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Review

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

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ABSTRACT

(Poly)phenols are a large group of compounds, found in food, beverages, dietary supplements and herbal medicines. Owing to interest in 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 exact pathways from substrate to final microbial 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.

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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 (poly)phenols 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

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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 (poly)phenol 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 (poly)phenol intakes because the EPIC study did not consider thearubigins. Black tea theaflavins account for some 3–5% of the beverage solids compared 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 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

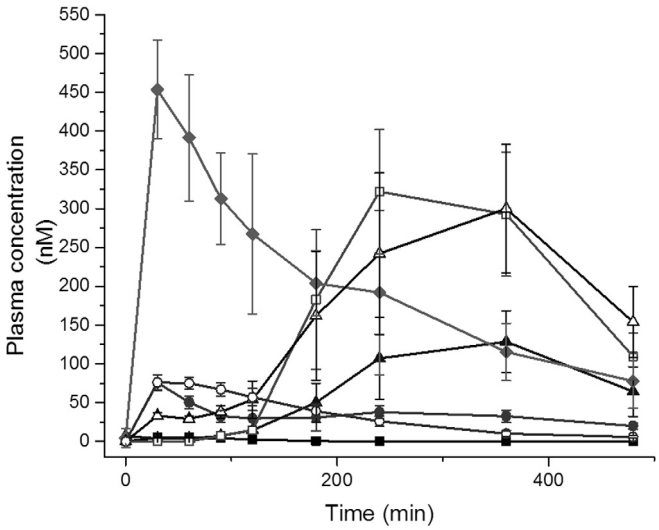

Fig. 1. Appearance of phenolic acids in plasma after consumption of chlorogenic acids from coffee. Healthy volunteers (n = 11) drank 3.4 g instant coffee in a 200 ml cup of coffee, or 4.0 g of instant coffee in 400 ml (n = 8) for the dimethoxycinnamic acid estimation. Dimethoxycinnamic acid (◆), dihydroferulic acid 4′-*O*-sulfate (▲), dihydroferulic acid (△), 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].

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 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] (Figs. 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