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Role of the small intestine, colon and microbiota in determining the metabolic fate of polyphenols

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1

Abstract

(Poly)phenols are a large group of compounds, found in food, beverages, dietary supplements and herbal medicines. Owing to their biological activities, absorption and metabolism of the most abundant compounds in humans are well understood. Both the chemical structure of the phenolic moiety and any attached chemical groups define whether the polyphenol is absorbed in the small intestine, or reaches the colon and is subject to extensive catabolism by colonic microbiota. Untransformed substrates may be absorbed, appearing in plasma primarily as methylated, sulfated and glucuronidated derivatives, with in some cases the unchanged substrate. Many of the catabolites are well absorbed from the colon and appear in the plasma either similarly conjugated, or as glycine conjugates, or in some cases unchanged. Although many (poly)phenol catabolites have been identified in human plasma and / or urine, the pathways from substrate to final catabolite, and the species of bacteria and enzymes involved, are still scarcely reported. While it is clear that the composition of the human gut microbiota can be modulated in vivo by supplementation with some (poly)phenol-rich commodities, such modulation is definitely not an inevitable consequence of supplementation, it depends on the treatment, length of time and on the individual metabotype, and it is not clear whether the modulation is sustained when supplementation ceases. Some catabolites have been recorded in plasma of volunteers at concentrations similar to those shown to be effective in in vitro studies suggesting that some benefit may be achieved in vivo by diets yielding such catabolites.

2

1. (Poly)phenols covered and main classes.

(Poly)phenols are a large group of compounds synthesised by plants for a variety of functions, such as protection against UV radiation, mechanical damage, and microbial infection [1, 2]. A wide variety of polyphenols are consumed as part of the normal diet [3], and are also present at high levels in supplements [4], and form essential components of many Chinese medicines [5]. A study within the European Prospective Investigation into Cancer and Nutrition (EPIC) using the Phenol Explorer database reported that the cohort studied consumed 427 different polyphenols including 94 that were consumed at a rate of >1 g/day. The estimated total polyphenol consumption ranged from 584 mg/day by Greek women to 1786 mg/day for men in Aarhus-Denmark. The corresponding data for Greek men and for Aarhus women were 744 mg/day and 1626 mg/ day, respectively, with all data adjusted for age and weighted by season and weekday of dietary recall [6]. This study defined the major contributors to be the phenolic acids (predominantly the caffeoylquinic acids (27–53%), also called chlorogenic acids) and flavonoids (predominantly flavanols (16–29%), proanthocyanidins (5–9%) and theaflavins (14–25%)) [6]. Hydroxybenzoic acids (3.3–7.4%), alkyl-phenols (1.6–4.)%), tyrosols (0.4–3.6%) and the glycosides of flavanones (2.6–4.0%), flavonols (2.8–5.1%) and flavones (0.7–1.5%) were also recorded along with smaller contributions from stilbenes (0.1–0.5%), lignans (0.1–0.7%) and trace amounts from several other subgroups. It is feasible that individuals with heavy coffee consumption might have greater total polyphenol intakes. Recent analyses of coffee beverage as sold in retail outlets can supply as much as 423 mg chlorogenic acids per cup [7]. Heavy consumers of black tea might also have greater total polyphenol intakes because the EPIC study did not consider thearubigins. Black tea theaflavins account for some 3–5% of the beverage solids compared

3

with some 17–20% for the extremely complex thearubigins which can equate to ≈100 mg/cup [8-10]. It has not been possible to trace consumption data for the transformed anthocyanins characteristic of matured red wines, but it is clear that an extensive range is present [11, 12], and regular consumers of red wine might have a significant intake. It has been demonstrated that foods also contain a significant amount of non-extractable (poly)phenols which, nevertheless, are gut microbiota substrates, and these will further raise the (poly)phenol intake [13]. The phenol-explorer database is a useful compilation of good quality compositional data [3], but lacks data for the transformed anthocyanins, thearubigins and unextractable (poly)phenols [14]. Since phenolic acids are mostly monomeric phenols, flavonoids contain 2 phenolic rings, and polymerised flavonoids contain multiple phenolic rings, we designate the term "(poly)phenols" to cover this group [15].

Flavonols, flavanones and anthocyanins exist in planta as glycosides, where the predominant attached sugars are glucose and rhamnose. Flavanols are present in planta mostly in their free forms, but can be galloylated (as in green tea) or polymerised to form proanthocyanidins, common components of many foods such as cocoa. Phenolic acids are usually attached to an organic acid, most commonly quinic acid, found at very high levels in coffee and at moderate levels in most fruits. Here, we will focus on the main compounds present in a normal diet, and only include those which have been well studied and understood. The route of absorption can be either through the stomach, small intestine or, if not absorbed at those sites, by the colon, after chemical modification by the colonic microbiota. During this process, the (poly)phenols become modified by various catabolic and conjugation reactions, appear in the blood, and are then excreted either in the urine or through the bile. Some unabsorbed substrate and catabolites are voided in the faeces. Recently, advances have been made

4

particularly in defining the effects of the microbiota on (poly)phenols, and how, in parallel, (poly)phenol-rich foods can affect the composition and activity of the microbiota.

2. Absorption into the bloodstream

Chlorogenic acid is a general term for the esters of a phenolic acid (e.g. ferulic, caffeic or dimethoxycinnamic acids) with quinic acid [16], and these classes are found at particularly high levels in coffee [17]. After reaching the small intestine, some hydrolysis of caffeoylquinic acid and of dimethoxycinnamoyl quinic acid occurs owing to the action of mammalian esterases, but the hydrolysis is relatively slow and only a proportion of the chlorogenic acid is hydrolysed [18]. The resulting free caffeic acid is absorbed through the small intestinal epithelium [19], and is rapidly sulfated, to form caffeic acid-3′-O-sulfate, or sulfated and methylated, resulting in ferulic acid-4′-O-sulfate, both with a T_{max} of ~30 min [20]. Dimethoxycinnamic acid is rapidly and efficiently absorbed after hydrolytic removal of its quinic acid moiety, and circulates in plasma as the unmodified dimethoxycinnamic acid, again with a T_{max} ~30 min [21, 22] (Fig. 1). Although dimethoxycinnamic acid is only a minor component of the coffee chlorogenic acids, it exhibits the highest concentration in plasma of all of the coffee-derived phenolic acids that are absorbed in the upper gastrointestinal tract. Feruloylquinic acids are not substrates for gut esterases, but a very small amount is absorbed intact, although the level only reaches low nM concentrations and this is not considered a major route of absorption and metabolism [23]. Most of the chlorogenic acids arrive in the colon intact. Here, the microbiota have abundant esterases for hydrolysing the phenolic-quinic acid linkage [24]. Released phenolic acids are readily converted by the microbiota to the dihydro forms, such as dihydroferulic acid and

5

dihydrocaffeic acid, and then absorbed through the colonic epithelium. Dihydroferulic, dihydroferulic acid-4′-O-sulfate and dihydrocaffeic acid-3′-O-sulfate circulate at relatively high concentrations [20] (Fig. 1 and 2). Some of the conjugated phenolic acids appear in the urine together with glycine conjugates such as feruloyl glycine. Having breakfast with coffee somewhat affected the timing of absorption, but not the overall amount absorbed [25], and non-dairy creamer, but not milk, has the same effect [26] and so the overall effect of food or beverages on the absorption and metabolism of chlorogenic acids appears to be minimal despite some reports to the contrary [27].

Hesperidin is found at high levels in citrus fruits and is the rutinoside of hesperetin (hesperetin-7-O-rhamnoglucoside) [28]. Intact hesperidin is not absorbed across the small intestine epithelium as shown by direct jejunal perfusion in humans [29] (Fig. 3). If the terminal rhamnose moiety is hydrolysed before consumption, the product, hesperetin-7-O-glucoside, becomes a substrate for the brush border enzyme lactase phloridzin hydrolase. After hydrolysis of the attached glucose at the surface of the intestine, the resulting hesperetin is efficiently absorbed with a T_{max} of ~30 min after direct perfusion of the pure compound into the jejunum [29] (Fig. 3). When hesperidin is consumed orally, hesperetin (conjugates of sulfate and glucuronide) appear in the plasma with a T_{max} of 4-6 h. When hesperetin-7-O-glucoside is consumed orally, the same conjugates appear in the plasma but with a much higher C_{max} and a much shorter T_{max} of ~ 30 min indicating absorption in the small intestine [30]. The microbiota, and not mammalian cells [31], can produce α-rhamnosidases which cleave the rhamnose from hesperidin [32], and this is a critical step in its absorption. Intact hesperidin is not absorbed passively, but hesperetin, after removal of the two sugars, is readily absorbed [33]. This is a clear cut example of where the attached sugars make an enormous and critical difference to both the site and extent of absorption. Consumption of

6

hesperidin with other foods can affect the shape of the pharmacokinetic curve [34]. The absorption, metabolism and excretion of hesperidin shows substantial inter-individual variation, but is only slightly affected by age [35]. Quercetin is found in nature attached to sugar moieties, such as the 3-O-glucoside and 3,4′-O-diglucoside forms found in onions [36], the 3-O-rutinoside as found in tea [37] and various glycosides in apple [38]. The same mechanism as described above for hesperidin applies to these quercetin derivatives, where the glucoside is absorbed rapidly and efficiently in the small intestine, but the rutinoside is absorbed only after hydrolysis of the sugars by the gut microbiota in the colon, leading to less efficient absorption with a longer T_{max} [39, 40]. The numerous conjugates of quercetin have been described [41, 42] and kinetic modelling has indicated that rats are not an adequate model to study quercetin pharmacokinetics since the pattern of conjugation is very different [43].

Epicatechin ((−)-epicatechin is the most abundant form) is found in most foods as the free form [44], is absorbed in the small intestine, and does not need prior action of the microbiota for absorption [45]. It undergoes sulfation, methylation and glucuronidation with typical T_{max} for conjugates of ~2 h [46, 47]. After both oral ingestion and direct jejunal perfusion, the EC-3′-O-glucuronide conjugate is the most abundant metabolite found in plasma [48] (Fig. 4). Despite reported differences in analytical procedures between laboratories [47], the consensus is that (−)-epicatechin-3′-O-glucuronide, (−)-epicatechin-3′-O-sulfate, and a 3′-O-methyl-(−)-epicatechin-5/7-sulfate are the predominant epicatechin metabolites in humans after oral consumption [47, 49]. The apparent half-life of most epicatechin conjugates is 2-4 h [49]. The proportion of epicatechin which is not absorbed in the small intestine will reach the colonic microbiota and will be converted to lower molecular weight compounds (see below) with no evidence for absorption of intact epicatechin in the colon.

Anthocyanins and proanthocyanidins are very poorly absorbed in the small intestine in their intact forms [50, 51]. These classes of compound are subject to extensive catabolism by the gut microbiota, and the products absorbed through the colon (see below). Anthocyanins are relatively unstable in the gastrointestinal tract, even in the conditions of the small intestine [52], and phenolic products from anthocyanins could arise from chemical degradation. Any anthocyanins which survived the small intestine would be rapidly transformed into lower molecular weight compounds by the colonic microbiota (see below [53, 54]). Proanthocyanidins are oligomeric flavanols, and there are no mammalian enzymes reported which have the ability to degrade the high molecular weight molecules into forms which are small enough to be easily absorbed in the small intestine. Only the dimer of epicatechin (procyanidin B2) is absorbed intact, but to a very limited extent [51]. The majority of the procyanidin B2, and other oligomers with a degree of polymerisation ≥2 reach the colon and are extensively metabolised by colonic microbiota. In rats, 80% of the microbiota-catalysed catabolites of radiolabelled procyandin B2 were absorbed [55].

3. Conversion of polyphenols by the gut microbiota

In the last decade there have been major advances in defining the composition of the gut microbiota with greatly increased use of methods based on the detection and sequencing of 16S rDNA and MALDI-TOF MS replacing conventional culture methods, but these diverse approaches remain complementary with more traditional methods required to determine microbiota functionality [56, 57]. The human gastro-intestinal tract hosts up to 100 trillion microbes [58], which have been assigned to well over 1000 species. The three most dominant bacterial phyla in the colon are the Firmicutes, Bacteroidetes, and Actinobacteria, with

Proteobacteria and Verrucomicrobia generally less abundant [56]. However, a detailed discussion of microbiota composition, reported variations therein with age [59, 60] or disease [61, 62], and the techniques used to determine it [56], are outside the scope of this review.

With regard to microbiota-catabolism, it is convenient to divide the substrates into three categories. There remain some for which no data are available, the transformed anthocyanins (pyranoanthocyanins) of red wines, plus some minor dietary components such as algal phlorotannins, alkyl resorcinols, urushiols, cardanols and anacardic acids, naphthoquinones and anthraquinones, coumarins, isocoumarins and furanocoumarins [63]. The second group are those substrates associated with unique catabolites, and the third group consists of some structurally diverse substrates that nevertheless yield essentially the same set of catabolites, this latter group predominating (see Figs. 2 and 5) [64]. The unique catabolites include 4-hydroxy-mandelic acid from p-sympatol [65], tyrosols from oleuropein and related compounds [66], S-equol, 5-hydroxy-equol and dihydrocinnamic acids with the aryl residue at C2 formed from isoflavones [67, 68], urolithins and nasutins from ellagitannins [69-71], diarylbutanes from lignans [32;35;36], and dihydroresveratrol from piceid and resveratrol [72]. The avenanthramides of oats, N-cinnamoyl conjugates of anthranilic acids, yield some unique catabolites, e.g. the corresponding dihydrocinnamoyl conjugates and 5-hydroxy-anthranilic acid (2-amino-5-hydroxybenzoic acid) in addition to the much commoner cinnamic and dihydrocinnamic acids [73]. Many of these unique catabolites have received considerable attention but their precursors are minor components of the diet, albeit with some considerable variation between subgroups [6], depending upon whether they consume significant amounts of oranges [65], olive oil [74], soya beans [75, 76], nuts, pomegranates, strawberries and raspberries [71, 77, 78], whole grains [76], wine and peanuts [79, 80] or oats [81], respectively.

9

The predominant catabolites generated by the microbiota from (poly)phenols are the aromatic and phenolic acids with zero to three aromatic hydroxyls, or their mono- or di-methoxy analogues, possessing also a sidechain of one to five carbons which might bear an aliphatic hydroxyl [64]. These are formed from the diet predominantly from chlorogenic acids, flavanols, proanthocyanidins, theaflavins [6] and thearubigins [82-85], but also from many other minor components of the diet (Figs. 2 and 5). Human studies in vivo and gut microbiota-fermentation studies have demonstrated that at least some of these C_6–C_1 and possibly C_6–C_2 phenolic acids can be decarboxylated yielding the corresponding phenols (C_6) or methyl-phenols (C_6–C_1) [84, 86-90], respectively. Phloroglucinol can also be formed from chalcones and dihydrochalcones [91, 92], and at least certain rumen microorganisms can reduce this to non-aromatic dihydrophloroglucinol [93, 94]. There are several catabolites, for example some mandelic and phenylhydracrylic acids, for which the origin remains uncertain.

Although the microbiota-mediated transformation of dietary (poly)phenols is generally considered to occur in the colon, studies in vitro using ileostomy fluid establish that some transformation can be expected to occur in the small intestine [95]. Studies with ileostomists where plasma and / or urine have been analysed support this observation, but studies in which ileostomists are compared with volunteers having an intact colon indicate that microbiota catabolism in the small intestine is very much less than in the colon [23].

As a result of the catabolic reactions indicated above, the products appear in the blood and circulate either as free or conjugated forms. The concentration of microbiota catabolites are often much higher than the parent compounds. As an example, Fig. 6 shows the appearance of low molecular weight phenolics in the blood after consumption of a mixture of fruits.

10

Catechol and 1-methyl pyrogallol are not found in foods, but are products of catabolism by the gut microbiota. These are characterised by a high T_{max} and a late appearance in the urine. As for the parent compounds, the catabolic products are also sulfated, glucuronidated and methylated, and although this does not necessarily account for 100% of the catabolite absorbed, catechol-O-sulfate is highly abundant in plasma after consumption of polyphenol-rich foods.

4. Pharmacokinetic consequences of multiple doses

(Poly)phenols associated with coffee, green tea and black tea are generally consumed frequently during the waking day, with the result that the gut microbiota have access to several bolus doses for a prolonged period of time. This would predispose to a larger and more uniform concentration of the associated catabolites in the gastrointestinal tract and in plasma after absorption, but little attention has been given to how this would affect the levels of (poly)phenols in circulation in human intervention studies. Polyphenols undergo several reactions before this stage, such as deglycosylation, conjugation, ring cleavage, etc., and so are not amenable to classical pharmacokinetic analysis and modelling to assess the implications of frequent consumption. There are very few data on the human pharmacokinetics of intravenous doses, particularly for the gut flora catabolites, which are needed for such modelling. Volunteer studies of Chinese herbal preparations which contain preformed protocatechuic acid given intravenously have revealed a clearance half-life of ca 10–20 minutes [96, 97].

Such rapid clearance is potentially-misleading when the catabolite is not produced until the colon, and is then produced throughout the transit of the large bowel, resulting in absorption

11

over the same substantial time period and creating a plasma profile more closely resembling a prolonged intravenous infusion. There are no intravenous dosing data for the C_6–C_3, C_6–C_2 or C_6–C_5 catabolites. A study in which volunteers consumed a bolus dose of strawberry purée containing ellagitannins has demonstrated that the unique urolithin catabolites were still detected in urine 90 h later suggesting that the ellagitannin substrate may have bound to the mucosa and not moved with the unbound digesta [98]. It is likely that a similar phenomenon will occur with proanthocyanidins which also bind strongly to proteins, and possibly thearubigins, but because their catabolites are not unique it would be more difficult to demonstrate than with the ellagitannins (see Figs 2 and 5). Such binding would extend the period during which the microbiota could attack such substrates, potentially increasing the yield of catabolites and the area under the concentration–time curve for these catabolites in plasma.

Volunteer studies of typical real world repeated consumption would, if available, circumvent the lack of data for intravenous dosing, but in their absence we performed a simple additive modelling using real data from a single dose study in order to obtain an indication of the potential concentrations that might be attained. This approach assumes that the rate of clearance from plasma does not change with dose, and would only be true if clearance was saturated, and thus the estimate obtained is on the high side.

Figure 7 shows the data obtained for dihydroferulic acid in plasma after consumption of a single cup of coffee from the study of Stalmach et al [20]. We then used these data for estimating the consequences of human subjects consuming seven servings at two-hour intervals, by adding the values expected at different times using the single serving data. The C_{max} is ~300 nM after a single serving, but reaches a steady state of ~750 nM after multiple

12

doses spaced 2 h apart, an increase of ~2.5-fold, and the total area under the curve is also increased 7-fold with a much longer time of exposure. It would be interesting to determine how multiple doses of polyphenols affect the plasma concentrations throughout the day using healthy volunteers, since, for example, many free-living people consume multiple cups of coffee at regular intervals each day. In contrast, pre-existing (poly)phenols that are absorbed in the stomach or duodenum (e.g. caffeoylquinic acids) will for any bolus dose be absorbed only during the comparatively brief residence period, and if cleared rapidly from the plasma will not accumulate to the same extent even if consumed at 2-hour intervals.

5. Prebiotic effects

There have been many studies of the potential of (poly)phenols substrates and catabolites to modulate the composition of the gut microbiota. These include studies using animals, in vitro incubations with faecal flora, and volunteer studies. Animal studies permit strategies not otherwise available, for example introducing a particular organism into a germ-free animal with suitable controls to assess its impact on the health of the gnotobiotic animal [68, 99, 100]. Studies in which conventional animals are given an atypical, human-type diet (e.g. high fat, high sugar, obesogenic) supplemented with a substantial dose of a specific (poly)phenols-rich commodity (e.g. table grapes [101] or cocoa [102]) or test substance (e.g. trans-resveratrol [103]) have been used extensively. Often, such studies demonstrate that the test substance or (poly)phenol-rich commodity modulate the composition of the gut microbiota and / or ameliorate the harmful effects of the test animal's less than ideal diet [101-108]. However, as noted in an earlier review, the metabolic competence of the normal gut microbiota may vary markedly between species [64], and it is uncertain whether or not a similar effect would be achieved in humans following a realistically supplemented diet. For

13

example, after supplementation for three months in 58 volunteers with nine capsules of flavanol-rich green tea extract per day, equivalent to several 100 ml cups daily, there was no change in the gut microbiota [109]. The oral microbiota is also stable to red wine consumption [110]. Similarly, a study in which 25 volunteers consumed a (poly)phenol-rich boysenberry beverage, an apple fibre-rich beverage, or both in combination (2×175 ml/day), failed to establish any change in the gut microbiota or short chain fatty acid production [111]. The consumption of seven dates per day by 22 volunteers for 21 days also did not alter the composition of the faecal microbiota, but stool ammonia was significantly lowered [112]. Similarly, the consumption of 200 g per day of raspberry purée for 4 days by 10 free-living volunteers failed to produce a detectable change in the composition of the gut microbiota [53]. A 5×4 dietary crossover study in which 23 volunteers consumed whole apples (550 g/day), apple pomace (22 g/day), clear or cloudy apple juice (500 ml/day) as supplements to a restricted diet also did not elicit any change in the gut microbiota [113].

Daily consumption of 200 ml pomegranate juice for four weeks by 12 healthy adults did not produce any statistically significant change in the faecal microbiota possibly because of considerable inter-individual variation in profile at all time points, and such inter-individual variation is commonplace. However, the faecal concentration of catechol was positively correlated with Oscillospira spp. and negatively correlated with Paraprevotella spp., whereas the concentration of 3-phenylpropionic acid was positively correlated with Odoribacter spp, and these catabolites increased significantly over the four week study [114], but such correlations do not establish a simple cause and effect relationship.

In contrast, 20 volunteers consumed 1 g/day of pomegranate extract for four weeks resulting in significant changes in microbiota composition (increases in Bacteroidetes and Proteobacteria, decreases in Firmicutes and Actinobacteria) in those volunteers at zero time

14

excreting urolithins, and those who were excreting urolithins at the end of the treatment period. Over the test period, there were significant increases in Veillonella spp., Serratia spp., Prevotella spp., Lactobacillus spp., Escherichia spp., Enterobacter spp. and Butyrivibrio spp. and a decline in Collinsella spp. However, six volunteers were still not excreting urolithins after four weeks pomegranate supplementation, but a reduction in Actinobacteria was still detected and there was a marginally significant decline in Bifidobacteria spp., Actinobacillus spp. and Thermovenabulum spp. [115]. It was noted that urolithin producers hosted at zero time a 33-fold greater population of Akkermansia muciniphila (phylum Actinobacteria) than the non-producers, and that after supplementation this differential was 47-fold [115], whereas Romo-Vaquero et al. were able to associate urolithin A production with Gordonibacter [116], an organism not detected by Li et al. These studies clearly demonstrate that there are two metabotypes with regard to ellagitannin catabolism — one for whom urolithin production is easily inducible and the other for which induction by dietary supplementation is more difficult or impossible, and that certain transformations can be achieved by more than one genus.

Moreno-Indias et al. reported that red wine (poly)phenols, given either as red wine (272 ml/day) or dealcoholized red wine (272 ml/day) for one month, reduced the incidence of undesirable species (Escherichia coli and Enterobacter cloacae) and increased the incidence of desirable species (bifidobacteria, Lactobacillus spp., Faecalibacterium prausnitzii and Roseburia spp.) in 10 patients suffering from metabolic syndrome. There were some similar, but more modest, improvements in the faecal flora of the healthy controls. There were significant improvements also in systolic and diastolic blood pressure and blood glucose levels in the metabolic syndrome group [117]. It was noted that more 4′-hydroxyphenylacetic acid was excreted by the healthy metabotype than the obese-diabetic metabotype after red

wine consumption. Although 4′-hydroxyphenylacetic acid is associated with tyrosine and phenylalanine catabolism, it is also a gut microbiota catabolite of some dietary (poly)phenols and the greater excretion was ascribed provisionally to a greater proportion of Faecalibacterium prausnitzii, Bifidobacterium spp. and Lactobacillus spp. in the gut microbiota of the healthy metabotype both before and after red wine consumption [118]. This result confirmed earlier studies by this research group which showed increases in bifidobacteria and excretion of anthocyanin catabolites (syringic, p-coumaric, gallic acids and pyrogallol) and flavanol catabolites (hydroxyphenylvalerolactones) [119, 120]. The effect of consuming anthocyanin-rich blueberry-beverages on the faecal concentration of bifidobacteria has also been investigated, with consumption reported to produce significant increases in Bifidobacterium longum subsp. infantis averaged across 20 volunteers but with considerable inter-individual variation such that this organism sometimes declined [121].

In an 8-week placebo controlled study of 80 healthy overweight / obese volunteers with low intake of fruit and vegetables and a sedentary lifestyle, a subgroup of 40 where refined wheat products were replaced with isocaloric whole grain products, there were modest increases in Prevotella spp. and modest reductions in Dialister spp., Bifidobacterium spp., Blautia spp. and Collinsella spp., with reduction in markers of inflammation associated with whole grain consumption [122]. In 38 adult volunteers assessed by using an annual food frequency questionnaire followed by plasma and stool analysis, the regular consumers of red wine (100 ml/day) had lower levels of Bifidobacterium spp., B. coccoides, C. leptum, and Lactobacillus spp., albeit with lower serum malondialdehyde than those who did not consume red wine [123], in contrast to the results discussed above. The markedly lower wine consumption of the regular drinkers is likely one factor contributing to this difference, but other diet and life-

16

style factors, and differences in the particular species, strains and metabolic competence of bifidobacteria might also be important.

Studies on volunteers must be viewed as the gold standard but they are susceptible to several confounding factors, such as faulty recall when food frequency questionnaires are used, or non-compliance when supplements are provided, and to substantial inter-individual variation. It has been suggested that analysis of gut microbiota catabolites in urine collected over 24-hours, expressed relative to creatinine, can be used as a compliance monitor [124], but unless the supplement generates a unique catabolite there is a possibility that the consumption of for example coffee or tea, whether approved or not, can invalidate the compliance assessment by yielding the same catabolites. Even with a unique catabolite variation in yield with volunteer metabotype may also limit its power, especially as the yield of S-equol following soy-milk consumption is significantly increased when consumed with coffee [125], and Crozier et al. have demonstrated a more general modulating effect of cream consumed with strawberries and yogurt consumed with orange juice [126, 127]. Similarly consumption of coffee with either a high-fat or high-carbohydrate meal also delayed absorption of the gut microbiota catabolites [25], as did non-dairy creamer, but not milk [26]. Coupled with the noted inter-individual variability, these factors limit the power of such studies, especially those with few volunteers.

These limitations notwithstanding, collectively these studies demonstrate that the composition of the human gut microbiota can be modulated in vivo by supplementation with some (poly)phenol-rich commodities, but that modulation is not an inevitable consequence, depending at least in part on the individual metabotype. Because these supplements are chemically complex it is not possible to identify the substrate(s) and / or catabolite(s)

responsible, nor define the mechanisms responsible for any consequential changes such as reduction in blood pressure. It is also not possible at present to estimate the minimum effective dose necessary to achieve a beneficial effect in vivo, the percentage of the population to which it would apply, nor how long-lasting is the change in microbiota and any consequential benefit.

That differences in customary diet can markedly influence the composition of the gut microbiota is strikingly illustrated by Cavalieri *et al's* study of children in Europe and children in Burkina Faso. Compared with European children, those in Burkina Faso had far fewer Firmicutes (12% vs 51%) and far more Bacteroidetes (73% vs 27%), with a unique and significant content of Prevotella spp. and Xylanibacter spp. capable of degrading cellulose and xylans in dietary fibre. The Enterobacteriaceae (Shigella spp. and Escherichia spp) were significantly lower in the Burkina Faso children [128]. A comparison of their faecal and urinary (poly)phenols catabolites supported by in vitro faecal fermentation studies would make a fascinating study, as would determination of the age at which babies / young children acquired the (poly)phenol catabolising microbiota. In contrast to the results of this study, a comparison of the microbiota of urban-dwelling vegans and omnivores living in the same US environment revealed only modest differences, mainly in the Firmicutes (but not Prevotella spp.), Actinobacter and Proteobacter. There were much larger differences in the metabolome: The vegans had much higher plasma concentrations of 3-hydroxyhippuric acid, hippuric acid and catechol-O-sulfate. It was not possible to explain the small difference in microbiota composition associated with such substantial differences in diet, but it was suggested that if differences in microbiota observed in globally distinct human populations are attributable to differences in diet then possibly these differences take several generations to develop, or

possibly the young must be exposed to the different diet at an early age. Another possibility suggested was an environmental factor other than diet [129].

Although it has long been accepted that the gut microbiota can catabolise dietary (poly)phenols, remarkably little attention has been paid to exactly how this is achieved. Controls used in model fermentations have clearly demonstrated that the catabolites produced by live microbiota are not observed with microbiota inactivated by chloroform suggesting that enzymes released from dead cells were not responsible [130, 131], and implying that uptake mechanisms for the test substance were available to at least some of the organisms present. Escherichia coli and Bifidobacterium bifidum can, under anaerobic conditions, take up quercetin much more efficiently than either rutin or quercetin-3-O-glucoside, and B. bifidum took up twice as much as E. coli. Both organisms could hydrolyse the glucoside. Under anaerobic conditions, E. coli mutants lacking either porins 0mpF and 0mpC or the efflux transporter multi-drug efflux pump AcrAB, did not differ in behaviour relative to the wild type [132], and so the precise uptake mechanisms remain unclear.

Some dietary (poly)phenols are unstable under the conditions employed for in vitro fermentations, and it is important to use an uninoculated control to detect purely chemical transformations. In vitro studies have demonstrated that not all species of Lactobacillus and Bifidobacterium found in the human microbiota are resistant to (poly)phenols and / or able to metabolise them. For example, L. fermentum, L. acidophilus and L. vaginalis were very intolerant even of (+)-catechin and (−)-epicatechin, whereas L. plantarum, L. casei and L. bulgaricus grew best in the presence of oligomeric procyanidins. L. plantarum was able also to produce non-aromatic catabolites from flavanol monomers [133]. Exposure of L. acidophilus to rutin initiated stress response mechanisms that protected the integrity of the

19

cell boundary, modulated protein and carbohydrate metabolism, and triggered enzymes involved in oxidation–reduction mechanisms, indicating that adaptation is possible [134]. Similar studies have demonstrated that individual flavonoid aglycone substrates such as (+)-catechin, quercetin and puerarin have significantly different effects on the microbiota composition. Catechin and quercetin induced significant increases in Bifidobacterium whereas puerarin was ineffective, and while quercetin and puerarin produced significant reductions in Bacteroides, catechin was much more potent [135]. Intact cell walls might significantly limit access of (poly)phenols-catabolising bacteria to their substrates [136], and Mosele et al. demonstrated that a simulated duodenal digestion that degraded pectin increased microbial access to many substrates [137]. It has also been demonstrated in vitro that the exposure to (poly)phenols modulates the ability of the microbiota to metabolise fructo-oligosaccharides and to generate short chain fatty acids [138, 139]. It must be anticipated that interactions absent from in vitro fermentations are likely in the gastrointestinal tract in vivo.

In vitro fermentation studies of flavanol diastereoisomers have demonstrated how subtle differences in structure and culture media can influence the catabolism. For example, Adlercreutzia equolifaciens JCM 14793 removed the 4′-OH from (−)-epigallocatechin but did not produce diaryl-propanols when stimulated by hydrogen, whereas with (−)-gallocatechin, diaryl propanol production dominated, with the 4′-dehydroxylation being a secondary pathway. However, with Ad. equolifaciens MT4s-5, the diaryl-propanol pathway predominated with both substrates [140]. Ad. equolifaciens JCM 14793 was able to ring open (+)-catechin and (−)-epicatechin more efficiently than (−)-catechin and (+)-epicatechin. Access to hydrogen dramatically increased ring opening with (−)-catechin, more modestly increased ring opening with (+)-catechin and (−)-epicatechin, but had little effect with (+)-

20

epicatechin [141]. This research group has obtained similar data for several other organisms, some of which are also stimulated by formate [142].

One must accept that human faecal samples do not truly represent the microbiota composition or the metabolic competence of the gastrointestinal tract from which it was voided. Some species are strongly bound to the gut surface and may not be voided. Some are very sensitive to oxygen and may not survive transfer to the culture medium, and the medium may be less than ideal for some species. Examples of tightly bound microorganisms are Lactobacillus gasseri, L. mucosae [143] and a strain of the facultative anaerobe Bacillus subtilis isolated from the human gastrointestinal tract [144]. When faecal samples from 10 healthy individuals were applied to the in vitro catabolism of black tea or a mixture of red wine and grape juice considerable variation was observed, each individual's microbiota producing a distinct catabolite profile [89]. Interestingly, all 10 microbiota were more efficient at producing 3-(3′-hydroxyphenyl) propionic acid from red wine than from black tea, whereas the yield of 3′-hydroxyphenylacetic acid was much greater from black tea than red wine. While vanillic acid (a known peonidin catabolite) was easily produced from red wine, three microflora were able to produce it from black tea, which typically does not contain peonidin [89], and the immediate precursor is not known. In contrast, pyrogallol, a known catabolite of delphinidin and gallate, both of which are typically found in red wine, and 2,6-dihydroxybenzoic acid, were produced only from black tea. Polyphenols with a 2,6-substitution are comparatively rare (minor components of olives, olive oil and beer) and the immediate precursor of this catabolite is uncertain. Thearubigins formed either by the route described in the oxidative cascade hypothesis [145-148], or by peroxidase [149], are possibilities.

Following the development of powerful LC–MS procedures, increasingly complex mixtures of catabolites are reported in both in vitro fermentation and volunteer studies, and it is routine to propose one or more pathways to account for the multiple transformations that have been detected. While plausible, in the absence of detailed studies using labelled substrates and putative intermediates, these pathways are speculative. In view of the very large number of discrete species, and the recognised potential for subtle variations in metabolic competence of even closely related species, it is almost certain that there is more than one organism capable of effecting any particular transformation, and probably more than one route connecting substrate to any catabolite. It is routine to assume that the conversion of a C_6–C_5 phenylvaleric acid proceeds via the C_6–C_3 phenylpropionic and C_6–C_2 phenylacetic intermediates en route to the C_6–C_1 benzoic acid and C_6 phenol products. However, the absence of the C_6–C_4 intermediate argues against repeated α-oxidations and might suggest that the C_6–C_2 intermediate arises from a flavonoid ring scission different from that providing the C_6–C_5 and / or C_6–C_3 catabolites. This inference is supported by the failure to detect the C_6–C_2 intermediate during in vitro fermentation of chlorogenic acids [150], or after dosing rats with dihydrocaffeic acid [151], although it is possible that its turnover is too rapid for it to be detected. The construction of these complex pathways is further complicated because some transformations can be made after absorption, such as hydrogenation and β-oxidation. It is also clear from numerous studies on volunteers that both C_6–C_5 and C_6–C_3 catabolites can be absorbed but it is not possible to define where the β-oxidation occurs.

The chemistry of many transformations has been reviewed [152], but some transformations are still poorly understood. Several mandelic acids have sometimes been considered as gut microbiota catabolites, but it is now recognised that 4-hydroxymandelic acid is a metabolite of p-sympatol (p-synephrine) and / or p-octopamine, which in dietary terms are known only

from citrus fruit [65, 153]. However, the rapid appearance of 4′-hydroxymandelic acid in plasma ($C_{max} \approx$ 1 h) indicates that the gut microbiota are not necessarily involved [65, 154]. The origin of 3′-methoxy-4′-hydroxymandelic acid and 4′-hydroxymandelic acid after the consumption of raspberries [71], or red wine and grape juice [155], remains uncertain but might be a consequence of dietary (poly)phenols or associated gut microbiota catabolites modulating endogenous mammalian catecholamine metabolism. Catecholamines are endogenous precursors of mandelic acids, and the potential for such interactions are receiving increasing attention [156]. The precise origin of phenylhydracrylic acids also is unclear. Despite being strongly associated with citrus flavanone consumption, phenylhydracrylic acids do not seem to be produced during in vitro flavanone fermentations [15, 157], suggesting that they are either purely endogenous metabolites, or at least a microbial catabolite which requires further metabolism after absorption. Volunteer studies have established that oral neohesperidin dihydrochalcone [92] and hesperetin [29] yield 3′-hydroxy-4′-methoxyphenylhydracrylic acid, i.e. the B-ring hydroxylation pattern is retained. This catabolite and 3′-hydroxyphenylhydracrylic acid have both been reported in urine samples from volunteers after orange juice consumption [65, 158]. 3′-Hydroxyphenylhydracrylic acid excretion increased also after consumption of a red wine-grape juice mixture [159], and raspberries [71], both of which lack flavanones, and there are older studies on volunteers which report its production from rutin [160], and after consumption of coffee [161]. Plausible immediate precursors are the appropriate cinnamic acid plus hydratase enzyme or dihydrocinnamic (3-(phenyl)propionic) acid plus P450 mono-oxygenase enzyme, but such precursors arise from so many substrates that many more reports of the analogous phenylhydracrylic acids would be expected. It has been suggested that an alternative route might be the formation of a phenylhydracrylic acid from a flavonoid by an unusual C-ring fission in which the pyran oxygen is retained on the side chain of the B-ring fragment rather

23

than on the A-ring fragment (see Fig. 8) [157]. 3′-Hydroxy-phenylhydracrylic acid has been

identified as a phenylalanine catabolite of incompletely characterised metronidazole-sensitive

bacteria [162], and proposed as a urinary biomarker for a range of neurological,

gastrointestinal, and psychiatric disorders [163], and 4′-hydroxyphenylhydracrylic acid has

been observed in patients with gastro-intestinal disease [164]. The definitive answer to the

origin and significance of phenylhydracrylic acids is still lacking, but it has been shown that

some Bacillus spp. can produce 3′-methoxy-4′-hydroxyphenylhydracrylic acid from ferulic

acid [165], and it is now recognised that some distinctive strains of Bacillus are found in the

human gut, including some B. subtilis that bind strongly to the mucosa possibly precluding

their elimination in faeces and availability in in vitro fermentations [144, 166].

6. **Effects of catabolites**

In vitro cell culture studies are probably the most effective way to investigate the effects of

gut microflora catabolites because when effects are observed there is the potential also to

investigate the underlying mechanism(s). Choice of tissue, choice of catabolite, concentration

and time of exposure, and choice of biomarker require careful consideration.

The chosen biomarker(s) must be biologically relevant, but more importantly, those selected

should be measurable with precision. Many biomarkers can only be quantified with a

replicate precision in excess of 10%, or even 20%, and such imprecision makes it very

difficult to detect small treatment-related changes in the biomarker. This constraint can be

circumvented by increasing the catabolite concentration, but when the catabolite is used at

even 10-fold the likely sustained plasma concentration, the relevance to real diets is

questionable. Certainly extrapolation to lower concentrations is unreliable as effects are likely to reach a threshold.

Ideally, the range of catabolite concentration should centre on plasma / tissue concentrations associated with real-world diets perceived as being beneficial to health. Such data for tissues are conspicuous by their absence but plasma C_{max} values for metabolites absorbed in the proximal gastrointestinal tract rarely exceed a transient 50 nM for a single bolus dose (unless supplemented) [25, 167-169] although 3′,4′-dimethoxycinnamic acid (ca 0.6 µM after ca two cups of coffee) [170] and 4-hydroxybenzoic acid (2.5 µM after 300 g fresh strawberries) [22] are notable exceptions. In contrast, as discussed above, for those catabolites derived from multiple substrates, and especially those associated with commodities consumed repeatedly at short intervals during the day, a higher C_{max} is to be expected, in some cases exceeding 1 µM. More importantly, a concentration of at least 0.5 C_{max} will be maintained for a considerable period, possibly overnight. Concentrations in human faecal water can be much higher [171], exposing the gut microbiota and the colonic mucosa to individual catabolites at concentrations in excess of 10 µM. Because multiple catabolites are present simultaneously, the total exposure in the plasma might realistically be some 2–3-fold higher and in the colon in excess of 1 mM. The relatively long-term exposure, possibly every day, give these catabolites a real potential to exert biological effects in vivo.

Data for a range of C_6–C_1, C_6–C_2, and C_6–C_3 phenolic acids are presented in Table 1, so far as possible comparing concentrations achieved in plasma and the colon following unsupplemented diets with data for biological effects observed in vitro. None of these studies observed any effect below 0.1 µM — indeed none even investigated a lower concentration. A simple comparison of plasma and colon concentrations with concentrations shown to be

25

effective in vitro suggests that there is potential for protocatechuic acid, vanillic acid, gallic acid and 3′,4′-dihydroxyphenylacetic acid to exert modest effects in vivo in at least some volunteers on real-world diets. 3′-Hydroxyphenylpropionic acid, 3′,4′-dihydroxyphenylpropionic and 3′-methoxy-4′-hydroxyphenylpropionic might also achieve sufficient concentrations following repeated consumption of coffee or tea. Note, however, that at least some of these catabolites, including protocatechuic and vanillic acids, bind to human serum albumin [172], and this might reduce their potency in vivo.

There has been limited investigation of mixtures of (poly)phenolic catabolites. In one study there was no evidence of any additive effect [173], but in another study a mixture of 4-hydroxybenzoic acid, protocatechuic acid and vanillic acid (albeit each at 0.33 µM) was more effective than any component alone at the same concentration [174], suggesting that there is some synergy rather than merely an additive effect. There is clearly a potential for some carefully-targeted studies seeking synergy, and these should not be restricted to (poly)phenol mixtures but embrace other classes of phytonutrients, and a need for further data on plasma and tissue catabolite concentrations in free-living volunteers, preferably in association with their dietary history. The development of biomarker assays, and the requisite reagents, to facilitate the generation of more precise replicate data would be a distinct advantage.

7. Summary, conclusions and recommendations for future research

The colonic microbiota transform a very complex range of (poly)phenol substrates with coffee chlorogenic acids, black tea theaflavins and thearubigins often dominating, with substantial contributions from proanthocyanidins and flavanols, flavonols, flavanones and anthocyanin glycosides, and unextractable (poly)phenols, from many fruit and vegetables. These transformations can be extensive, and while some microbial catabolites are substrate-specific (e.g. equol, urolithins and nasutins, mammalian lignans, hydroxyanthranilic acid), certain catabolites are common to many of the major substrates, implying that the spectrum of catabolites produced is less complex and qualitatively less variable than the spectrum of substrates consumed. The catabolites most likely to dominate are the C_6 phenols, C_6–C_1, C_6–C_2 and C_6–C_3 dihydro acids derived from chlorogenic acids/cinnamates, and most flavonoids including black tea thearubigins and theaflavins (see Figs. 2 and 5).

There is growing evidence from in vitro and intervention studies that some of these catabolites are biologically active, and that, along with untransformed substrates, may function as prebiotics capable of modulating the human gut microbiota composition. In addition, some catabolites after absorption may have further beneficial effects provided that sufficient concentrations are achieved for a sufficient time in the relevant tissue(s). Consumption of multiple doses, such as regular coffee and tea drinking throughout the day, have the potential to increase the plasma concentration of catabolites, especially for those derived from microbial action.

Future investigations must address the minimum effective dose of potentially prebiotic substrates, determine what percentage of the population are susceptible, whether susceptibility can be induced, and how long any associated benefits persist, especially if the supplementation is subsequently curtailed. There is also a pressing need for investigation of free-living volunteers who regularly consume (poly)phenol-rich beverages at frequent intervals to better define the circulating catabolite profiles after consumption of multiple doses. The answers to these are crucial if we are to understand and fully exploit the effect of (poly)phenol consumption on human health.

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Figure legends

Figure 1. Appearance of phenolic acids in plasma after consumption of chlorogenic acids from coffee. Healthy volunteers (n=11) drank 3.4 g instant coffee a 200 ml cup of coffee, or 4.0 g of instant coffee in 400 ml (n=8) for the dimethoxycinnamic acid estimation. Dimethoxycinnamic acid (♦), dihydroferulic acid 4′-O-sulfate (▲), dihydroferulic acid (Δ), ferulic acid 4′-O-sulfate (●), 5-feruloylquinic acid (■), dihydrocaffeic acid-3′-O-sulfate (□), caffeic acid-3′-O-sulfate (○). Redrawn using data from [20] and [22].

Figure 2: Diagrammatic illustration of the conversion of three classes of dominant dietary (poly)phenols to a series of common catabolites

Figure 3. Appearance of hesperetin in plasma after consumption of hesperetin 7-O-glucoside or 7-O-rhamnoside. Oral consumption (Panel A) or direct jejunal perfusion (Panel B) of hesperidin (hesperetin-7-O-rhamnoside), and oral consumption (Panel C) or direct jejunal perfusion (Panel D) of hesperetin-7-O-glucoside. Hesperetin after absorption is found as glucuronidated and sulfated conjugates, but here enzymic deconjugation was used to convert them back to hesperetin aglycone. Redrawn using data from [30] and [29].

Figure 4. Appearance of epicatechin conjugates in plasma. Upper panel: after oral administration of chocolate containing 79 mg of epicatechin; n=5. Lower panel: after direct jejunal perfusion of 50 mg pure epicatechin; n=6; EC-3′-O-glcA (■) EC-3′-O-sulfate (●) Me-EC-5-O-sulfate (▲). Redrawn from [48, 49]. Note that the y axes are different scales.
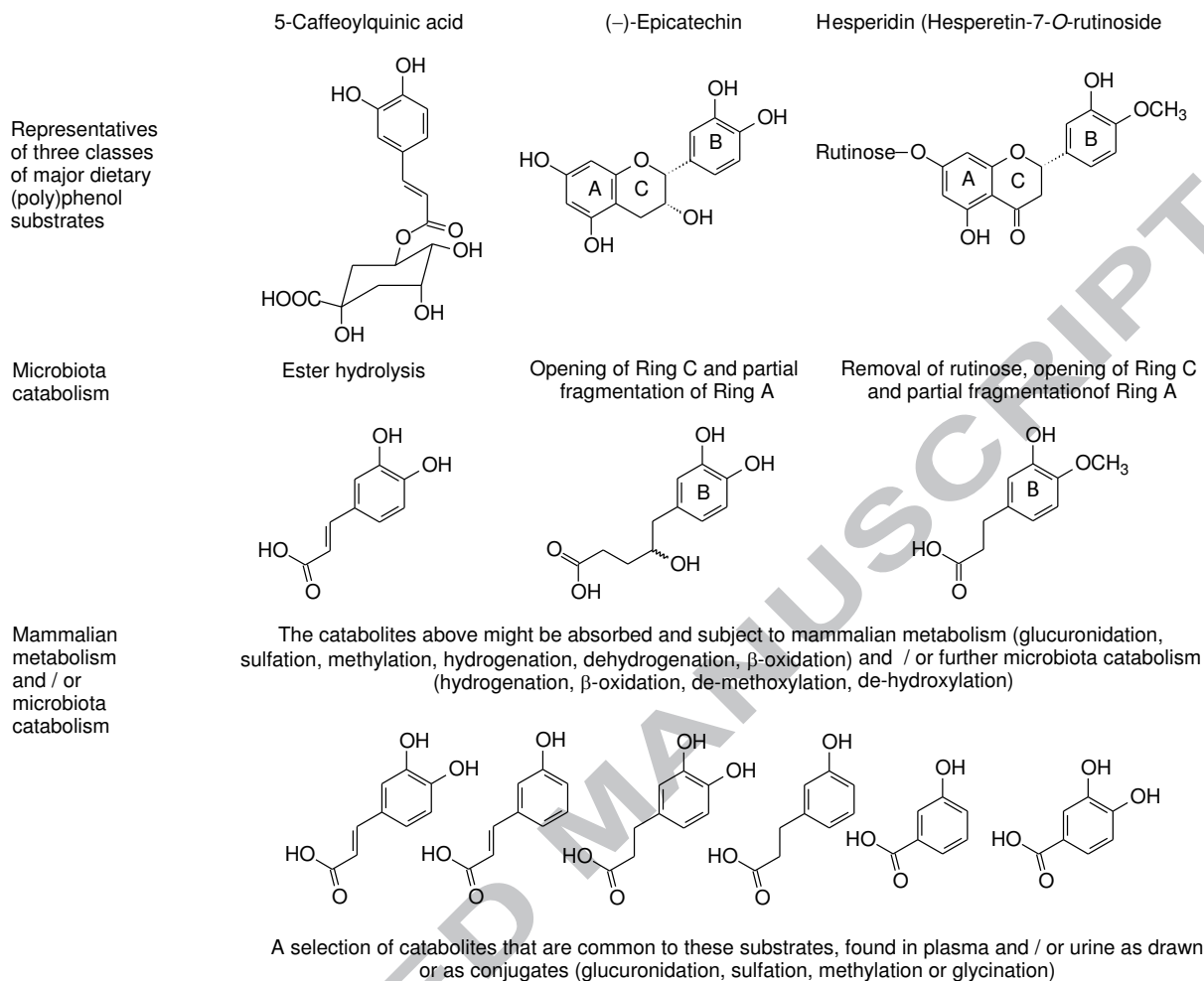
Figure 5: Specimen structures for two classes of dominant dietary (poly)phenol substrates that are not absorbed unchanged. The initial catabolic steps are not precisely known but lead to products similar to those shown in Fig. 2 for (–)-epicatechin.
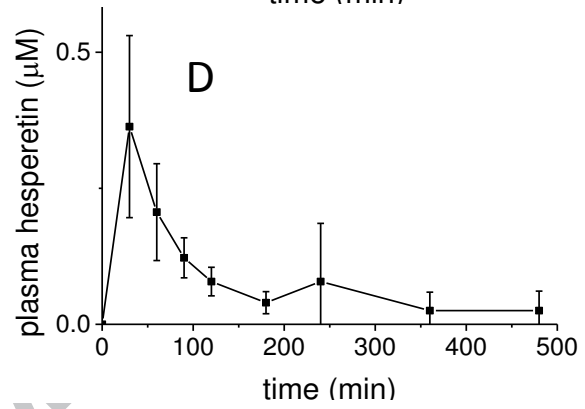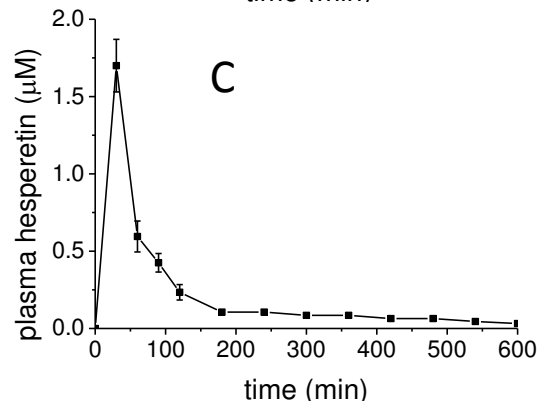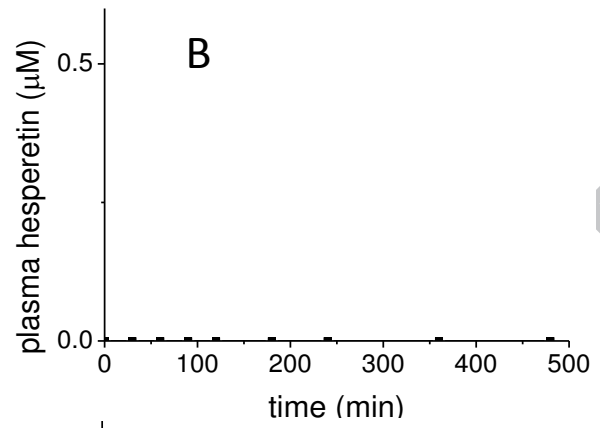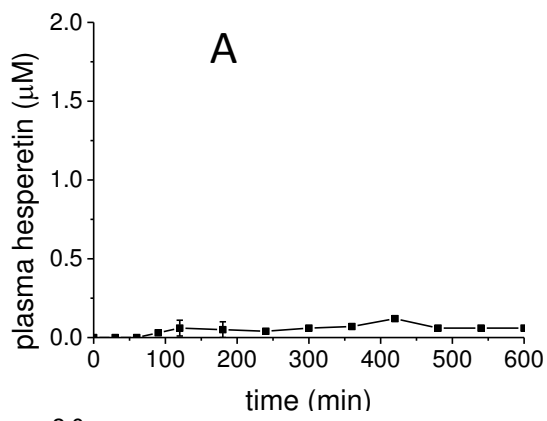
Figure 6. Appearance of microbial metabolites in the plasma and urine from volunteers (n=22) after consumption of mixed berry fruits. Top panel: Plasma catechol-O-sulfate (■) and 1-methyl-pyrogallol-O-sulfate (●). Lower panel: Urinary catechol-O-sulfate. Redrawn from [87] and [86]. *, $p < 0.05$; **, $p < 0.01$ relative to time zero. Relative quantification is based on peak area corrected for the internal standard.
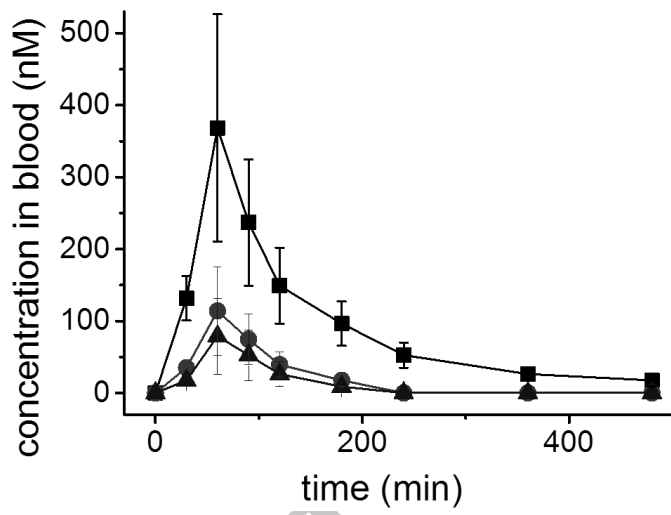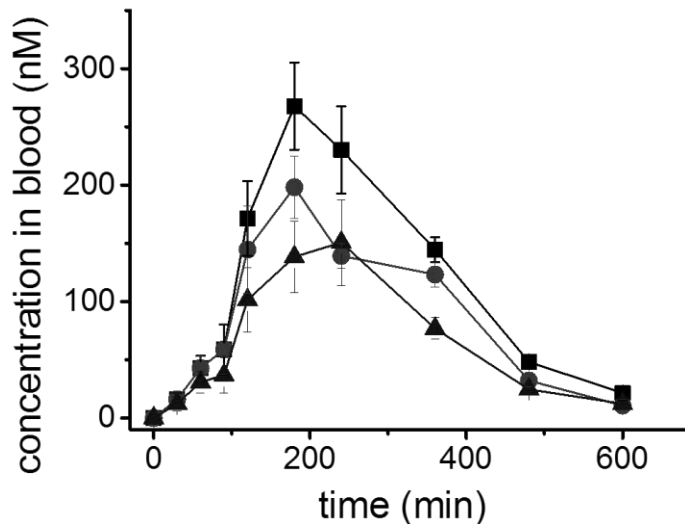
Figure 7. Measured concentration of 3′-methoxy-4′-hydroxy-phenylpropionic acid (DHFA) in plasma after consumption of one cup of coffee (●), or calculated after seven cups, one every 120 min (■). Data on single dose taken from [20] and is the DHFA plasma concentration after consumption of a cup of coffee (3.4 g of instant coffee in 200 ml water) containing 412 mg total chlorogenic acid. Effect of seven doses consumed every 120 min was calculated using simple addition of each time point from the single dose data, without taking into account any change in plasma clearance as a function of plasma concentration.

Figure 8: Possible catabolic routes from hesperetin to hydroxyphenylhydracrylic acids.
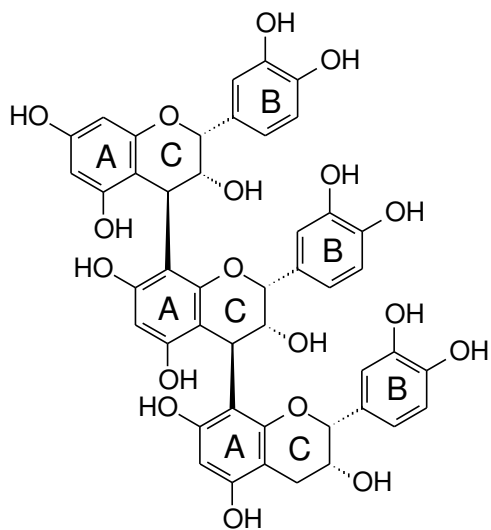
5-Caffeoylquinic acid   (−)-Epicatechin   Hesperidin (Hesperetin-7-*O*-rutinoside)

Representatives of three classes of major dietary (poly)phenol substrates

Microbiota catabolism

| Ester hydrolysis | Opening of Ring C and partial fragmentation of Ring A | Removal of rutinose, opening of Ring C and partial fragmentation of Ring A |

Mammalian metabolism and / or microbiota catabolism

The catabolites above might be absorbed and subject to mammalian metabolism (glucuronidation, sulfation, methylation, hydrogenation, dehydrogenation, β-oxidation) and / or further microbiota catabolism (hydrogenation, β-oxidation, de-methoxylation, de-hydroxylation)

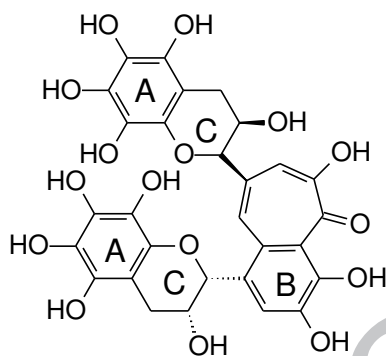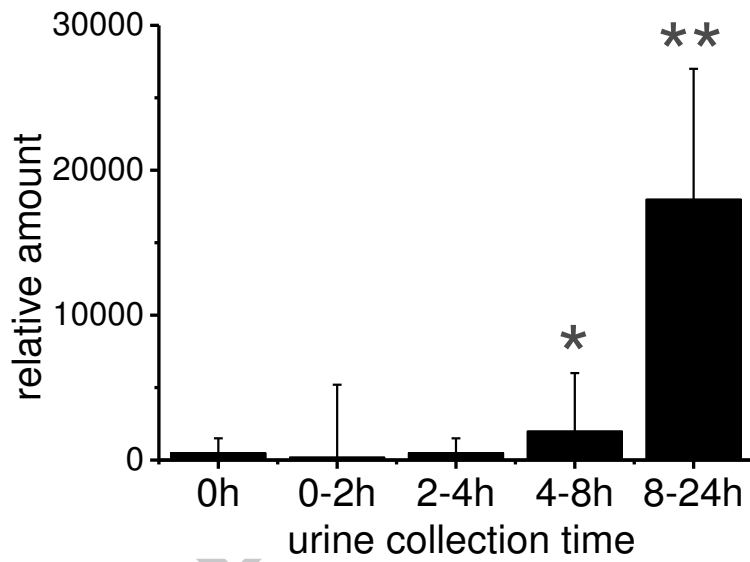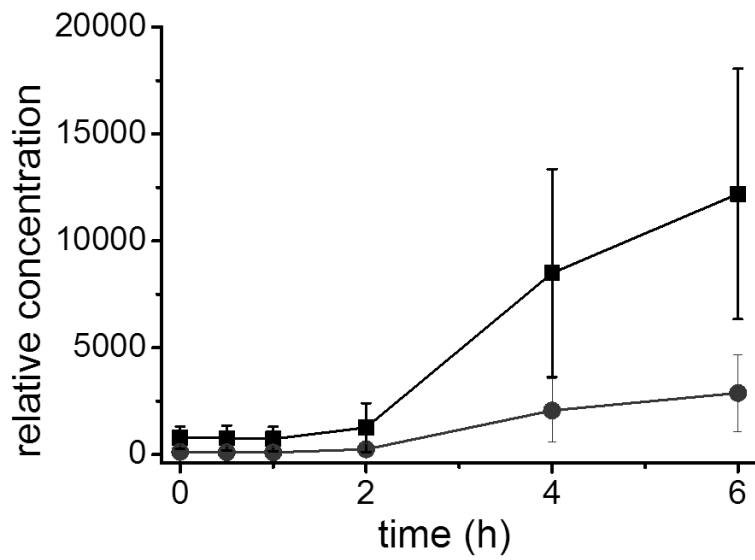A selection of catabolites that are common to these substrates, found in plasma and / or urine as drawn or as conjugates (glucuronidation, sulfation, methylation or glycination)

Specimen Proanthocyanidin

Specimen Thearubigin
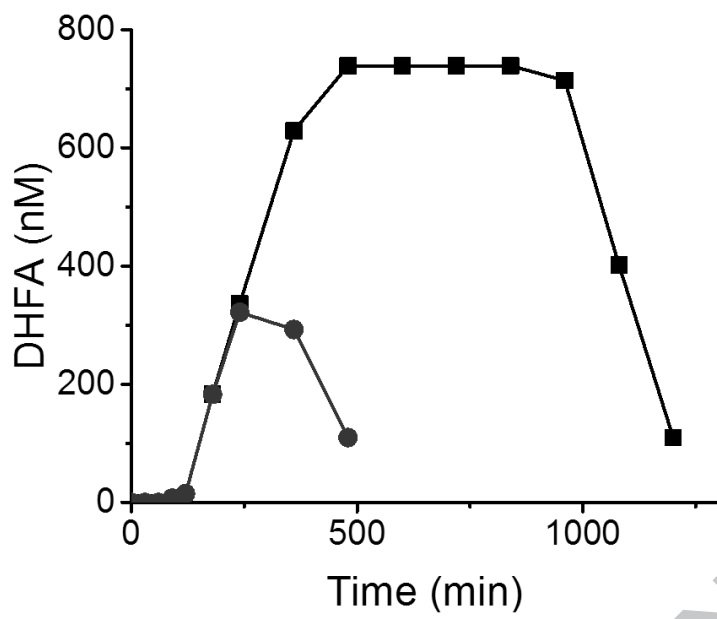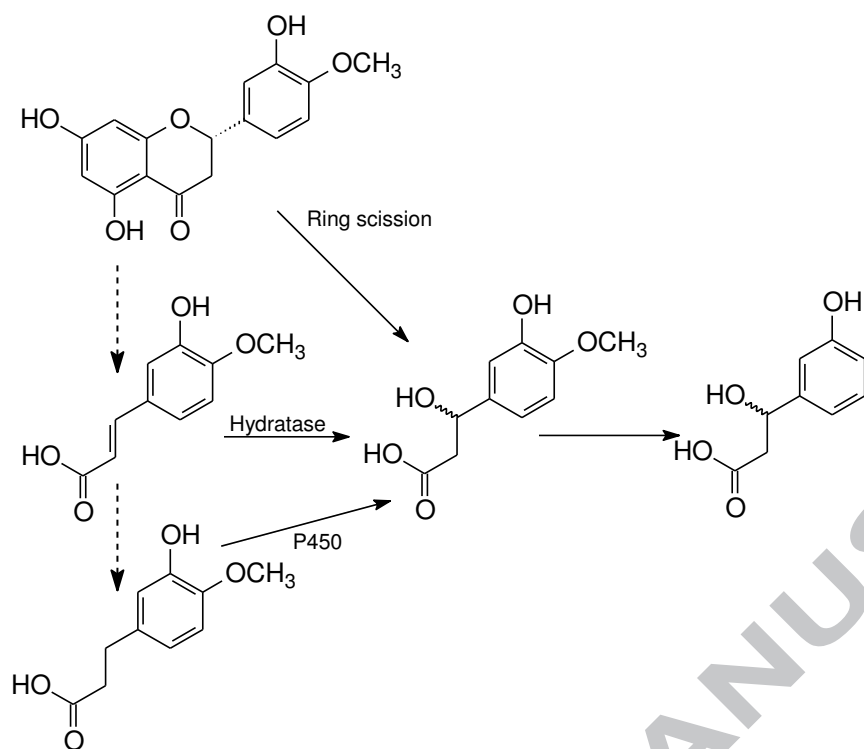
Table 1: Comparison of plasma and colon catabolite concentrations in volunteers compared to concentrations shown to be effective in in vitro

Catabolite	Plasma C_{max} concentration	Faecal water [171] or faeces concentration [175]	Concentration range used	Tissue	Minimum effective concentration	IC$_{50}$	Biomarker	Reference
Protocatechuic acid	0.17 µM [22] mean 0.16 µM, range 0.02–0.64 µM [176]	0.32–1.41 µM [171] 0.28 – 0.54 µmol/g [175]	0–100 µM	LPS-stimulated macrophages	10 µM		Suppression of NO	[177]
Protocatechuic acid				Human prostate cancer cells		43 µM	Eph tyrosine kinase receptors	[178]
Protocatechuic acid				TNF-α-stimulated HUVECs.	1 µM		VCAM-1 protein secretion	[173]
Protocatechuic acid			0–10 µM	Oxidatively challenged HUVECs	0.1 µM		IL6 production	[179]
Protocatechuic acid				HUVECs	0.2 µM		monocyte adhesion to TNFα-stimulated HUVEC	[180]
Protocatechuic acid-3′-O--sulfate			0–10 µM	Oxidatively challenged HUVECs	0.1 µM		IL6 production	[179]
Protocatechuic acid-3′-O-sulfate				TNF-α-stimulated HUVECs.	1 µM		VCAM-1 protein secretion	[173]
Protocatechuic acid-4′-O-sulfate				TNF-α-stimulated HUVECs.	1 µM		VCAM-1 protein secretion	[173]
Vanillic	Mean	0.11–1.24	0–10 µM	Oxidativ	0.1 µM		IL6	[179]

acid	0.02 µM, range 0.006–0.08 µM [176]	µM		ely challenged HUVECs			production	
Gallic acid	1.2 ± 1.0 µM [82]	0.17–1.40 µM		Human prostate cancer cells		0.26 µM	Eph tyrosine kinase receptors	[178]
3′,4′-Dihydroxy-phenylacetic acid		0.47–16.43 µM		Human prostate cancer cells		13 µM	Eph tyrosine kinase receptors	[178]
3′,4′-Dihydroxy-phenylacetic acid			0–10 µM	Pancreatic β-cells	1 µM		Insulin secretion	[181]
3′-hydroxy-phenylpropionic acid		6.9–43.7 µmol/g [175]	0–10 µM	Pancreatic β-cells	1 µM		Insulin secretion	[181]
3′,4′-dihydroxy-phenylpropionic acid (DHCA)	49–72 nM [167] 41 ± 10 nM [20]	0.67–9.42 µM	0.2–10 µM	t-BOOH-stimulated HepG2 cells	1 µM		GSH content	[182]
3′-methoxy-4′-hydroxy-phenylpropionic acid (DHFA)	385 ± 86 nM [20] 0.88 ± 0.64 µM [168][a]		0.2–10 µM	t-BOOH-stimulated HepG2 cells	0.2 µM		GSH content	[182]

Notes: a: Dose given equivalent to ca two cups of coffee. HUVEC, human umbilical cord endothelial cell