Polyphenols: do they play a role in the prevention of human pathologies?

H. Tapiero1*, K.D. Tew2, G. Nguyen Ba1, G. Mathé1

1Faculté de Pharmacie, Université de Paris, CNRS UMR 8612, 5, rue Jean Baptiste Clément, 94200 Chatenay Malabry, France; 2Fox Chase Cancer Center, Philadelphia, PA, USA

(Received 5 February 2002; accepted 16 February 2002)

Summary – Polyphenols are the most abundant antioxidants in our diets. The main classes of polyphenols are phenolic acids (mainly caffeic acid) and flavonoids (the most abundant in the diet are flavanols (catechins plus proanthocyanidins), anthocyanins and their oxidation products), which account for one- and two-thirds, respectively. Polyphenols are reducing agents, and together with other dietary reducing agents, such as vitamin C, vitamin E and carotenoids, referred to as antioxidants, protect the body’s tissues against oxidative stress and associated pathologies such as cancers, coronary heart disease and inflammation. The biological properties, bioavailability, antioxidant activity, specific interactions with cell receptors and enzymes, are related to the chemical structure of polyphenols. It is, therefore, essential to know the nature of the main polyphenols ingested, their dietary origin, the amounts consumed in different diets, their bioavailability and the factors controlling their bioavailability.

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Phenolic acids / Flavonoids / Distribution / Intake / Bioavailability

NATURE OF DIETARY POLYPHENOLS

The main classes of polyphenols are: phenolic acids (Fig. 1), flavonoids (Fig. 2) and the less common stilbenes and lignans (Fig. 3). The consumption of polyphenol-rich foods or beverages has been reported to play a role in cancers [1-3], stroke [4] and coronary heart disease [5-7].

Phenolic acids

A major class of phenolic compounds is the hydroxycinnamic acids, which are found in almost every plant. The major representative of hydroxycinnamic acids is caffeic acid, which occurs in foods mainly as an ester with quinic acid called chlorogenic acid (5-caffeoylquinic acid; Fig. 1B) [8,9]. Coffee is a major source of chlorogenic acid in the human diet; daily intake in coffee drinkers is 0.5–1 g, while coffee abstainers will usually ingest <100 mg/d. Most of the chlorogenic acid from foods will reach the colon and part will enter into the blood circulation [10]. Chlorogenic acid and caffeic acid are antioxidants in vitro [11] and they might inhibit the formation of mutagenic and carcinogenic N-nitroso compounds because they are inhibitors of the N-nitrosation reaction in vitro [12]. Further, chlorogenic acid can inhibit DNA damage in vitro [13,14]. Therefore, the inverse association between coffee intake and colon cancer in some epidemiologic studies might be explained in part by the chlorogenic acid present in coffee [15-17].

Flavonoids

Flavonoids (Fig. 2) are the most abundant polyphenols in human diets and represent a subclass of
Polyphenols with a C6–C3–C6 backbone structure. They can be divided into several classes according to the degree of oxidation of the oxygen heterocycle.

Flavonols are mainly represented by myricetin, fisetin, quercetin and kaempferol. Quercetin is present in many fruits, vegetables and beverages. It is the main flavonol in our diet and is particularly abundant in onions (0.3 mg/g fresh weight) [18] and tea (10–25 mg/l) [19].

Isoflavones are mainly represented by daidzein and genistein. The main source is soy, which contains ~1 mg of genistein and daidzein per gram dry bean [20]. These two isoflavones have received considerable attention due to their estrogenic properties and their suggested role in the prevention of breast cancer and osteoporosis [3,21].

Catechins are the main flavanols, which are very abundant in tea. Young shoots contain 200–340 mg of catechin, gallocatechin and their galloylated derivatives per gram of dry leaves [22]. An infusion of green tea contains 1 g/l catechins [23]. In black tea their content is reduced to about half this value due to their oxidation into more complex polyphenols during fermentation [24]. Other sources are red wine (270 mg/l) [25] and chocolate [26].

Flavanones are mainly represented by taxifolin, naringenin and hesperitin. The main source of flavanone is citrus fruits and the most widely consumed is hesperidin from oranges (125–250 mg/l of juice) [27].
Flavones, mainly represented by luteolin, wogonin and apigenin, are less common and were identified in sweet red pepper (luteolin) and celery (apigenin) [18].

In addition to these simple flavonoids and phenolic acids, other classes of polyphenols such as anthocyanins (pigments of red fruits with contents varying from 0.15 mg/g in strawberries, 4.5 mg/g in cherries to 26 mg/l in red wines [25]) or proanthocyanidins composed of flavan-3-ol monomers and their respective oligomers have been investigated. Others such as stilbenes are not widespread in food plants. Nevertheless, one of them, resveratrol (Fig. 3), has recently received great attention for its presence in wine and its anticarcinogenic properties [28,29]. However, its very low concentration in wine (0.3–2 mg/l in red wines) [25] makes the attribution of protective effects to this molecule unlikely.

Enterodiol and enterolactone were shown to be derived from dietary plant lignans, especially secoisolariciresinol diglucoside, by the action of intestinal microflora (Fig. 3). They have been identified in human plasma and urine [2]. The only foods that contain considerable quantities of lignans are flaxseed and flaxseed oil [30]. Lignans are recognized as phytostrogens due to their estrogen agonist and antagonist properties, and have been implicated as contributing to lower levels of breast cancer amongst vegetarians.

In conclusion, flavonoids are intensively studied because of their proposed protective effects to atherosclerosis and certain cancers [31,32]. They are ubiquitously present in plant foods and several beverages, such as tea and wine, mainly as β-glycosidic conjugates [33]. In food, they are present mainly as β-glycosides, and the nature of glycosylation markedly influences the efficiency of their absorption, which is thought to occur in the small intestine or in the large intestines after bacterial deconjugation [34].

**INTAKE OF PHENOLIC ACIDS AND FLAVONOIDS**

Provided that interference with other reducing agents is eliminated, total phenols were estimated by colorimetric methods based on the reducing capacity of phenolic groups [35]. Nevertheless, specific compounds such as chlorogenic acid, quercetin or catechins in tea were estimated individually by chromatographic techniques. Polyphenol intake depends to a large extent on dietary habits and preferences. Phenolic acids account for approximately one-third of the total phenols, and flavonoids account for two-thirds. Heavy coffee drinkers will likely consume more polyphenolic acids than flavonoids. Intake of flavonols, flavones and isoflavones is relatively low compared with that of polyphenolic acids and other flavonoids, and oxidized polyphenols. Chocolate is also very rich in polyphenols, and a minor consumption of chocolate may significantly contribute to total polyphenol intake and more particularly to the catechin [26] and proanthocyanidin intake. For isoflavones, an average dietary intake of 30–40 mg/d was determined for the Japanese [36,37]. Consumption in Western countries is significantly lower due to the limited consumption of soy products [38]. A major source of polyphenols is beverages (red wine, coffee, tea and fruit juices). Orange juice is not as rich in polyphenols.

Although the precise mechanism of absorption by the intestinal cells is presently unknown, the sugar moiety of the glycoside seems to be an important determinant for the site of absorption. Sugar-conjugated flavonoids may be hydrolyzed by the intestinal microflora [39,40] or by hydrolases located at the intestinal brush border membrane (e.g., lactase phlorizin hydrolase (LPH)) [41], after which the
aglycone may diffuse across the membrane into the cell. Alternatively, flavonoids may enter the cell as intact glycosides via the sodium-dependent glucose transporter (SGLT1) [42]. However, inside the enterocyte, cytosolic β-glucosidases may cleave the glycosides [43,44]. Glucuronidation, sulfation and methylation of the absorbed polyphenols have been shown to occur in humans [45-47].

**BIOAVAILABILITY OF POLYPHENOLS**

Biological properties of polyphenols depend on their bioavailability. The chemical properties of polyphenols determine their rate and extent of intestinal absorption, and of the metabolites circulating in the plasma, low for catechins in green tea, high for catechins in tea, high for quercetin-3-O-glucoside (quercetin), low for quercetin and rutin (a glycosylated flavonoid, polyphenol. Thus, the biological, physical and biological properties of the polyphenol. Thus, the flavonol quercetin has a partition coefficient (log octanol/water) of 1.2 ± 0.1, whereas for a glycoside, quercetin-3-O-rhamnoglucoside, the value is lower (0.37 ± 0.06), which suggests a greater hydrophilicity [59]. For glycosylated flavonoids, removal of the sugar by enzymes (glycosidases) and consequently of the hydrophilic moiety will usually be necessary for passive diffusion across the small intestine brush border to occur. Glycosidase activities can occur in the food itself (endogenous or added during processing) or in the cells of the gastrointestinal mucosa or can be secreted by the colon microflora. Human cells express some β-glucosidases, but the expression pattern is tissue-specific and often regulated during development. Polyphenols with attached glucose, arabinose or xylose are potential substrates for endogenous human enzymes. Attached rhamnose is not a substrate for human β-glucosidases and so is only cleaved by colon microflora-rhamnosidases. The activity of the liver extracts and of the small intestine is due to cytosolic β-glucosidase (CBG)—a soluble enzyme found in many tissues.

Flavanols such as (−)-epicatechin are often acylated, especially by gallic acid. Galloyl substitutions result in only a small change in the partition coefficients of flavanols and do not influence the bioavailability of polyphenols as dramatically as glycosylation. Flavanols appear to pass through biological membranes and to be absorbed without deconjugation or hydrolysis [60,61]. Hydroxycinnamates such as caffeic acid are also commonly esterified to sugars, organic acids and lipids. Chlorogenic acid is caffeic acid ester linked to quinic acid, and this compound is found at very high levels in coffee [8]. Since there are no esterases in human tissues able to release caffeic acid from chlorogenic acid [62], the only significant site for chlorogenic acid metabolism is the colonic microflora [63]. After hydrolysis to the free aglycone, polyphenols are conjugated by methylation, sulfation, glucuronidation or a combination. Much of the information available on the metabolism of polyphenols derives from comparisons with drug metabolism. The formation of conjugates can alter the biological properties of the circulating metabolites. In contrast to drug administration that can saturate the metabolic pathways (since it is usually administered in concentrated dose), polyphenols in food will probably not saturate the metabolic pathways, and the circulating species are expected to conjugate. Thus, after intake of a large dose (2 g) of (+)-catechin, free and methyl-catechin were detected in the plasma after 30 and 120 min, respectively. After 8 h, 40% of the catechin found in urine was methylated, sulfated and glucuronidated. In contrast, after the consumption of only a few milligrams of (+)-catechin, all the catechin found in the plasma was conjugated and no free polyphenol could be detected
Following high-dose polyphenol administration, metabolism occurred primarily in the liver whereas when smaller doses were administered, metabolism took place first at the intestinal mucosa, the liver playing a secondary role to further modify the conjugated polyphenol [65]. This implies that the intestine is an important site for metabolism of food-derived polyphenols. In studies using rats or isolated rat intestine as models, polyphenol glycosides such as phloridzin [66], luteolin-7-O-glucoside [67], quercetin glycosides [68], kaempferol-3-O-glucoside [69], genistin and daidzin [70] are first deglycosylated and then converted to glucuronides or sulfates with or without methylation. Few human studies carried out with quercetin and kaempferol [71,72] or naringin (naringenin-7-rhamnoglucoside) [73] are in support of these data. Polyphenols that are absorbed, metabolized in the liver and excreted in the bile or directly from the enterocyte back to the small intestine will reach the colon in a different chemical form (such as a glucuronide) than polyphenols that are not absorbed in the stomach or small bowel and carried to the colon. The colon, which contains important colonic microflora, has enormous catalytic and hydrolytic potential to catalyze the breakdown of the polyphenol to more simple compounds, such as phenolic acids. The polyphenol concentration in the gut should be much higher than in the plasma. For example, the dilution of 500 mg of polyphenols with the digestive bolus in the colon would give a local concentration of 3 mM.

**ENZYMES INVOLVED IN POLYPHENOL METABOLISM: DISTRIBUTION AND INDUCIBILITY**

CBG is found in a wide variety of tissues but especially in liver. It is thought to catalyze the hydrolysis of a wide variety of xenobiotic glycosides [74,75].

LPH is found only in the small intestine. LPH has recently been suggested to play an important role in the metabolism of polyphenol glucosides, because it catalyzes the hydrolysis of a wide range of polyphenol glucosides, including quercetin-3-O-glucoside, which is not a substrate for CBG [76]. Five percent of Europeans and 90% of Africans and Asians have LPH deficiency in adulthood.

Catechol-O-methyltransferase (COMT) methylates polyphenols and occurs in a wide range of tissues. The specificity for polyphenols will determine which hydroxyl groups on the polyphenol ring are methylated. However, cytochrome P450 demethylates flavonols at the 4’ position and not at the 3’ position, and the specificity of methylation of quercetin could be defined by specificity of demethylation by cytochrome P450, not methylation by COMT [77].

UDP glucuronosyltransferase (UDPGT, UGT) catalyzes the conjugation of polyphenols to glucuronic acid. It is situated in the endoplasmic reticulum and exists as a large family of related enzymes. Glucuronidation is modified by the environment, diet and genetic polymorphisms, which could explain interindividual differences observed in the glucuronidation of catechin [78]. The effect of glucuronidation at exact locations on most polyphenols is not known. The biological activities of metabolites is an important area for further research. Glucuronidation of polyphenols is predominantly by the UGT1A family, which occurs in intestine, liver and kidney. UGT1A1, -1A3, -1A4, -1A6 and -1A9 are found in human liver; UGT1A1, -1A3, -1A4, -1A6, -1A8, -1A9 and -1A10 are expressed in human colon; and UGT1A9 is high in kidney.

Liver has the greatest capacity for glucuronidation [79-81]. Drugs, alcohol and smoking induce UGT1A. Furthermore, diet affects the levels of UGT [82,83].

Phenol sulfotransferases (P-PST, SULT) are a small group of cytosolic enzymes that are widely distributed. The endogenous substrates are iodothyronines, although other substrates include 4-nitrophenol, phenols and hydroxyarylamines [84]. Generally, sulfotransferases are not induced by diet, xenobiotics or the environment [82]. Some sulfotransferases are inhibited by polyphenols. Quercetin inhibited human SULT1A1 [85].

**CONCLUSIONS**

With regard to other dietary antioxidants, measurement of the total antioxidant capacity of plasma after ingestion of polyphenols suggests that metabolites formed in our tissues and/or by the colon microflora significantly contribute to the antioxidant capacity, to the prevention of oxidative stress and their role in the prevention of diseases. Thus, better knowledge of the consumption and bioavailability of dietary polyphenols would be desirable.
ACKNOWLEDGEMENTS

This work was partly supported by PharmaForum and P.A.C. Companies. This review is part of the dossier on ‘Polyphenols: Diversity and Bioavailability’ that will be published in the next issue of Biomedicine and Pharmacotherapy.

REFERENCES


