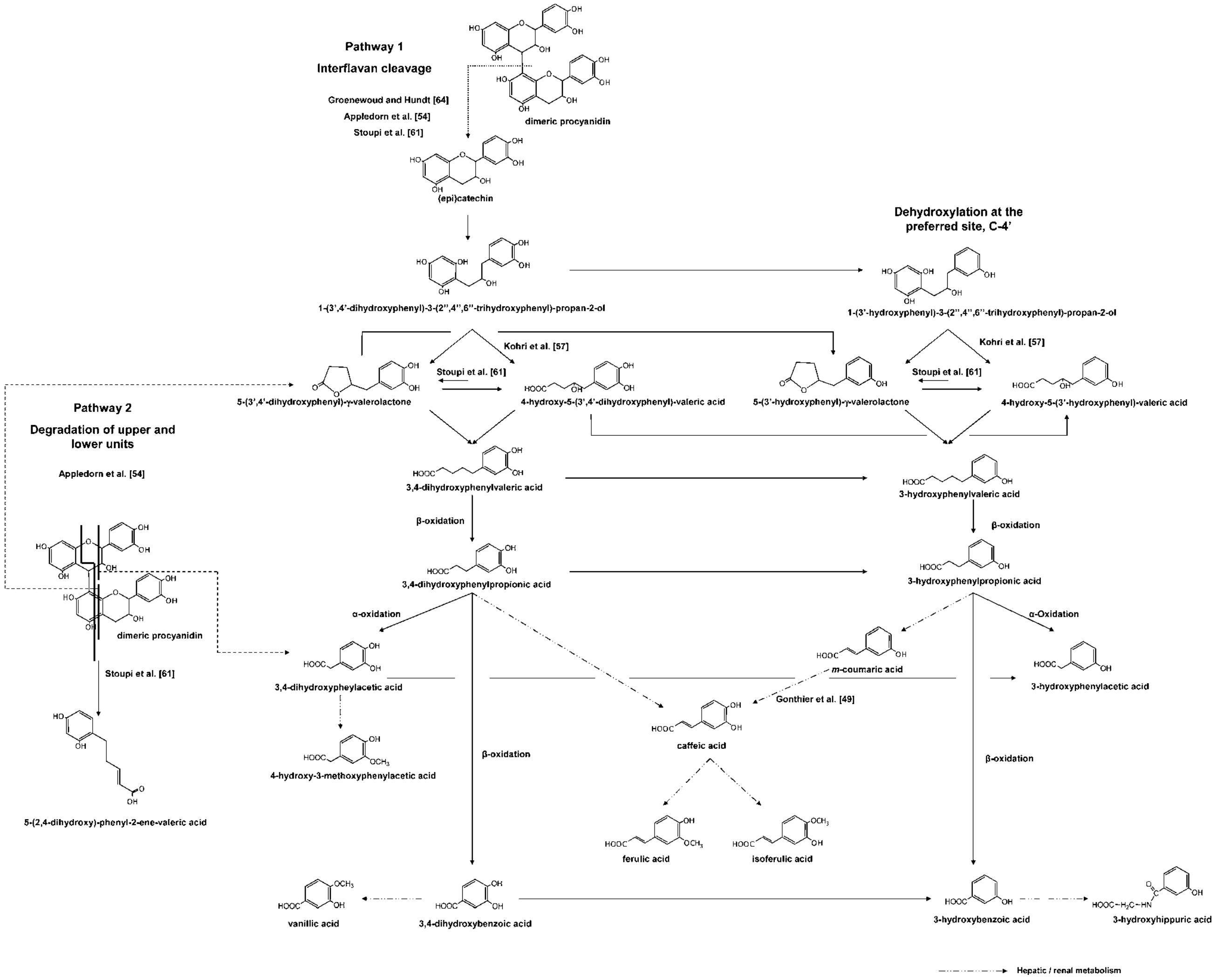


Fig. 3 Metabolic pathway tentatively proposed for the catabolism of monomeric flavan-3-ols and dimeric procyanidins by the intestinal microbiota.

microbiota[8,10] and their implication in the overall bioavailability and bioactivity of polyphenols.

III.1 First steps of the catabolism of flavan-3-ols: formation of hydroxyphenylvalerolactones and valeric acids

The complex catabolism of B-type proanthocyanidins involves C-ring opening, followed by lactonization, decarboxylation, dehydroxylation, and oxidation reactions, among others.[10] Although numerous *in vitro* fermentation and *in vivo* studies have been carried out in recent years, the accumulated knowledge has only led to partial elucidation of the catabolic route of monomeric and B-type dimeric structures[49,52–55] (Fig. 3). In the case of galloylated monomeric flavan-3-ols (ECG and EGCG), the microbial catabolism usually starts with the rapid cleavage of the gallic acid ester moiety by microbial esterases, giving rise to gallic acid which is further decarboxylated into pyrogallol.[56–58] The C-ring is subsequently opened, giving rise to diphenylpropan-2-ol, which is later converted into 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone (in the case of (epi)catechins) or 5-(3′,4′,5′-trihydroxyphenyl)-γ-valerolactone (in the case of (epi)gallocatechins).[56,58,59] The valerolactone ring later breaks, giving rise to 5-(3′,4′-dihydroxyphenyl)valeric acid and/or 4-hydroxy-5-(3′,4′-dihydroxyphenyl)valeric acid. The identification of this latter compound was firstly proposed by Khori *et al.*[57] and recently confirmed by Llorach *et al.*[60] in urine samples collected after cocoa consumption in humans, as well as by Stoupi *et al.*[61] after *in vitro* fermentations carried out with human faeces in the presence of (−)-epicatechin and procyanidin B2.

Although it was first proposed that 4-hydroxy-5-(hydroxyphenyl)valeric acids could arise from the degradation of diphenylpropan-2-ols, concurrently with hydroxyphenyl-γ-valerolactones[57] (Fig. 3), it has recently been suggested that they are formed instead from hydroxyphenyl-γ-valerolactones, and that an interconversion between both forms [4-hydroxy-5-(hydroxyphenyl)valeric acids and 5-(hydroxyphenyl)-γ-valerolactones] may exist, but is largely displaced towards the formation of the formers.[61] Subsequent biotransformations of these valeric acids give rise to hydroxyphenylpropionic and hydroxybenzoic acids by successive loss of carbon atoms from the side chain through β-oxidation.[56]

III.2 Metabolites arising from the catabolism of dimeric procyanidins

The possible formation of 3,4-dihydroxyphenylacetic acid *via* α-oxidation of 3,4-dihydroxyphenylpropionic acid (as described for tyrosine[49,62]) in the microbial catabolism pathway of monomeric flavan-3-ols, has been widely debated. Firstly, it was thought that 3,4-dihydroxyphenylacetic acid was only characteristic of the catabolism of dimeric procyanidins;[63] however, other authors have recently proposed α-oxidation as a possible pathway for the formation of this compound in the case of both monomers and dimers,[61] without discarding other possible pathways, as proposed by Appeldoorn *et al.*[54] in the case of dimers. According to these latter authors, 3,4-dihydroxyphenylacetic acid results from the cleavage of the upper unit of dimeric procyanidins, whereas the lower unit gives rise to 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone and to the triggering of the rest of the previously described route (Fig. 3). The possible depolymerization of dimeric structures into monomeric units, firstly proposed by Groenewoud *et al.*,[64] has been recently confirmed to occur but to a lesser extent,[54,61] representing less than 10% in the case of procyanidin B2.[61] Other microbial metabolites arising exclusively from the catabolism of dimeric procyanidins have recently been identified, such as 5-(2′,4′-dihydroxyphenyl)-2-ene-valeric acid, as well as other compounds which have been tentatively identified as derivatives from the A-ring of the upper unit, including the interflavanic bond.[61]

III.3 Last steps of the catabolism of flavan-3-ols

Finally, the last steps of the microbial catabolism of (epi)catechin involve dehydroxylation of 3,4-dihydroxylated phenolic acids at C-4′ (preferentially), and C-3′, resulting in 3- and 4-monohydroxylated phenolic acids, respectively.[53,61] In the case of (epi)gallocatechins, dehydroxylation preferentially occurs at C-5, resulting in 3,4-dihydroxylated phenolic acids which undergo further dehydroxylation at C-4 and C-3, as mentioned above. However, in the case of hydroxyphenylvalerolactones, the 3,5-dihydroxylated derivative arising from the dehydroxylation of 5-(3′,4′,5′-trihydroxyphenyl)-γ-valerolactone has also been identified, indicating that dehydroxylation at C-4′ occurs.[20] Once absorbed, the microbial metabolites from flavan-3-ols are mainly metabolized in the liver by phase II enzymes as conjugated derivatives that are subsequently eliminated in urine. At the same time, a portion of microbial metabolites (non-conjugated microbial metabolites) is eliminated in the faeces.

Several microbe-derived metabolites that have been detected in urine in their actual conjugated form by targeted analysis including: monoglucuronide and monosulfate of 5-(3′,4′- and 3′,5′-dihydroxyphenyl)-γ-valerolactone, in addition to the methyl-sulfate derivatives of 5-(3′,4′,5′-trihydroxyphenyl)-γ-valerolactone.[20,22,30,65] In the case of phenolic acids, monoglucuronide and monosulfate conjugates of mono- and di-hydroxyphenylpropionic and *p*-coumaric acids have been reported.[66] Other reactions occurring in the liver and kidney include: glycine conjugation, dehydrogenation, hydroxylation and methylation.[53] The excretion of microbial metabolites varies markedly between subjects, and for some individuals it may also vary with the substrate, reaching a very high proportion (up to 50%) of the intake of polyphenols.[8]

III.4 Main microbial phenolic metabolites found in urine

Several feeding studies have revealed significant changes in the urinary excretion of microbe-derived phenolic acids after the intake of rich sources of flavan-3-ols. Among phenolic acids, mono- and di-hydroxylated phenylpropionic and phenylacetic acids, together with hydroxyhippuric acids, have been found as main urinary microbial phenolic acids derived from flavan-3-ol intake.

With regard to cocoa and cocoa-derived products, Gonthier *et al.*[49] reported an increase in 3,4-dihydroxyphenylacetic and 3-hydroxyphenylacetic acids in urine after the administration of procyanidin B3 to rats. Similarly, Rios *et al.*[67] reported a significant increase in the urinary excretion of these compounds, as well as in 3-hydroxyphenylpropionic and 3-hydroxybenzoic acids in healthy humans after acute consumption of flavanol-rich chocolate. Recently, Urpi-Sarda *et al.*[68] also found increased urinary levels of 3,4-dihydroxyphenylacetic and 3-hydroxyphenylacetic acids in humans after chronic consumption of cocoa powder with milk. Other studies have reported an increased urinary excretion of 3-hydroxypropionic and 3-hydroxyphenylacetic acids after human consumption of grape seed polyphenols.[69] In the case of green tea, 3-hydroxyphenylpropionic and 4-hydroxyphenylacetic and 4-hydroxyphenylacetic acids significantly increased in human urine.[58] Finally, 3-hydroxyphenylpropionic and 3-hydroxybenzoic acids were also reported to increase in the urine of rats fed wine polyphenols.[53]

Besides these phenolic acids, which are also common to the microbial catabolism of other flavonoids,[10] 5-(3′,4′,5′-trihydroxyphenyl)-γ-valerolactone and 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone are considered important microbial metabolites and potential biomarkers of flavan-3-ol consumption in humans, as has been confirmed after the intake of green tea,[20,22,23,58] cocoa products[30] and almond skins.[65,70]

IV. Intestinal bacteria with ability to catabolize flavan-3-ols

It is important to mention that the above difference of opinions concerning the possible catabolic route of monomeric and dimeric flavan-3-ols could be partly attributed to differences in the microbiota composition of faecal samples used in the different studies, suggesting that different pathways could coexist or one predominate over the others, depending on the catabolic capacity of the microbiota. An important limitation in this area is that bacteria belonging to human microbiota with the capacity to catabolize flavan-3-ols have still not been identified. To date, only bacteria with the capacity to catabolize other types of flavonoid compounds, mainly flavonols and flavones, have been described. These bacteria, in general, belong to the *Clostridium* and *Eubacterium* groups.[10]

Among the factors that may limit the identification of flavan-3-ol catabolic bacteria, it is important to highlight the well-known growth inhibitory effects of proanthocyanidins. Another factor that deserves consideration is the structural features of flavan-3-ols as complex non-planar molecules.

IV.1 Inhibitory effects of proanthocyanidins and "tannin-resistant" bacteria

The growth-inhibitory effects of proanthocyanidins on bacteria have been reviewed by Smith *et al.*[71] Tannins are capable of complexing with polymers and minerals, making nutrients unavailable. In addition, they could have a direct effect by interacting with membranes, cell walls, and/or extracellular proteins. "Tannin-resistant" bacteria have been defined as those bacteria that are able to withstand the inhibitory effect of tannins. "Resistance" implies that some action is required on the part of the organism to withstand the inhibitory effect of tannins, including inducible adaptation or even gene transfer.[71] Tannin-resistance may also depend on the tannin concentration, structural composition and DP. It is important to highlight the fact that bacteria which are predominant in tannin-rich mediums may not be resistant *per se*, but are less affected by nutrient limitations or are better able to access limiting nutrients.

"Tannin-resistant" Gram-negative species (Enterobacteriaceae and *Bacteriodes*) have been isolated from rat faecal samples after prolonged administration of condensed tannins from *Acacia angustissima*, a forage legume.[72] "Tannin-resistant" Gram-positive bacteria have also been identified. Brooker *et al.*[73] isolated a *Streptococcus* strain (named *S. caprinus* and close to *S. bovis*) from the rumen of goats which was able to grow at 2.5% of condensed tannins. A *Streptococcus* strain (close to *S. bovis* and *S. gallolyticus*) has also been isolated from the rumen of sheep, goats and deer.[74] Later, Molina *et al.*[75] has also isolated a *Eubacterium* strain (close to *E. cellulosolvens*) from the rumen of moose, able to tolerate 0.5 g/L of condensed tannins.

Some mechanisms by which bacteria can overcome inhibition by tannins include: modification/degradation of the substrate, dissociation of tannin–substrate complexes, cell membrane modification/repair and metal ion sequestration. It has been reported that *Bifidobacterium infantis* and *Lactobacillus acidophilus* are not inhibited by tannins because lactic acid bacteria do not require iron as they do not depend on metal-chelating enzymes, in particular heme enzymes.[76] Moreover, *in vivo* studies have revealed that consumption of grape seed extract, containing 40% of condensed tannins, produced an increase in the bifidobacteria population in healthy individuals.[77] Although tannin resistance is the first step in order for bacteria to metabolize condensed tannins, resistance does not guarantee metabolic activity, and the biodegradation pathway of "tannin-resistant" bacteria has not yet been described.

IV.2 Structural features of flavan-3-ols limiting bacterial catabolism

There is some evidence that the structural characteristics and stereochemistry of flavan-3-ols could be limiting factors for intestinal bacteria to be able to degrade these types of compounds. It has been reported that *Eubacterium ramulus* was unable to degrade (+)-catechin because of the absence of a functional group at C-4 in this flavonoid structure.[78] Similarly, the human bacterium *Eubacterium* sp. (SDG-2) was able to open the ring of the 3*R* [(−)-catechin and (−)-epicatechin] and the 3*S* [(+)-catechin and (+)-epicatechin] forms of monomeric flavan-3-ols into 1,3-diphenylpropan-2-ols (Fig. 3), but was incapable of producing the same fission in their galloylated esters.[79] However, in no instance was this bacteria able to continue the catabolism up to the formation of 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone. Another characteristic of this bacterium was the ability to dehydroxylate the OH groups in the B ring of 1,3-diphenylpropan-2-ols, but only of the *R* forms.[79] This fact, together with the inability to catabolize the gallate esters, suggests that the spatial configuration of both the original flavan-3-ol molecule and intermediate metabolites may limit the microbial degradation of flavan-3-ols. In fact, in a recent *in vitro* fermentation study with human faeces it was found that (+)-catechin (2*R*,3*S*) was firstly converted into (+)-epicatechin (2*S*,3*S*) by intestinal microbiota in order for the biotransformation process to proceed.[80]

Taken together, these findings suggest that it may be difficult to identify a single bacterium capable of exhibiting the whole catabolic pathway proposed in Fig. 3, but rather the catabolism may be carried out by different bacteria with specific catabolic activities that work in sequential form on the appearance of the different intermediate metabolites. Among the different phases of the catabolic pathway, formation of 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone seems to be a limiting step.

V. Bioactivity of flavan-3-ol metabolites

As a consequence of their extensive metabolism in the human organism, the original flavan-3-ol structures present in food are not present in plasma and urine (with the exception of small amounts of gallate ester of monomeric flavan-3-ols and dimeric procyanidins that appear unmetabolized, as mentioned above) but rather appear as a complex series of phase II or tissular metabolites and, particularly, of microbe-derived phenolic metabolites. Therefore, both types of circulating metabolites should be responsible for the health benefits associated with the consumption of dietary sources rich in flavan-3-ols.

V.1 Bioactivity of phase II or tissular metabolites derived from small-intestine and liver metabolism

One of the limitations of many *in vitro* and *ex vivo* studies which have tried to unravel the health effects of flavan-3-ols has been the use of unconjugated structures, as well as the use of test concentrations (mM) at a much higher range than that found in biological fluids (μM range). Taking this into consideration, this section will only try to cover the results of studies performed with conjugated metabolites in the micromolar or submillimolar range (0.5–30 μM) found in plasma (Table 1).

In general, the conjugation process (glucuronidation, sulfation and methylation) affects the physico-chemical properties of flavan-3-ols and, in turn, their residence in plasma, their excretion rate, and finally the bioactive properties of the parent compound.[13] In particular, sulfation and glucuronidation involve a considerable attenuation of biological activity. The case of methylation seems to be more complex because the incorporation of methyl groups reduces the number of available OH groups, but at the same time increases the lipophilic nature of the compound, which can be advantageous for cellular uptake by passive diffusion.[13]

Antioxidant activity. The antioxidant activity of flavonoid metabolites has been widely studied, considering the fact that oxidative stress is implicated in the initiation and progression of chronic diseases. In the case of flavonoid compounds (*i.e.* quercetin), it has been observed that glucuronidation at C-3′ and C-4′ of the B ring (catechol-type structure) produces a greater loss of antioxidant capacity than when it occurs at C-3 of the C ring.[81–83] In contrast, glucuronidation at C-7 (A ring) seems to produce a slight increase in antioxidant activity.[83] In the case of flavan-3-ol metabolites, (−)-epicatechin and its 7-*O*-glucuronide presented a similar delay of Cu^{2+}-induced LDL oxidation, whereas the activities of the 3′-*O*-glucuronide and the 4′-*O*-methyl-3′-*O*-glucuronide were significantly lower.[84] However, in the case of galloylated (epi)gallocatechins, the position of glucuronidation affected the anti-radical capacity against DPPH differently to the other flavonoids, since EGCG-7-*O*-glucuronide and EGCG-4″-*O*-glucuronide (galloylation in the gallic acid ring) were less active than the aglycone, whereas the 3′- and 3″-*O*-glucuronides showed the same activity as the aglycone.[24] For non-galloylated (epi)gallocatechins, EGC-7-*O*-glucuronide and -3′-*O*-glucuronide were more active than the aglycone.[24]

In the case of *O*-methylation, Cren-Olivé *et al.*[85] also reported that the catechol B-ring was also the active moiety of (+)-catechin, since the 3′- and 4′-*O*-methyl ethers and 3′,4′-di-*O*-methyl ether showed a much lower inhibition of Cu^{2+}-induced LDL oxidation than the aglycone, but the activity was recovered when these positions were free, as in the 5,7-di-*O*-methyl analogue. The C-3′ and C-4′-*O*-methyl ethers of (−)-epicatechin also showed a lower inhibition of peroxynitrite-induced tyrosine nitration than the parent compound.[86] Similarly, *O*-methylation at position C-3′ in (−)-epicatechin, (−)-epigallocatechin and (−)-epicatechin-3-*O*-gallate elicited a potential inhibition of lipid oxidation of canola oil in comparison to the aglycone.[87] In a recent study, C-3′ and C-4′-*O*-methyl ethers of (+)-catechin and (−)-epicatechin showed a lower antioxidant capacity than the parent compound, as measured by the ferric-reducing power (FRAP) and by the ability to scavenge the $ABTS^+$ radical cation.[88] Moreover, the antioxidant activity of these metabolites was found to be pH dependent, but significant radical scavenging activity was found to be retained at pH 7.4, suggesting that they could act as potential antioxidants under physiological conditions.[88]

Vascular effects. Epicatechin and its metabolite, epicatechin-7-*O*-glucuronide, have been identified as independent predictors of the vascular effects observed after flavanol-rich cocoa intake.[19]

Anti-inflammatory effects. In the case of EGCG metabolites, glucuronidation at C-7 affected the ability to inhibit the production of NO or the arachidonic acid metabolism in HT29 cells compared to the aglycone, but it was not affected in the case of glucuronidation at C-3′, C-3″, C-4″.[24] Conversely, in the case of ECG, glucuronidation at C-3′ decreased such capacity by 20% compared to the aglycone, but it was not affected in the case of the 7-*O*-glucuronide.[24]

Inhibition of cellular growth. The effectiveness of (−)-epicatechin metabolites on the inhibition of cellular growth has been studied in various types of cell lines. In the case of neuronal cells, it has been reported that 3′-*O*-methyl-epicatechin was as effective as (−)-epicatechin in the inhibition of apoptosis induced by oxidized LDL.[89] Similarly, it has been reported that 3′-*O*-methyl-epicatechin was as efficient as (−)-epicatechin in protecting human fibroblasts against cell death induced by oxidative stress.[90] In the case of galloylated flavan-3-ol metabolites, methylation at C-4′ and C-4″ in (−)-epigallocatechin-3-*O*-gallate (EGCG) produced a 50% decrease in the growth-inhibitory and pro-apoptotic activities of murine osteoclasts, compared to EGCG.[91] In another study, methylated derivatives of EGCG at positions C-4″ and C-4′-4″ (dimethyl derivative) presented less inhibitory capacity than EGCG of the enzyme 20S proteasome, which catalyzes the degradation of intracellular proteins and is associated with cancer.[92]

Table 1 Biological activity of phase II or tissular metabolites of flavan-3-ols.[a,b,e]

Reference	Test	Metabolite	Concentration	Result
Antioxidant effects				
Cren-Olivé *et al.*[85]	Inhibition of Cu^{2+}-induced LDL oxidation	C-3′-*O*-methyl C-4′-*O*-methyl C-5,7-di-*O*-methyl C-3′,4′-di-*O*-methyl C-3′,4′,5,7-tetra-*O*-methyl 4-Methylcatechol	EC$_{50}$ = 15.2 ± 1.0 µM EC$_{50}$ = 11.7 ± 0.9 µM EC$_{50}$ = 0.63 ± 0.02 µM EC$_{50}$ = 3.80 ± 0.08 µM EC$_{50}$ > 100 µM EC$_{50}$ = 1.00 ± 0.05 µM	• The order of activity was: C-5,7-di-*O*-methyl > C > 4-methylcatechol > C-3′,4′-di-*O*-methyl > C-4′-*O*-methyl ~ C-3′-*O*-methyl > C-3′,4′,5,7-tetra-*O*-methyl.
Lu *et al.*[24]	DPPH radical scavenging activity	EGCG-4″-*O*-glucuronide EGCG-7-*O*-glucuronide EGCG-3″-*O*-glucuronide EGCG-3′-*O*-glucuronide EGC-3′-*O*-glucuronide EGC-7-*O*-glucuronide	EC$_{50}$ = 0.084[c] EC$_{50}$ = 0.081 EC$_{50}$ = 0.035 EC$_{50}$ = 0.037 EC$_{50}$ = 0.19 EC$_{50}$ = 0.11	• The order of activity was: EGCG-3″-*O*-glucuronide = EGCG-3′-*O*-glucuronide = EGCG > ECG > EGC > EGCG-7-*O*-glucuronide = EGCG-4″-*O*-glucuronide > EGC-7-*O*-glucuronide = EC > EGC-3′-*O*-glucuronide.
Natsume *et al.*[84]	Inhibition of Cu^{2+}-induced LDL oxidation	EC-7-*O*-glucuronide EC-3′-*O*-glucuronide EC-4′-*O*-methyl-3′-*O*-glucuronide	0.5 µg/mL	• The order of activity was: EC ≅ EC-7-*O*-glucuronide (~3% lower) ≫ EC-4′-*O*-methyl-3′-glucuronide ≅ EC-3′-*O*-glucuronide.
Su *et al.*[87]	Inhibition of lipid oxidation of canola oil	EC-3′-*O*-methyl EGC-3′-*O*-methyl EGCG-3′-*O*-methyl	0.5 mM	• The three metabolites were less effective than EC.
Pollard *et al.*[86]	Inhibition of peroxynitrite-induced tyrosine nitration	EC-*O*-methyl (1 : 1 mix)[d] EC-5-*O*-glucuronide	IC$_{50}$ = 19.0 µM IC$_{50}$ = 30.7 µM	• The order of activity was: EC > EC-*O*-methyl mix > EC-5-*O*-glucuronide.
Pollard *et al.*[86]	TEAC assay	EC-*O*-methyl (1 : 1 mix)[d] EC-5-*O*-glucuronide	0.1–500 µM	• The order of activity was: EC > EC-5-*O*-glucuronide > EC-*O*-methyl EC.
Dueñas *et al.*[88]	ABTS/peroxide assay; ABTS/persulfate assay; Ferric-reducing power (FRAP) assay	C-3′-*O*-methyl C-4′-*O*-methyl EC-3′-*O*-methyl EC-4′-*O*-methyl	n.a.	• C and EC > 3′- and 4′-*O*-methyl metabolites of C or EC. • Methylated metabolites still retain significant radical scavenging activity at pH 7.4. • Relatively high antioxidant activity was found in the case of C-3′-*O*-methyl catechin at pH 7.4 compared to C.
Vascular effects				
Schroeter *et al.*[19]	FMD after flavanol-rich cocoa ingestion	EC-7-*O*-glucuronide	C_{max} ~ 200 nM (2 h)	• EC and EC-7-*O*-glucuronide are predictors of the increase of FMD.

Table 1 (*Contd.*)

Reference	Test	Metabolite	Concentration	Result
Anti-inflammatory effects				
Lu *et al.*[24]	Inhibition of release of arachidonic acid from HT-29 human colon cancer cells	EGCG-7-*O*-glucuronide, EGCG-3′-*O*-glucuronide, EGCG-3″-*O*-glucuronide, EGCG-4″-*O*-glucuronide, EGC-3′-*O*-glucuronide, EGC-7-*O*-glucuronide, EC-5-*O*-glucuronide EC-4′-*O*-methyl-*O*-β-D-glucuronides (7, 5 and 3′) EC-7-*O*-glucuronide	2 and 10 µM C_{max} ~ 1450 nM (2 h) C_{max} ~ 210 nM (2 h)	• At 2 and 10 µM, the order of activity was: EGC-3′-*O*-glucuronide < EGC-7-*O*-glucuronide= EGC. • At 2 µM, the order of activity was: EGC-7-O-glucuronide < EGCG = three glucuronide derivatives of EGCG (3′, 3″ and 4″).
Inhibition of cellular growth				
Schroeter *et al.*[89]	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay Activation of signalling pathways ERK1/2 and JNK	EC-3′-*O*-methyl	30 µM	• EC-3′-*O*-methyl protects against oxLDL-induced neuronal injury. MTT reductions: EC (90%) and EC-3′-*O*-methyl-(93%). • Inhibition of the oxLDL-mediated activation of ERK1/2 and JNK
Basu-Modak *et al.*[90]	Modulation of UVA-induced cell death (FEK4 cells)	EC-3′-*O*-methyl	1 µM 30 µM	• No effects of 3′-*O*-methyl-EC at 1 µM. • Significant protection against cell death in live-cell population, similar to epicatechin at 30 µM.
Nakagawa *et al.*[91]	Cytotoxicity to murine osteoclasts Fe^{3+} reduction activity	EGCG-3′-*O*-methyl EGCG-4′-*O*-methyl EGCG-3″-*O*-methyl EGCG-4″-*O*-methyl	$EC_{50} = 87 \pm 2.2$ µM $EC_{50} > 100$ µM $EC_{50} = 70 \pm 11$ µM $EC_{50} > 100$ µM	• Methylated metabolites at position 4′ (B ring) or at position 4″ (D-ring) showed markedly cytotoxicity to osteoclasts. • EGCG-4′-O-Me also showed the lowest Fe^{3+}-reducing activity among EGCGs.
Landis-Piwowar *et al.*[92]	Inhibition of purified 20S proteasome assessed by using a chymotrypsin-like specific fluorogenic substrate	ECG-3″-*O*-methyl ECG-4″-*O*-methyl ECG-3″,4″-di-*O*-methyl EGCG-3″-*O*-methyl EGCG-4″-*O*-methyl EGCG-3″,4″-di-*O*-methyl EGCG-4′-*O*-methyl EGCG-4′,4″-di-*O*-methyl EGCG-4′-3″,4″-tri-*O*-methyl	$IC_{50} = 3.43 \pm 1.3$ µM $IC_{50} = 19.12 \pm 1.88$ µM $IC_{50} = 48.25 \pm 0.64$ µM $IC_{50} = 5.63 \pm 0.03$ µM $IC_{50} = 6.91 \pm 0.40$ µM $IC_{50} = 9.81 \pm 0.15$ µM $IC_{50} = 2.45 \pm 0.30$ µM $IC_{50} = 8.23 \pm 0.07$ µM $IC_{50} = 43.03 \pm 1.98$ µM	• The proteasome inhibitory activity for the ECG series was: ECG > ECG-3″-methyl > ECG-4″-methyl > ECG-3″,4″-dimethyl. • The proteasome inhibitory activity for the ECG series was: EGCG > EGCG-3″-*O*-methyl > EGCG-4″-*O*-methyl > EGCG-3″,4″-di-*O*-methyl. • And: EGCG > EGCG-4′-methyl > EGCG-4′,4″-dimethyl > EGCG-4′-3″,4″-tri-*O*-methyl.

[a] C: (+)-catechin; EC: (−)-epicatechin; ECG: (−)-epicatechin-3-*O*-gallate; EGC: (−)-epigallocatechin; EGCG: (−)-epigallocatechin-3-*O*-gallate. [b] DPPH: 2,2,-diphenylpicrylhydrazyl; ERK1/2: extracellular signal-regulated kinases 1/2; JNK: c-Jun N-terminal kinase; FMD: flow-mediated vasodilation. [c] EC_{50} expressed as molar ratio compound/DPPH. [d] 3′-*O*-methyl and 4′-*O*-methyl-epicatechin (1 : 1 mixture). [e] n.a.: not available.

Table 2 Biological activity of phenolic metabolites derived from the catabolism of flavan-3-ols by the intestinal microbiota.[a]

Reference	Test	Metabolite	Concentration	Result
Hydroxyphenyl-γ-valerolactones				
Antioxidant effect				
Grimm *et al.*[99]	Ferric-reducing antioxidant potential (FRAP)	5-(3′,4′-Dihydroxyphenyl)-γ-valerolactone	$EC_{50} = 10.64 \pm 0.42$ µM	• The order of activity was: 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone > (+)-catechin > ascorbic acid > 5-(3′-methoxy-4′-hydroxyphenyl)-γ-valerolactone.
		5-(3′-Methoxy-4′-hydroxyphenyl)-γ-valerolactone	$EC_{50} = 19.65 \pm 0.75$ µM	
Grimm *et al.*[99]	Radical scavenging test against superoxide radicals	5-(3′,4′-Dihydroxyphenyl)-γ-valerolactone	$EC_{50} \sim 25$µM	• The order of activity was: 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone > (+)-catechin ≅ ascorbic acid > taxifolin > vitamin E.
		5-(3′-Methoxy-4′-hydroxyphenyl)-γ-valerolactone	No effect	
Anti-proliferative activity				
Lambert *et al.*[100]	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyletarazolium bromide (MTT) assay on: esophageal squamous carcinoma cells (KYSE150); human colon adenocarcinoma cells (HT-29 and HCT-116); immortalized human intestinal cells epithelial cells (INT-407); immortalized rat intestinal epithelial cell line (IEC-6)	(−)-5-(3′,4′,5′-Trihydroxyphenyl)-γ-valerolactone	$IC_{50} = 15–73$ µM	• 5-(3′,4′,5′-trihydroxyphenyl)-γ-valerolactone was the most active metabolite tested in carcinoma and intestinal cells, whereas 5-(3′,4′,5′-trimethoxyphenyl)-γ-valerolactone only inhibited cell growth by less than 20% at concentrations up to 50 µM.
		(−)-5-(3′,4′-dihydroxyphenyl)-γ-valerolactone; (3-hydroxy-4′-methoxyphenyl)-γ-valerolactone; 5-(3′,4′,-dimethoxyphenyl)-γ-valerolactone; 5-(3′,4′,5′-Trimethoxyphenyl)-γ-valerolactone	>50 µM	• 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone, and its mono- and di-methoxylated derivates were significantly less potent than 5-(3′,4′,5′-trihydroxyphenyl)-γ-valerolactone and inhibited growth of KYSE150 cells by 20–40% at 50µM, but had no effect on HT-29 cells.
Anti-inflammatory effect				
Grimm *et al.*[99]	Inhibition of the enzymatic activity of MMP-1, MMP-2 and MMP-9 (matrix metalloproteinases)	5-(3′,4′-Dihydroxyphenyl)-γ-valerolactone	$IC_{50} = 10–23$ µg/mL (MMP-1); $IC_{50} \sim 13–23$ µg/mL (MMP-2); $IC_{50} \sim 4–19$ µg/mL (MMP-9)	• The metabolites were more effective than their metabolic precursor (+)-catechin in MMP inhibition.
		5-(3′-Methoxy-4′-hydroxyphenyl)-γ-valerolactone	$IC_{50} = 10–23$ µg/mL (MMP-1); $IC_{50} \sim 20–22$ µg/mL (MMP-2); $IC_{50} \sim 9–10$ µg/mL (MMP-9)	
	Inhibition of MMP-9 secretion	5-(3′,4′-Dihydroxyphenyl)-γ-valerolactone; 5-(3′-methoxy-4-hydroxyphenyl)-γ-valerolactone	$IC_{50} = 0.5$ µg/mL	• Highly potent prevention of MMP-9 release by both metabolites.
Lambert *et al.*[100]	Inhibition of the release of arachidonic acid and production of nitric oxide (NO) by LPS-stimulated murine macrophages (RAW264.7)	5-(3′,4′,5′-Trihydroxyphenyl)-γ-valerolactone	$IC_{50} = 20$ µM	• Neither compound inhibited the release of arachidonic acid. Only 5-(3′,4′,5′-trihydroxyphenyl)-γ-valerolactone inhibited NO production by 50% at 20 µM.
		5-(3′,4′,5′-Trimethoxyphenyl)-γ-valerolactone	No effect	

Table 2 (*Contd.*)

Reference	Test	Metabolite	Concentration	Result
Phenolic acids				
Effect of intestinal microbiota				
Lee *et al.*[101]	Growth inhibition of pathogenic and non-pathogenic human intestinal bacteria	3-*O*-Methyl gallic acid; gallic acid; caffeic acid; 4-hydroxyphenylpropionic acid; phenylpropionic acid; 4-hydroxyphenylacetic acid	1.0 mg/mL	• Significant inhibition of the growth of: – *Clostridium perfringens* by 3-*O*-methyl gallic acid and gallic acid; – *Staphylococcus* spp. by caffeic acid, 4-hydroxyphenylpropionic acid, phenylpropionic acid, 4-hydroxyphenylacetic acid; – *E. coli* and *Salmonella* spp. by 4-hydroxyphenylpropionic acid, phenylpropionic acid, 4-hydroxyphenylacetic acid. • Lower inhibition of commensal bacteria and probiotics: *Clostridium* spp; *Bidobacterium* spp.; *Lactobacillus* spp.
Alakomi *et al.*[102]	Permeability assay through 1-*N*-phenylnaphthylamine uptake assay	3,4-Dihydroxyphenylacetic acid; 3-hydroxyphenylacetic acid; 3,4-dihydroxyphenylpropionic acid; 4-hydroxyphenylpropionic acid; phenylpropionic acid; 3-hydroxyphenylpropionic acid	2.5 mM	• 3,4-Dihydroxyphenylacetic acid, 3-hydroxyphenylacetic acid, 3,4-dihydroxyphenylpropionic acid, 4-hydroxyphenylpropionic acid, phenylpropionic acid, and 3-hydroxyphenylpropionic acid efficiently destabilized the outer membrane (OM) of *Salmonella enterica* subsp. *enterica* serovar Typhimurium and *S. enterica* subsp. *enterica* serovar Infantis. Their activity is based on removal of OM-stabilizing divalent cations.
Cueva *et al.*[103]	Growth inhibition of pathogenic and non-pathogenic human intestinal bacteria	Benzoic acid; 3-hydroxybenzoic acid; 4-hydroxybenzoic acid; 3,4-dihydroxybenzoic acid; 4-hydroxy-3-methoxybenzoic acid; phenylacetic acid; 3-hydroxyphenylacetic acid; 4-hydroxyphenylacetic acid; 3,4-dihydroxyphenylacetic acid; phenylpropionic acid; 3-hydroxyphenylpropionic acid; 4-hydroxyphenylpropionic acid; 3,4-dihydroxyphenylpropionic acid	62.5–1000 μg/mL	• For *E.coli* strains, the order of activity was: – Benzoic and phenylacetic acids: non-substituted ≫ 4-hydroxy-3-methoxy- > 3-hydroxy- > 4-hydroxy- > 3,4-dihydroxy-substituted. – Phenylpropionic acids: non-substituted ≫ 4-hydroxy- > 3-hydroxy- > 3,4-dihydroxy-substituted. • For *Lactobacilli* and *Staphylocccus aureus* strains, the order of activity was: – Benzoic acids: 4-hydroxy- > 3-hydroxy- > non-substituted > 4-hydroxy-3-methoxy- > 3,4-dihydroxy-substituted. – Phenylacetic acids: non-substituted > 3-hydroxy- > 4-hydroxy- > 3,4-dihydroxy-substituted. – Phenylpropionic acids: non-substituted > 4-hydroxy- > 3-hydroxy > 3,4-dihydroxy-substituted. – Phenolic acids failed to inhibit the growth of the Gram-negative bacterium *P. aeruginosa* PAO1 at any concentration tested.

Table 2 (*Contd.*)

Reference	Test	Metabolite	Concentration	Result
Antioxidant effect				
Gläßer *et al.*[104]	DPHH radical scavenger assay in cultured rat hepatocytes	3,4-Dihydroxyphenylacetic acid 4-Hydroxyphenylacetic acid 3-Hydroxyphenylacetic acid Homovanillic acid 3,4-Dihydroxytoluene Hippuric acid	$EC_{50} = 4.6$ µM $EC_{50} > 500$ µM $EC_{50} > 500$ µM $EC_{50} = 17$ µM $EC_{50} = 7.3$ µM $EC_{50} > 500$ µM	• The radical scavenging activity decreased in the order: 3,4-dihydroxyphenylacetic acid > 3,4-dihydroxytoluene > homovanillic acid ≫ hippuric acid = 4-hydroxyphenylacetic acid = 3-hydroxyphenylacetic acid.
	Lipid peroxidation in cultured rat hepatocytes challenged with tert-Butyl hydroperoxide (MDA assay)	3-Hydroxyphenylacetic acid Homovanillic acid 3,4-Dihydroxytoluene Hippuric acid 3,4-Dihydroxyphenylacetic acid 4-Hydroxyphenylacetic acid	No effect $EC_{50} > 100$ µM $EC_{50} = 30$ µM No effect $EC_{50} > 150$ µM No effect	• None of the studied metabolites was effective up to 70 µM, with the exception of 3,4-dihydroxytoluene.
Anti-thrombotic activity				
Rechner *et al.*[105]	Platelet aggregation induced by TRAP, ADP and collagen	Polyphenol mix 1[b]; 3-hydroxyphenylpropionic acid; 4-hydroxyphenylpropionic acid; 3,4-dihydroxyphenylpropionic acid; dihydroferulic acid; homovanillic acid; 3-hydroxyphenylacetic acid; homovanillyl alcohol; 3-hydroxybenzoic acid; phloroglucinol; hippuric acid	10 µM	• For the agonist TRAP the threshold concentration to induce aggregation increased following the incubation of platelet-rich plasma with dihydroferulic acid (+1.0 µM), homovanillyl alcohol (+0.84 µM) and Polyphenol mix 1 (+1.93 µM). Other compounds tested showed no significant effect on the platelets' sensitivity towards the agonist TRAP. • None of the tested polyphenol metabolites affected ADP- and collagen-induced platelet aggregation at concentrations up to 100 µM.
	P-selectin expression			• Reduction of P-selectin expression on resting platelets was observed following incubation with dihydroferulic acid (−29 ± 14%), dihydrocaffeic acid (−20 ± 18%), 3-hydroxyphenylpropionic acid (−19 ± 11%), and Polyphenol mix 1 (−16 ± 9%).
	TRAP-induced platelet activation			• Activation of platelet with TRAP increased the P-selectin expression (from 0.5 to 15%). The activation was reduced with dihydroferulic acid (−20 ± 17%), 3-hydrophenylpropionic acid (−21 ± 17%), and Polyphenol mix 1 (−12 ± 6).
	TRAP-induced platelet activation under oxidative stress			• H_2O_2 increased response to TRAP. This response was partly reversed with dihydroferulic acid (−12 ± 19%), 3-hydrophenylpropionic acid (−16 ± 10%), and Polyphenol mix 1(−13 ± 10).
	Effects on epinephrine-stressed platelets			• Only 3-hydroxyphenylpropionic acid (−11 ± 12%) significantly reversed epinephrine-induced increase in P-selectin expression. • Epinephrine-induced glycoprotein CD63

Table 2 (*Contd.*)

Reference	Test	Metabolite	Concentration	Result
				expression decreased following incubation with 3-hydroxyphenylpropionic acid (−15 ± 9%), 3-hydroxyphenylacetic acid (−12 ± 7%), 3-hydroxybenzoic acid (−17 ± 12%), phloroglucinol (−20 ± 7), and Polyphenol mix 1 (−13 ± 11%).
Anti-inflammatory effect				
Karlsson *et al.*[106]	Inhibition of COX-2 protein levels in TNF-α- induced HT-29 cells	Phenylpropionic acid; 3-hydroxyphenylacetic acid; 4-hydroxyphenylpropionic acid	250–500 μmol/L	• % COX-2 inhibition at 250 and 500 μmol/L, respectively was: – phenylpropionic acid: 29.5 ± 14% and 35 ± 7%. – 3-hydroxyphenylacetic acid: 14.7 ± 15% and 39.9 ± 5%. – 4-hydroxyphenylpropionic acid: 61.9 ± 8% and 67 ± 6%.
Russell *et al.*[107]	Inhibition of prostanoid biogenesis	2-Hydroxybenzoic acid; 4-hydroxybenzoic acid; 2,3-dihydroxybenzoic acid; 2,4-dihydroxybenzoic acid; 2,5-dihydroxybenzoic acid; 2,6-dihydroxybenzoic acid; protocatechuic acid; 3,5-dihydroxybenzoic acid; gallic acid; vanillic acid; acetovanillone; vanillin; vanillyl alcohol; homovanillic acid; eugenol; cinnamic acid; *o*-coumaric acid; *m*-coumaric acid; *p*-coumaric acid; caffeic acid; 3-(3,4,5-trihydroxyphenyl)-acrylic acid; ferulic acid; sinapic acid; ethyl ferulate; coniferyl alcohol; curcumin	0.1 μmol/L	• Compounds inhibiting prostanoid production presented the following structure: – 4-hydroxy and 3-methoxyl aromatic substitution pattern (vanillic acid, acetovanillone, vanillin, vanillyl alcohol, homovanillinc acid, ferulic acid, sinapic acid, ethyl ferulate), which significantly inhibited prostanoid biogenesis by up to 81% (vanillin). – 3-carbon side chain (eugenol, cinnamic acid, *o*-coumaric acid, *m*-coumaric acid, *p*-coumaric acid,3-(3,4,5-trihydroxyphenyl)acrylic acid, coniferyl alcohol), which significantly inhibited prostanoid biogenesis by up to 75% (coniferyl alcohol).
Monagas *et al.*[108]	Production of pro-inflammatory cytokines (TNF-α, IL-1β and IL-6) in LPS-stimulated peripheral blood mononuclear cells (PBMC) pretreated with the phenolic metabolites	3,4-Dihydroxyphenylpropionic acid; 3-hydroxyphenylpropionic acid; 3,4-dihydroxyphenylacetic acid; 3-hydroxyphenylacetic acid; 4-hydroxybenzoic acid; 4-hydroxyhippuric acid	1 μM	• With the exception of 4-hydroxyhippuric acid for TNF-α secretion, only the dihydroxylated compounds, 3,4-dihydroxyphenylpropionic acid and 3,4-dihydroxyphenylacetic acid significantly inhibited the secretion of these pro-inflammatory cytokines in LPS-stimulated PBMC (84.9 and 86.4%, respectively). • The concentrations of IL-6 were reduced by 88.8 and 92.3% with 3,4-dihydroxyphenylpropionic acid and 3,4-dihydroxyphenylacetic acid, respectively. • Inhibition for IL-1β was 93.1% for 3,4-

Table 2 (*Contd.*)

Reference	Test	Metabolite	Concentration	Result
				dihydroxyphenylpropionic acid and 97.9% for 3,4-dihydroxyphenylacetic acid.
Larrosa *et al.*[109]	DSS-induced colitis model	3,4-Dihydroxyphenylpropionic acid	50 mg/kg	• 3,4-dihydroxyphenylpropionic acid reduced the expression of the cytokines IL-1β, IL-8, and TNF-α, malonyldialdehyde levels and oxidative DNA damage (measured as 8-oxo-2′-deoxyguanosine levels) in distal colon mucosa.
Larrosa *et al.*[109]	Measure of prostaglandin E_2 (PGE_2) production by CCD-18 colon fibroblast cells stimulated with IL-1β	3-Hydroxybenzoic acid; 4-hydroxybenozoic acid; protocatechuic acid; vanillic acid; phenylacetic acid; 3-hydroxyphenylacetic acid; 4-hydroxyphenylacetic acid; homoprotocatechuic acid; homovanillic acid; hippuric acid; 3-hydroxyhippuric acid; 3,4-dihydroxyphenylpropionic acid; 3-phenylpropionic acid; 3-hydroxyphenylpropionic acid; 4-hydroxyphenylpropionic acid; hydroferulic acid; ferulic acid; valeric acid	100 µM	• 3,4-dihydroxyphenylpropionic, hydroferulic and 3,4-dihydroxyphenylacetic acids inhibited PGE_2 production more than 50%.
Larrosa *et al.*[109]	Writhing and paw pressure test in rats	3,4-Dihydroxyphenylpropionic acid; hydroferulic acid; 3,4-dihydroxyphenylacetic acid	30 mg/kg	• The writhing number was reduced by 27, 35 and 40% by hydroferulic acid, 3,4-dihydroxyphenylpropionic acid and 3,4-dihydroxyphenyl acetic acid. • The three compounds increased the tolerance to the applied pressure of the inflamed paw, but 3,4-dihydroxyphenylpropionic acid was the most potent.
Anti-proliferative activity and cytotoxicity				
Tanaka *et al.*[111]	Tumor incidence and multiplicity induced by azoxymethane	Protocatechuic acid	500–1000 ppm	• Protocatechuic acid administration at 500 ppm and 1000 ppm during the initiation and post-initiation stage significantly inhibited intestinal carcinogenesis.
Krajka-Kúzniak *et al.*[112]	Effect on murine hepatic and renal cytochrome P450 and phase II enzymes	Protocatechuic acid	80–800 mg/kg	• Decrease in hepatic and renal EROD and MROD by ~20–30% at high concentration (400 and 800 mg/kg). • Decrease in renal PNPH by 28%. • Similar results on the expression of hepatic CYP1A1/1A2 and CYP2E1 proteins.

Table 2 (*Contd.*)

Reference	Test	Metabolite	Concentration	Result
				• The activity of phase II enzyme GST was increased at 80 mg/kg. • Inhibition of hepatic NQO1 by 70% at 800 mg/kg • No effects on renal NQO1.
Gao *et al.*[110]	Incubation with LNCaP prostate cell line, HCT116 colonic cell line, and IEC6; normal intestinal epithelial cell line	3-Methoxy-4-hydroxyphenylacetic acid	LNCaP: $IC_{50} > 200$ µM; HCT116: $IC_{50} > 200$ µM	• 3,4-Dihydroxyphenylacetic acid exhibited anti-proliferative activity in prostate and colon cancer cells. • 3,4-Dihydroxyphenylacetic acid was significantly more inhibitory in colon cancer cells (HCT116) compared with an immortalized normal intestinal epithelial cell line (IEC6).
		4-Hydroxyphenylacetic acid	LNCaP: $IC_{50} > 200$ µM	
		3,4-Dihydroxyphenylacetic acid	LNCaP: $IC_{50} = 135$ µM; HCT116: $IC_{50} = 90$ µM	
		3-Hydroxyphenylpropionic acid	LNCaP: $IC_{50} > 200$ µM; HCT116: $IC_{50} > 200$ µM	
		2,4,6-Trihydroxybenzoic acid	LNCaP: $IC_{50} > 200$ µM	
		3-Hydroxyphenylacetic acid	LNCaP: $IC_{50} > 200$ µM	
		Hippuric acid	LNCaP: $IC_{50} > 200$ µM; HCT116: $IC_{50} > 200$ µM	
Yip *et al.*[114]	Cytotoxicity on HepG2 hepatocellular cells	Protocatechuic acid	100 µmol/L ($IC_{50} = 60$ µmol/L)	• Cell viability was reduced by ~70% at 100 µmol/L. • Dose-dependent cytotoxicity resulted in a $IC_{50} = 60$ µmol/L.
Yip *et al.*[114]	Activation of signalling pathways involved in cancer (MAPK cascades)	Protocatechuic acid	3–300 µmol/L	• Detectable activation of the JNK and p38 subgroups of MAPK in HepG2 hepatocellular carcinoma cells at 30 µmol/L; maximum activation was observed at 100–300 µmol/L
Liu *et al.*[116]	Effects on rotenone-induced apoptosis in PC12 cells	Protocatechuic acid	0.1–1.0 mM	• Increase in cell viability by 71.15% at 1.0 mM. • Reduction by 12% in the total number of early apoptosis and late apoptosis/necrosis cells at 1.0 mM. • Significant supression of mitochondrial ROS, total glutathione, transmembrane potential, caspase-3-activity at 0.5 and 1.0 mM.

Table 2 (*Contd.*)

Reference	Test	Metabolite	Concentration	Result
				• Up-regulation of Bcl-2 gene family protein levels from 0.2–1.0 mM.
Modulation of lipid metabolism				
Gläßer *et al.*[104]	Effects on cholesterol biosynthesis in cultured heptocytes and HepG2 cells by the incorporation of radiolabeled acetate into the fraction of non-saponifiable neutral lipids	3,4-Dihydroxytoluene	$EC_{50} = 50$ µM	• 3,4-Dihydroxytoluene mimicked the effect of quercetin in primary rat hepatocytes, but much less so in HepG2 cells.

[a] ADP: adenosine 5′-diphosphate; DDS: dextran sodium sulfate; DPPH: 2,2,-diphenylpicrylhydrazyl; EROD: ethoxyresorufin-*O*-deethylase; JNK: c-Jun *N*-terminal kinase; GST: glutathione *S*-transferase; LPS: lipopolysaccharide; MAPK: mitogen-activated protein kinase; MMP: matrix metalloproteinases; MROD: methoxyresorufin *O*-demethylase; NQO1: NAD(P)H:quinone oxidoreductase; ROS: reactive oxygen species; TRAP: thrombin-receptor-activating peptide. [b] Polyphenol mix 1: 1 µM dihydroferulic acid, 1 µM 3-(3-hydroxyphenyl)propionic acid, 1 µM homovanillic acid, 1 µM 3-hydroxyphenylacetic acid, 0.1 µM delphinidin-3-rutinoside, 0.1 µM cyanidin-3-rutinoside, 0.1 µM cyanidin-3-glucoside, 0.1 µM malvidin-3-glucoside.

Interaction with cellular signalling pathways. In recent years, it has been suggested that polyphenols may exert their health effects *via* a mechanism of action beyond their antioxidant activity, and which is more related to its ability to generate an adaptive response at the cellular level that involves interaction with certain key proteins in triggering cell signalling pathways of oxidative stress and exposure to environmental toxins.[9] In the case of flavan-3-ols, most studies have been performed mainly with the non-conjugated forms. It has been reported that EGCG induces apoptosis and causes cell-cycle arrest in tumor cells – but not in non-transformed normal cells – through the modulation of nuclear factor kappa-B (NF-κB). NF-κB is a redox-sensitive transcription factor which regulates the expression of pro-inflammatory cytokines, iNOS, COX-2, growth factors and inhibitors of apoptosis, and is related to inflammatory diseases (atherosclerosis, ulcerative colitis and rheumatoid arthritis), as well as neurodegenerative diseases and cancer.[93,94] In another study, EGCG was also found to down-regulate NF-κB-inducing kinase (NIK), death-associated protein kinase (DPAK 1), and rho B and tyrosine protein kinase in PC-9 human lung cancer cells.[95] A down-regulation of genes involved in a wide range of physiological functions was found in the mucosa of rats with induced colon carcinogenesis that had been fed wine polyphenols for 16 weeks, being the major pathways down-regulated those involved in the inflammatory response and steroid metabolism.[96]

With regards to genes involved in relevant process of atherosclerosis, red wine polyphenols were also found to significantly inhibit the proliferation of human vascular smooth muscle cells – but not of human vascular endothelial cells – by reducing the promoter activity and expression of the cyclin A gene.[97] Green tea polyphenols have been shown to modulate the regulation of the transcriptional expression of proatherogenic molecules, including the sterol-response element binding protein (SREBP), PPAR-γ, IL-8, and apoprotein-E.[98]

V.2 Bioactivity of microbe-derived phenolic metabolites

The biological activities of microbial metabolites derived from the catabolism of flavan-3-ols are still largely unknown, but in recent years those of hydroxyphenyl-γ-valerolactones, and especially of phenolic acids (di- and mono- hydroxylated phenylproponic, phenylacetic, benzoic acids and derivatives) formed from the subsequent catabolism of the former, have started to be unravelled. In contrast to phase II or tissular metabolites derived from small-intestine and liver metabolism as described above, to date, *in vitro* studies performed with microbe-derived phenolic metabolites have been carried out with unconjugated metabolites (with the exception of hippuric acids) (Table 2).

V.2.1 Hydroxyphenyl-γ-valerolactones

Antioxidant activity. The antioxidant activity of 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone and its methyl derivative 5-(3′-methoxy-4′-hydroxyphenyl)-γ-valerolactone has been tested against superoxide radicals, as well as by the ferric-reducing antioxidant potential (FRAP) test.[99] In the radical scavenging test, 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone was more effective than (+)-catechin, ascorbic acid and trolox, whereas 5-(3′-methoxy-4′-hydroxyphenyl)-γ-valerolactone did not exhibit antioxidant activity. In the reducing test, the order of values was: 5-(3,4-dihydroxyphenyl)-γ-valerolactone > (+)-catechin > ascorbic acid > 5-(3-methoxy-4-hydroxyphenyl)-γ-valerolactone.[99]

Anti-proliferative activity. 5-(3′,4′,5′-Trihydroxyphenyl)-γ-valerolactone was more effective in the inhibition of the growth of a series of immortalized and malignant human cell lines than its trimethoxylated derivative, with the exception of HCT-116 colon cancer cells, and immobilized human (INT407) and rat (IEC-6) intestinal cells, which were not sensitive to the growth-inhibitory effects of this compound.[100] 5-(3′,4′,5′-Trihydroxyphenyl)-γ-valerolactone was also more effective in the inhibition of the growth of colon (HT-29) and oesophagus (KYSE150) cancer cells than 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone and its mono- and di-methoxylated derivatives.[100] However, the growth-inhibitory effects of this metabolite were lower than that of the aglycone, EGCG. Treatment of KYSE150 with 5-(3′,4′,5′-trihydroxyphenyl)-γ-valerolactone at 50 μM resulted in a 40% cell-growth inhibition after 48 h, whereas EGCG resulted in a 50% inhibition at 20 μM.[100]

Anti-inflammatory effects. The inhibition of NO production in murine macrophage cells (RAW264.7) by 5-(3′,4′,5′-trihydroxyphenyl)-γ-valerolactone and its trimethoxylated derivative has also been described.[100] Whereas the former metabolite had $IC_{50} = 20$ μM, the latter metabolite did not present any activity. However, none of the metabolites had inhibitory activity towards arachidonic acid metabolism in the same cell model.[100] On the other hand, 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone and its methyl derivative 5-(3′-methoxy-4′-hydroxyphenyl)-γ-valerolactone had similar inhibitory activity of the enzymatic activity of matrix metalloproteinases (MMP-1, MMP-2 and MMP-9).[99] Both metabolites also had similar efficacy in the inhibition of the secretion of MMP-9 from LPS-stimulated human monocytes.[99]

V.2.2 Phenolic acids

Effects on intestinal microbiota. Some phenolic acids, including 3-*O*-methyl gallic, gallic, caffeic, 4-hydroxyphenylpropionic, phenylpropionic, and 4-hydroxyphenylacetic acids derived from the microbial degradation of tea catechins, were able to inhibit the growth of several pathogenic and non-beneficial intestinal bacteria without significantly affecting the growth of beneficial bacteria (*Lactobacillus* spp. and *Bifidobacterium* spp.).[101] Other studies have revealed that dihydroxylated forms (*i.e.* 3,4-dihydroxyphenylacetic and 3,4-dihydroxyphenylpropionic acids) efficiently destabilize the outer membrane of *Salmonella*.[102] Recently, Cueva *et al.*[103] found that the number and position of substitutions in the benzene ring of phenolic acids and the saturated side chain length influenced the antimicrobial potential of phenolic acids against different microorganisms (*Escherichia coli*, *Lactobacillus* spp., *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*), although it was strain-dependent. In general, non-hydroxylated and monohydroxylated phenolic acids were more potent than dihydroxylated or disubstituted phenolic acids. With regard to the saturated side chain, the order of potency, for the same benzene ring-substitution, was benzoic > phenylacetic > phenylpropionic acid. Moreover, *Lactobacillus* spp. and *S. aureus* (Gram-positive) appeared more susceptible to the action of a series of microbial phenolic acids than Gram-negative bacteria, such as *E. coli* and *P. aeruginosa*.[103]

Antioxidant activity. Among a series of microbe-derived phenolic acids, 3,4-dihydroxyphenylacetic and 3,4-dihydroxytoluene exhibited the highest radical scavenging activity against DPPH in cultured rat hepatocytes.[104] However, only the latter metabolite was found to be effective against the lipid peroxidation of rat hepatocytes challenged with *tert*-butyl-hydroperoxide.[104]

Anti-thrombotic activity. Rechner *et al.*[105] studied the effect of several microbe-derived phenolic acids and their mixture on platelet function through several tests, including: platelet aggregation, P-selectin expression on resting platelets, effect on TRAP-induced platelet activation and epinephrine-stressed platelets. Dihydrocaffeic acid (3,4-dihydroxyphenylpropionic acid), dihydroferulic acid (4-hydroxy-3-methoxyphenylpropionic acid) and 3-hydroxyphenylpropionic acid, as well as the polyphenol mixture, were among the metabolites with the best activity in all tests performed.[105]

Anti-inflammatory activity. Studies carried out by Karlsson *et al.*[106] showed that faecal samples containing microbial phenolic acids affected cyclooxygenase-2 (COX-2) protein levels in colon cancer cells (HT-29) stimulated with TNF-α. Recently, Russell *et al.*[107] reported that phenolic acids presenting 4-hydroxy-3-methoxy substitution and a one-carbon side chain such as vanillic acid and its derivatives (vanillin, vanillyl alcohol and acetovanillone), as well as a three-carbon side chain (cinnamic, *o*-, *m*- and *p*-coumaric acid, and caffeic acid), inhibited cytokine-induced prostanoid biogenesis in human colonic fibroblasts. A structure–activity relationship has been observed between phenolic acids and their anti-inflammatory effects, since only dihydroxylated phenolic acids (*i.e.* 3,4-dihydroxyphenylpropionic and 3,4-dihydroxyphenylacetic acids) significantly inhibited the production of pro-inflammatory cytokines TNF-α, IL-1β, IL-6 in peripheral blood mononuclear cells (PBMC) stimulated with LPS, whereas no significant effect was found for the monohydroxylated ones.[108] Similarly, Larrosa *et al.*[109] recently found that these dihydroxylated phenolic acids provided the best inhibition of prostaglandin E2 production in cancer cells of fibroblast (CCD-18) stimulated with IL-1β. *In vivo* experiments with rats have also shown that 3,4-dihydroxypropionic acid was the most potent metabolite in writhing and paw pressure tests in rodents and reduced the expression of cytokines TNF-α, IL-1β, IL-8, as well as the levels of malonaldehyde and oxidative damage to DNA in the distal mucosa of rats with dextran sodium sulfate (DSS)-induced colitis.[109]

Anti-proliferative activity and cytotoxicity. Among a series of microbial phenolic metabolites, 3,4-dihydroxyphenylacetic acid presented anti-proliferative activity in prostate (LNCaP) and, in particular, in colon cancer (HCT116) cells.[110] *In vivo* studies have also revealed that protocatechuic acid reduces the incidence and multiplicity of cancerous tumors in the colon of rats.[111] The modulation of cytochrome P450 and enzymes involved in xenobiotic activation and/or detoxification pathways (phase II enzymes) by protocatechuic acid in mouse liver and kidney has also been reported.[112] Moreover, protocatechuic acid affected the level of rat hepatic and renal glutathione S-transferase (GST) isoenzymes.[113] Cytotoxicity assays have also shown that protocatechuic acid effectively kill the HepG2 hepatocellular carcinoma cells by stimulating the c-Jun N-terminal kinase (JNK) and p38 subgroups of the mitogen-activated protein kinase (MAPK) family.[114] A similar signalling pathway has been reported to be involved in the apoptosis of human gastric adenocarcinoma cells by protocatechuic acid.[115] In a recent study, protocatechuic acid has also shown significant neuroprotective effects on retenone-induced apoptosis in PC12 cells by ameliorating the mitochondrial dysfunction.[116]

Modulation of lipid metabolism. It has been reported that 3,4-dihydroxytoluene acid inhibits the synthesis of heptocellular cholesterol by inhibiting the incorporation of acetate into HepG2 liver cells.[104]

VI. Concluding remarks

Over the last decade, a large number of epidemiological and interventional studies have demonstrated that there may be an association between flavonoid consumption and human health. Mechanistic studies trying to determine flavan-3-ol health effects have revealed that these polyphenols exhibit a wide range of biological effects. Despite the enormous effort devoted to this area, some results may be misleading, since polyphenol metabolism as xenobiotics has not been considered in a large number of studies which employed structural forms and concentration ranges not found *in vivo*. Therefore, polyphenol bioavailability is a key issue in the link between polyphenol and human health. In comparison to other micronutrients, knowledge about polyphenol bioavailability is advancing with the progress of analytical instrumentation which allows the identification of new metabolites *in vivo*. The recognition that some polyphenols, in particular proanthocyanidins, are extensively metabolized by the intestinal microbiota into low molecular weight compounds, and that these metabolites represent a very large percentage of the amount ingested, is bringing into consideration the inclusion of microbial metabolism as part of the bioavailability concept currently adopted for polyphenols. On the basis of these facts, interest is now focused on the study of the bioactivity of microbe-derived metabolites, in addition to phase II or tissular metabolites, as compounds responsible for the health effects of flavan-3-ols. Although advances are being made in the determination of the bioactivity of microbe-derived metabolites, most studies carried out until now have failed, again by not testing the conjugated forms found *in vivo*. With regards to the bioactivity of actual conjugated forms derived from flavan-3-ol *in vivo* metabolism, research carried out in the last decade has revealed that flavan-3-ols are multifunctional compounds that may display effects by mechanism(s) of action beyond their antioxidant activity.

The health effects derived from the interaction between flavan-3-ols and the intestinal microbiota should be a subject of increasing interest. Although some authors have pointed out that polyphenols may be beneficial to gut health by increasing the population of potentially beneficial bacteria or exerting prebiotic actions, the effects that the interaction between flavan-3-ols and intestinal microbiota may have on the functionality of the metabolic activity of the microbiota and overall gastrointestinal health still remains largely unknown. In fact, for flavan-3-ols to function as a prebiotic, intestinal bacteria with such metabolic capacity should exist in the colon, but they are difficult to identify due to direct or indirect factors inherent in flavan-3-ols. The identification of flavan-3-ol-metabolizing bacteria and their possible use as a probiotic could be a good strategy for increasing the bioavailability and potential bioactivity of proanthocyanidins.

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