Polyphenols: food sources and bioavailability[1,2]

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ABSTRACT

Polyphenols are abundant micronutrients in our diet, and evidence for their role in the prevention of degenerative diseases such as cancer and cardiovascular diseases is emerging. The health effects of polyphenols depend on the amount consumed and on their bioavailability. In this article, the nature and contents of the various polyphenols present in food sources and the influence of agricultural practices and industrial processes are reviewed. Estimates of dietary intakes are given for each class of polyphenols. The bioavailability of polyphenols is also reviewed, with particular focus on intestinal absorption and the influence of chemical structure (eg, glycosylation, esterification, and polymerization), food matrix, and excretion back into the intestinal lumen. Information on the role of microflora in the catabolism of polyphenols and the production of some active metabolites is presented. Mechanisms of intestinal and hepatic conjugation (methylation, glucuronidation, sulfation), plasma transport, and elimination in bile and urine are also described. Pharmacokinetic data for the various polyphenols are compared. Studies on the identification of circulating metabolites, cellular uptake, intracellular metabolism with possible deconjugation, biological properties of the conjugated metabolites, and specific accumulation in some target tissues are discussed. Finally, bioavailability appears to differ greatly between the various polyphenols, and the most abundant polyphenols in our diet are not necessarily those that have the best bioavailability profile. A thorough knowledge of the bioavailability of the hundreds of dietary polyphenols will help us to identify those that are most likely to exert protective health effects. *Am J Clin Nutr* 2004;79:727–47.

KEY WORDS Polyphenols, flavonoids, phenolic acids, food sources, dietary intake, intestinal absorption, metabolism, bioavailability

INTRODUCTION

Over the past 10 y, researchers and food manufacturers have become increasingly interested in polyphenols. The chief reason for this interest is the recognition of the antioxidant properties of polyphenols, their great abundance in our diet, and their probable role in the prevention of various diseases associated with oxidative stress, such as cancer and cardiovascular and neurodegenerative diseases (Scalbert A, Manach C, Morand C, Rémésy C, Jiménez L. Crit Rev Food Sci Nutr, in press). Furthermore, polyphenols, which constitute the active substances found in many medicinal plants, modulate the activity of a wide range of enzymes and cell receptors (1). In this way, in addition to having antioxidant properties, polyphenols have several other specific biological actions that are as yet poorly understood. Two aims of research are to establish evidence for the effects of polyphenol consumption on health and to identify which of the hundreds of existing polyphenols are likely to provide the greatest protection in the context of preventive nutrition. If these objectives are to be attained, it is first essential to determine the nature and distribution of these compounds in our diet. Such knowledge will allow evaluation of polyphenol intake and enable epidemiologic analysis that will in turn provide an understanding of the relation between the intake of these substances and the risk of development of several diseases. Furthermore, not all polyphenols are absorbed with equal efficacy. They are extensively metabolized by intestinal and hepatic enzymes and by the intestinal microflora. Knowledge of the bioavailability and metabolism of the various polyphenols is necessary to evaluate their biological activity within target tissues. The types and distribution of polyphenols in foods and the bioavailability of polyphenols are the topics of the present review.

TYPES AND DISTRIBUTION OF POLYPHENOLS IN FOODS

Several thousand molecules having a polyphenol structure (ie, several hydroxyl groups on aromatic rings) have been identified in higher plants, and several hundred are found in edible plants. These molecules are secondary metabolites of plants and are generally involved in defense against ultraviolet radiation or aggression by pathogens. These compounds may be classified into different groups as a function of the number of phenol rings that they contain and of the structural elements that bind these rings to one another. Distinctions are thus made between the phenolic acids, flavonoids, stilbenes, and lignans (**Figure 1**). The flavonoids, which share a common structure consisting of 2 aromatic rings (A and B) that are bound together by 3 carbon atoms that form an oxygenated heterocycle (ring C), may themselves be divided into 6 subclasses as a function of the type of heterocycle involved: flavonols, flavones, isoflavones, flavanones, anthocyanidins, and flavanols (catechins and proanthocyanidins) (**Figure 2**). In addition to this diversity, polyphenols may be associated with various carbohydrates and organic acids and with one another.

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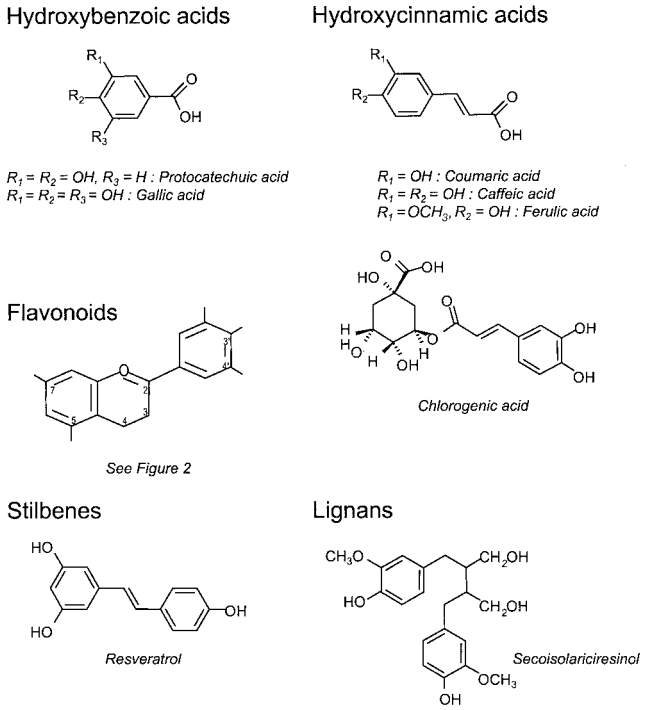

FIGURE 1. Chemical structures of polyphenols.

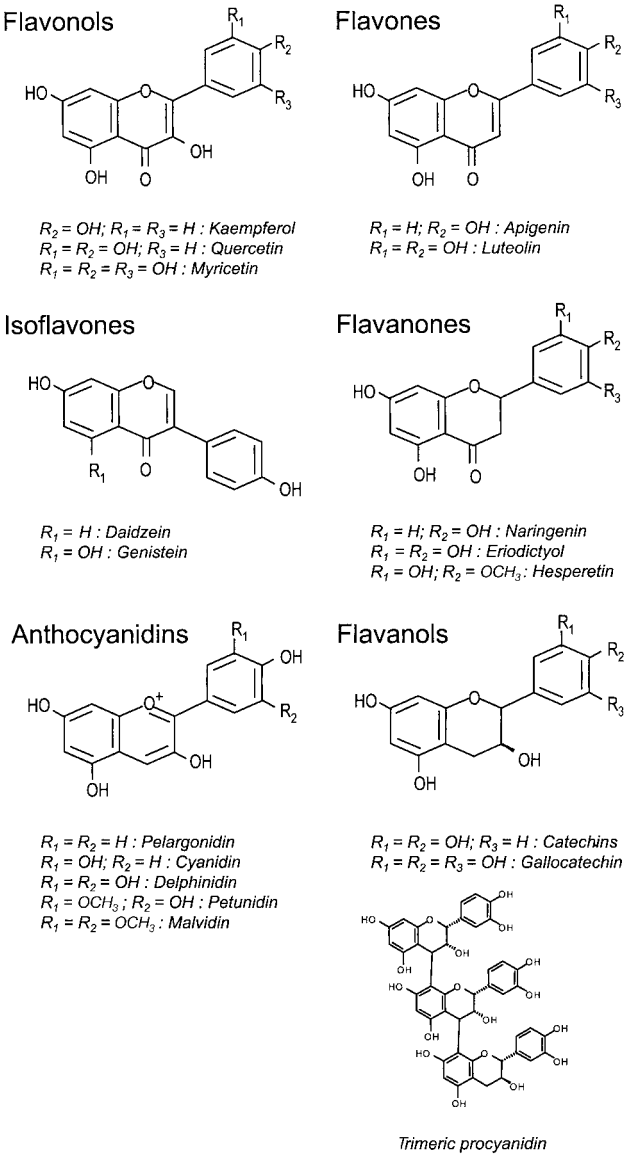

FIGURE 2. Chemical structures of flavonoids.

Phenolic acids

Two classes of phenolic acids can be distinguished: derivatives of benzoic acid and derivatives of cinnamic acid (Figure 1). The hydroxybenzoic acid content of edible plants is generally very low, with the exception of certain red fruits, black radish, and onions, which can have concentrations of several tens of milligrams per kilogram fresh weight (2). Tea is an important source of gallic acid: tea leaves may contain up to 4.5 g/kg fresh wt (3). Furthermore, hydroxybenzoic acids are components of complex structures such as hydrolyzable tannins (gallotannins in mangoes and ellagitannins in red fruit such as strawberries, raspberries, and blackberries) (4). Because these hydroxybenzoic acids, both free and esterified, are found in only a few plants eaten by humans, they have not been extensively studied and are not currently considered to be of great nutritional interest.

The hydroxycinnamic acids are more common than are the hydroxybenzoic acids and consist chiefly of *p*-coumaric, caffeic, ferulic, and sinapic acids. These acids are rarely found in the free form, except in processed food that has undergone freezing, sterilization, or fermentation. The bound forms are glycosylated derivatives or esters of quinic acid, shikimic acid, and tartaric acid. Caffeic and quinic acid combine to form chlorogenic acid, which is found in many types of fruit and in high concentrations in coffee: a single cup may contain 70–350 mg chlorogenic acid (5). The types of fruit having the highest content (blueberries, kiwis, plums, cherries, apples) contain 0.5–2 g hydroxycinnamic acids/kg fresh wt (**Table 1**) (6).

Caffeic acid, both free and esterified, is generally the most abundant phenolic acid and represents between 75% and 100% of the total hydroxycinnamic acid content of most fruit. Hydroxycinnamic acids are found in all parts of fruit, although the highest concentrations are seen in the outer parts of ripe fruit. Concentrations generally decrease during the course of ripening, but total quantities increase as the fruit increases in size.

Ferulic acid is the most abundant phenolic acid found in cereal grains, which constitute its main dietary source. The ferulic acid content of wheat grain is ≈0.8–2 g/kg dry wt, which may represent up to 90% of total polyphenols (28, 29). Ferulic acid is found chiefly in the outer parts of the grain. The aleurone layer and the pericarp of wheat grain contain 98% of the total ferulic acid. The ferulic acid content of different wheat flours is thus directly related to levels of sieving, and bran is the main source of polyphenols (30). Rice and oat flours contain approximately the same quantity of phenolic acids as wheat flour (63 mg/kg), although the content in maize flour is about 3 times as high (2). Ferulic acid is found chiefly in the *trans* form, which is esterified to arabinoxylans and hemicelluloses in the aleurone and pericarp. Only 10% of ferulic acid is found in soluble free form in wheat bran (29). Several dimers of ferulic acid are also found in cereals and form bridge structures between chains of hemicellulose.

TABLE 1
Polyphenols in foods

		Polyphenol content	
	Source (serving size)	By wt or vol	By serving
		mg/kg fresh wt (or mg/L)	*mg/serving*
Hydroxybenzoic acids (2, 6)	Blackberry (100 g)	80–270	8–27
Protocatechuic acid	Raspberry (100 g)	60–100	6–10
Gallic acid	Black currant (100 g)	40–130	4–13
p-Hydroxybenzoic acid	Strawberry (200 g)	20–90	4–18
Hydroxycinnamic acids (2, 5–7)	Blueberry (100 g)	2000–2200	200–220
Caffeic acid	Kiwi (100 g)	600–1000	60–100
Chlorogenic acid	Cherry (200 g)	180–1150	36–230
Coumaric acid	Plum (200 g)	140–1150	28–230
Ferulic acid	Aubergine (200 g)	600–660	120–132
Sinapic acid	Apple (200 g)	50–600	10–120
	Pear (200 g)	15–600	3–120
	Chicory (200 g)	200–500	40–100
	Artichoke (100 g)	450	45
	Potato (200 g)	100–190	20–38
	Corn flour (75 g)	310	23
	Flour: wheat, rice, oat (75 g)	70–90	5–7
	Cider (200 mL)	10–500	2–100
	Coffee (200 mL)	350–1750	70–350
Anthocyanins (8–10)	Aubergine (200 g)	7500	1500
Cyanidin	Blackberry (100 g)	1000–4000	100–400
Pelargonidin	Black currant (100 g)	1300–4000	130–400
Peonidin	Blueberry (100 g)	250–5000	25–500
Delphinidin	Black grape (200 g)	300–7500	60–1500
Malvidin	Cherry (200 g)	350–4500	70–900
	Rhubarb (100 g)	2000	200
	Strawberry (200 g)	150–750	30–150
	Red wine (100 mL)	200–350	20–35
	Plum (200 g)	20–250	4–50
	Red cabbage (200 g)	250	50
Flavonols (11–18)	Yellow onion (100 g)	350–1200	35–120
Quercetin	Curly kale (200 g)	300–600	60–120
Kaempferol	Leek (200 g)	30–225	6–45
Myricetin	Cherry tomato (200 g)	15–200	3–40
	Broccoli (200 g)	40–100	8–20
	Blueberry (100 g)	30–160	3–16
	Black currant (100 g)	30–70	3–7
	Apricot (200 g)	25–50	5–10
	Apple (200 g)	20–40	4–8
	Beans, green or white (200 g)	10–50	2–10
	Black grape (200 g)	15–40	3–8
	Tomato (200 g)	2–15	0.4–3.0
	Black tea infusion (200 mL)	30–45	6–9
	Green tea infusion (200 mL)	20–35	4–7
	Red wine (100 mL)	2–30	0.2–3
Flavones (11–12, 14, 18)	Parsley (5 g)	240–1850	1.2–9.2
Apigenin	Celery (200 g)	20–140	4–28
Luteolin	Capsicum pepper (100 g)	5–10	0.5–1
Flavanones (19–21)	Orange juice (200 mL)	215–685	40–140
Hesperetin	Grapefruit juice (200 mL)	100–650	20–130
Naringenin	Lemon juice (200 mL)	50–300	10–60
Eriodictyol			
Isoflavones (22–25)	Soy flour (75 g)	800–1800	60–135
Daidzein	Soybeans, boiled (200 g)	200–900	40–180
Genistein	Miso (100 g)	250–900	25–90
Glycitein	Tofu (100 g)	80–700	8–70
	Tempeh (100 g)	430–530	43–53
	Soy milk (200 mL)	30–175	6–35
Monomeric flavanols (6, 17, 26, 27)	Chocolate (50 g)	460–610	23–30
Catechin	Beans (200 g)	350–550	70–110
Epicatechin	Apricot (200 g)	100–250	20–50
	Cherry (200 g)	50–220	10–44
	Grape (200 g)	30–175	6–35
	Peach (200 g)	50–140	10–28
	Blackberry (100 g)	130	13
	Apple (200 g)	20–120	4–24
	Green tea (200 mL)	100–800	20–160
	Black tea (200 mL)	60–500	12–100
	Red wine (100 mL)	80–300	8–30
	Cider (200 mL)	40	8

Flavonoids

Flavonols are the most ubiquitous flavonoids in foods, and the main representatives are quercetin and kaempferol. They are generally present at relatively low concentrations of ≈15–30 mg/kg fresh wt. The richest sources are onions (up to 1.2 g/kg fresh wt), curly kale, leeks, broccoli, and blueberries (Table 1). Red wine and tea also contain up to 45 mg flavonols/L. These compounds are present in glycosylated forms. The associated sugar moiety is very often glucose or rhamnose, but other sugars may also be involved (eg, galactose, arabinose, xylose, glucuronic acid). Fruit often contains between 5 and 10 different flavonol glycosides (6). These flavonols accumulate in the outer and aerial tissues (skin and leaves) because their biosynthesis is stimulated by light. Marked differences in concentration exist between pieces of fruit on the same tree and even between different sides of a single piece of fruit, depending on exposure to sunlight (31). Similarly, in leafy vegetables such as lettuce and cabbage, the glycoside concentration is ≥10 times as high in the green outer leaves as in the inner light-colored leaves (14). This phenomenon also accounts for the higher flavonol content of cherry tomatoes than of standard tomatoes, because they have different proportions of skin to whole fruit.

Flavones are much less common than flavonols in fruit and vegetables. Flavones consist chiefly of glycosides of luteolin and apigenin. The only important edible sources of flavones identified to date are parsley and celery (Table 1). Cereals such as millet and wheat contain *C*-glycosides of flavones (32–34). The skin of citrus fruit contains large quantities of polymethoxylated flavones: tangeretin, nobiletin, and sinensetin (up to 6.5 g/L of essential oil of mandarin) (2). These polymethoxylated flavones are the most hydrophobic flavonoids.

In human foods, flavanones are found in tomatoes and certain aromatic plants such as mint, but they are present in high concentrations only in citrus fruit. The main aglycones are naringenin in grapefruit, hesperetin in oranges, and eriodictyol in lemons. Flavanones are generally glycosylated by a disaccharide at position 7: either a neohesperidose, which imparts a bitter taste (such as to naringin in grapefruit), or a rutinose, which is flavorless. Orange juice contains between 200 and 600 mg hesperidin/L and 15–85 mg narirutin/L, and a single glass of orange juice may contain between 40 and 140 mg flavanone glycosides (20). Because the solid parts of citrus fruit, particularly the albedo (the white spongy portion) and the membranes separating the segments, have a very high flavanone content, the whole fruit may contain up to 5 times as much as a glass of orange juice.

Isoflavones are flavonoids with structural similarities to estrogens. Although they are not steroids, they have hydroxyl groups in positions 7 and 4' in a configuration analogous to that of the hydroxyls in the estradiol molecule. This confers pseudohormonal properties on them, including the ability to bind to estrogen receptors, and they are consequently classified as phytoestrogens. Isoflavones are found almost exclusively in leguminous plants. Soya and its processed products are the main source of isoflavones in the human diet. They contain 3 main molecules: genistein, daidzein, and glycitein, generally in a concentration ratio of 1:1:0.2. These isoflavones are found in 4 forms: aglycone, 7-*O*-glucoside, 6″-*O*-acetyl-7-*O*-glucoside, and 6″-*O*-malonyl-7-*O*-glucoside (35). The 6″-*O*-malonylglucoside derivatives have an unpleasant, bitter, and astringent taste. They are sensitive to heat and are often hydrolyzed to glycosides during the course of industrial processing, as in the production of soya milk (36). The fermentation carried out during the manufacturing of certain foods, such as miso and tempeh, results in the hydrolysis of glycosides to aglycones. The aglycones are highly resistant to heat. The isoflavone content of soya and its manufactured products varies greatly as a function of geographic zone, growing conditions, and processing. Soybeans contain between 580 and 3800 mg isoflavones/kg fresh wt, and soymilk contains between 30 and 175 mg/L (25, 37).

Flavanols exist in both the monomer form (catechins) and the polymer form (proanthocyanidins). Catechins are found in many types of fruit (apricots, which contain 250 mg/kg fresh wt, are the richest source; Table 1). They are also present in red wine (up to 300 mg/L), but green tea and chocolate are by far the richest sources. An infusion of green tea contains up to 200 mg catechins (38). Black tea contains fewer monomer flavanols, which are oxidized during "fermentation" (heating) of tea leaves to more complex condensed polyphenols known as theaflavins (dimers) and thearubigins (polymers). Catechin and epicatechin are the main flavanols in fruit, whereas gallocatechin, epigallocatechin, and epigallocatechin gallate are found in certain seeds of leguminous plants, in grapes, and more importantly in tea (27, 39). In contrast to other classes of flavonoids, flavanols are not glycosylated in foods. The tea epicatechins are remarkably stable when exposed to heat as long as the pH is acidic: only ≈15% of these substances are degraded after 7 h in boiling water at pH 5 (40).

Proanthocyanidins, which are also known as condensed tannins, are dimers, oligomers, and polymers of catechins that are bound together by links between C4 and C8 (or C6). Their mean degree of polymerization in foods has rarely been determined. In cider apples, the mean degree of polymerization ranges from 4 to 11 (41). Through the formation of complexes with salivary proteins, condensed tannins are responsible for the astringent character of fruit (grapes, peaches, kakis, apples, pears, berries, etc) and beverages (wine, cider, tea, beer, etc) and for the bitterness of chocolate (42). This astringency changes over the course of maturation and often disappears when the fruit reaches ripeness; this change has been well explained in the kaki fruit by polymerization reactions with acetaldehyde (43). Such polymerization of tannins probably accounts for the apparent reduction in tannin content that is commonly seen during the ripening of many types of fruit. It is difficult to estimate the proanthocyanidin content of foods because proanthocyanidins have a wide range of structures and molecular weights. The only available data concern dimers and trimers, which are as abundant as the catechins themselves (26).

Anthocyanins are pigments dissolved in the vacuolar sap of the epidermal tissues of flowers and fruit, to which they impart a pink, red, blue, or purple color (9). They exist in different chemical forms, both colored and uncolored, according to pH. Although they are highly unstable in the aglycone form (anthocyanidins), while they are in plants, they are resistant to light, pH, and oxidation conditions that are likely to degrade them. Degradation is prevented by glycosylation, generally with a glucose at position 3, and esterification with various organic acids (citric and malic acids) and phenolic acids. In addition, anthocyanins are stabilized by the formation of complexes with other flavonoids (copigmentation). In the human diet, anthocyanins are found in red wine, certain varieties of cereals, and certain leafy and root vegetables (aubergines, cabbage, beans, onions, radishes), but they are most abundant in fruit. Cyanidin is the most common anthocyanidin in foods. Food contents are generally proportional to color intensity and reach values up to 2–4 g/kg

fresh wt in blackcurrants or blackberries (Table 1). These values increase as the fruit ripens. Anthocyanins are found mainly in the skin, except for certain types of red fruit, in which they also occur in the flesh (cherries and strawberries). Wine contains ≈200–350 mg anthocyanins/L, and these anthocyanins are transformed into various complex structures as the wine ages (10, 44).

Lignans

Lignans are formed of 2 phenylpropane units (Figure 1). The richest dietary source is linseed, which contains secoisolariciresinol (up to 3.7 g/kg dry wt) and low quantities of matairesinol. Other cereals, grains, fruit, and certain vegetables also contain traces of these same lignans, but concentrations in linseed are ≈1000 times as high as concentrations in these other food sources (45). Lignans are metabolized to enterodiol and enterolactone by the intestinal microflora. The low quantities of secoisolariciresinol and matairesinol that are ingested as part of our normal diet do not account for the concentrations of the metabolites enterodiol and enterolactone that are classically measured in plasma and urine. Thus, there are undoubtedly other lignans of plant origin that are precursors of enterodiol and enterolactone and that have not yet been identified (46). Thompson et al (47) used an in vitro technique involving the fermentation of foods by human colonic microflora to quantitatively evaluate precursors of enterodiol and enterolactone. They confirmed that oleaginous seeds (linseed) are the richest source and identified algae, leguminous plants (lentils), cereals (triticale and wheat), vegetables (garlic, asparagus, carrots), and fruit (pears, prunes) as minor sources.

Stilbenes

Stilbenes are found in only low quantities in the human diet. One of these, resveratrol, for which anticarcinogenic effects have been shown during screening of medicinal plants and which has been extensively studied, is found in low quantities in wine (0.3–7 mg aglycones/L and 15 mg glycosides/L in red wine) (48–50). However, because resveratrol is found in such small quantities in the diet, any protective effect of this molecule is unlikely at normal nutritional intakes.

VARIABILITY OF POLYPHENOL CONTENT OF FOODS

Fruit and beverages such as tea and red wine constitute the main sources of polyphenols. Certain polyphenols such as quercetin are found in all plant products (fruit, vegetables, cereals, leguminous plants, fruit juices, tea, wine, infusions, etc), whereas others are specific to particular foods (flavanones in citrus fruit, isoflavones in soya, phloridzin in apples). In most cases, foods contain complex mixtures of polyphenols, which are often poorly characterized. Apples, for example, contain flavanol monomers (epicatechin mainly) or oligomers (procyanidin B2 mainly), chlorogenic acid and small quantities of other hydroxycinnamic acids, 2 glycosides of phloretin, several quercetin glycosides, and anthocyanins such as cyanidin 3-galactoside in the skin of certain red varieties. Apples are one of the rare types of food for which fairly precise data on polyphenol composition are available. Differences in polyphenol composition between varieties of apples have notably been studied. The polyphenol profiles of all varieties of apples are practically identical, but concentrations may range from 0.1 to 5 g total polyphenols/kg fresh wt and may be as high as 10 g/kg in certain varieties of cider apples (41, 51).

For many plant products, the polyphenol composition is much less known, knowledge is often limited to one or a few varieties, and data sometimes do not concern the edible parts. Some foods, particularly some exotic types of fruit and some cereals, have not been analyzed yet. Furthermore, numerous factors other than variety may affect the polyphenol content of plants; these factors include ripeness at the time of harvest, environmental factors, processing, and storage.

Environmental factors have a major effect on polyphenol content. These factors may be pedoclimatic (soil type, sun exposure, rainfall) or agronomic (culture in greenhouses or fields, biological culture, hydroponic culture, fruit yield per tree, etc). Exposure to light has a considerable effect on most flavonoids. The degree of ripeness considerably affects the concentrations and proportions of the various polyphenols (6). In general, phenolic acid concentrations decrease during ripening, whereas anthocyanin concentrations increase. Many polyphenols, especially phenolic acids, are directly involved in the response of plants to different types of stress: they contribute to healing by lignification of damaged areas, they possess antimicrobial properties, and their concentrations may increase after infection (2, 6, 52). Although very few studies directly addressed this issue, the polyphenol content of vegetables produced by organic or sustainable agriculture is certainly higher than that of vegetables grown without stress, such as those grown in conventional or hydroponic conditions. This was shown recently in strawberries, blackberries, and corn (53). With the current state of knowledge, it is extremely difficult to determine for each family of plant products the key variables that are responsible for the variability in the content of each polyphenol and the relative weight of those variables. A huge amount of analysis would be required to obtain this information. For example, determination of the *p*-coumaric acid content of >500 red wines showed that genetic factors were more important than was exposure to light or climate (54).

Storage may also affect the content of polyphenols that are easily oxidized. Oxidation reactions result in the formation of more or less polymerized substances, which lead to changes in the quality of foods, particularly in color and organoleptic characteristics. Such changes may be beneficial (as is the case with black tea) or harmful (browning of fruit) to consumer acceptability. Storage of wheat flour results in marked loss of phenolic acids (28). After 6 mo of storage, flours contained the same phenolic acids in qualitative terms, but their concentrations were 70% lower. Cold storage, in contrast, did not affect the content of polyphenols in apples (55, 56), pears (57), or onions (58). At 25 °C, storage of apple juice for 9 mo results in a 60% loss of quercetin and a total loss of procyanidins, despite the fact that polyphenols are more stable in fruit juices than is vitamin C (59, 60).

Methods of culinary preparation also have a marked effect on the polyphenol content of foods. For example, simple peeling of fruit and vegetables can eliminate a significant portion of polyphenols because these substances are often present in higher concentrations in the outer parts than in the inner parts. Cooking may also have a major effect. Onions and tomatoes lose between 75% and 80% of their initial quercetin content after boiling for 15 min, 65% after cooking in a microwave oven, and 30% after frying (18). Steam cooking of vegetables, which avoids leaching, is preferable. Potatoes contain up to 190 mg chlorogenic acid/kg, mainly in the skin (61). Extensive loss occurs during cooking,

and no remaining phenolic acids were found in French fries or freeze-dried mashed potatoes (54).

Industrial food processing also affects polyphenol content. As with fruit peeling, dehulling of legume seeds and decortication and bolting of cereals can result in a loss of some polyphenols. Grinding of plant tissues may lead to oxidative degradation of polyphenols as a result of cellular decompartmentation and contact between cytoplasmic polyphenol oxidase and phenolic substrates present in the vacuoles. Polyphenols are then transformed into brown pigments that are polymerized to different degrees. This unwanted process can occur, for example, during the process of making jam or compote from fruit. Production of fruit juice often involves clarification or stabilization steps specifically aimed at removing certain flavonoids responsible for discoloration and haze formation. Manufactured fruit juices thus have low flavonoid content. The pectinolytic enzymes used during such processing also hydrolyze the esters of hydroxycinnamic acid (62). Conversely, maceration operations facilitate diffusion of polyphenols in juice, as occurs during vinification of red wine. This maceration accounts for the fact that the polyphenol content of red wines is 10 times as high as that of white wines (63) and is also higher than that of grape juice (64).

Because of the wide range of existing polyphenols and the considerable number of factors that can modify their concentration in foods, no reference food-composition tables (as they exist for other micronutrients such as vitamins) have yet been drawn up. Only partial data for certain polyphenols, such as flavonols and flavones, catechins, and isoflavones, have been published on the basis of direct food analysis (11, 27) or bibliographic compilations (37, 65). Since March 2003, a database in which the flavonoid contents of 225 selected foods were compiled from 97 bibliographic sources has been available on the US Department of Agriculture website (66). A comprehensive composition table for polyphenols is essential; it should allow daily polyphenol consumption to be calculated from dietary questionnaires. Polyphenol intake could then be correlated with the incidence of certain diseases or early markers for these diseases in epidemiologic studies, which would permit investigations of the protective role of these micronutrients.

DIETARY INTAKE OF POLYPHENOLS

Only partial information is available on the quantities of polyphenols that are consumed daily throughout the world. These data have been obtained through analysis of the main aglycones (after hydrolysis of their glycosides and esters) in the foods most widely consumed by humans.

In 1976 Kuhnau (8) calculated that dietary flavonoid intake in the United States was ≈1 g/d and consisted of the following: 16% flavonols, flavones, and flavanones; 17% anthocyanins; 20% catechins; and 45% "biflavones." Although these figures were obtained under poorly detailed conditions, they continue to serve as reference data. Certain studies have subsequently provided more precise individual data concerning the intake of various classes of polyphenols. Flavonols have been more extensively studied. Consumption of these substances has been estimated at ≈20–25 mg/d in the United States, Denmark, and Holland (67–69). In Italy, consumption ranged from 5 to 125 mg/d, and the mean value was 35 mg/d (70). The intake of flavanones is similar to or possibly higher than that of flavonols, with a mean consumption of 28.3 mg hesperetin/d in Finland (71). Because citrus fruit is practically the sole source of flavanones, ingestion of these substances is probably greater in regions where these fruits are produced, such as southern Europe. Anthocyanin consumption was studied only in Finland, where high amounts of berries are eaten, and was found to be 82 mg/d on average, although some intakes exceeded 200 mg/d (72).

Consumption of soya in the Asian countries is ≈10–35 g/d, which is equivalent to a mean intake of 25–40 mg isoflavones/d, with a maximum intake of 100 mg/d (23, 73, 74). Americans and Europeans, who eat little soya, consume only a few milligrams of isoflavones per day. Nevertheless, the incorporation of growing quantities of soya extracts into manufactured food products could result in an increase in isoflavone intake. Women undergoing phytoestrogen replacement therapy for menopause consume between 30 and 70 mg isoflavones/d in the form of soya extract capsules (75).

In Spain the total consumption of catechins and proanthocyanidin dimers and trimers has been estimated at 18–31 mg/d, and the main sources are apples, pears, grapes, and red wine (76). Consumption of monomer flavonols in Holland is significantly higher (50 mg/d), and the principal sources are tea, chocolate, apples, and pears (27). Ingestion of more highly polymerized proanthocyanidins could be as high as several hundred milligrams per day as previously suggested (42), but there are still no reliable data.

Consumption of hydroxycinnamic acids may vary highly according to coffee consumption. Some persons who drink several cups per day may ingest as much as 500–800 mg hydroxycinnamic acids/d, whereas subjects who do not drink coffee and who also eat small quantities of fruit and vegetables do not ingest >25 mg/d (54). A German study estimated daily consumption of hydroxycinnamic acids and hydroxybenzoic acids at 211 and 11 mg/d, respectively. Caffeic acid intake alone was 206 mg/d, and the principal sources were coffee (which provides 92% of caffeic acid) and fruit and fruit juices combined (source of 59% of *p*-coumaric acid) (65).

Various authors have noted a high variability in polyphenol intake. Intake of phenolic acids ranged from 6 to 987 mg/d in Germany (65). The mean consumption of flavonols and flavones in the Dutch population was 23 mg/d; values at the 10th and 90th percentiles were 4 and 46 mg/d, respectively; and some subjects consumed up to 100 mg/d (69). The main reason for these variations is individual food preferences. When polyphenol content is expressed as the amount provided by a food serving, as in Table 1, the consumption of one particular food, such as berries for anthocyanins or coffee for hydroxycinnamic acids, clearly appears to be capable of markedly changing the total polyphenol intake. If mean values are required, the addition of the intakes of flavonols, flavanones, flavanols (monomers, dimers, and trimers), and isoflavones gives a total daily consumption of 100–150 mg in Western populations, to which must be added the considerably variable intake of hydroxycinnamic acids, anthocyanins, and proanthocyanidins. Finally, the total polyphenol intake probably commonly reaches 1 g/d in people who eat several servings of fruit and vegetables per day. Note that it is really difficult to follow a diet totally free of polyphenols. Because polyphenol intake is difficult to evaluate by using dietary questionnaires, biomarkers for polyphenol exposure would be very useful. A few studies have tried to correlate flavonol, flavanone, and isoflavone intakes with plasma concentrations or urinary excretion of metabolites (77–82), but we are not yet able to

propose a reliable measurement in urine or plasma samples that could reflect the long-term intake of the various polyphenols.

BIOAVAILABILITY OF POLYPHENOLS

It is important to realize that the polyphenols that are the most common in the human diet are not necessarily the most active within the body, either because they have a lower intrinsic activity or because they are poorly absorbed from the intestine, highly metabolized, or rapidly eliminated. In addition, the metabolites that are found in blood and target organs and that result from digestive or hepatic activity may differ from the native substances in terms of biological activity. Extensive knowledge of the bioavailability of polyphenols is thus essential if their health effects are to be understood.

Metabolism of polyphenols occurs via a common pathway (83). The aglycones can be absorbed from the small intestine. However, most polyphenols are present in food in the form of esters, glycosides, or polymers that cannot be absorbed in their native form. These substances must be hydrolyzed by intestinal enzymes or by the colonic microflora before they can be absorbed. When the flora is involved, the efficiency of absorption is often reduced because the flora also degrades the aglycones that it releases and produces various simple aromatic acids in the process. During the course of absorption, polyphenols are conjugated in the small intestine and later in the liver. This process mainly includes methylation, sulfation, and glucuronidation. This is a metabolic detoxication process common to many xenobiotics that restricts their potential toxic effects and facilitates their biliary and urinary elimination by increasing their hydrophilicity. The conjugation mechanisms are highly efficient, and aglycones are generally either absent in blood or present in low concentrations after consumption of nutritional doses. Circulating polyphenols are conjugated derivatives that are extensively bound to albumin. Polyphenols are able to penetrate tissues, particularly those in which they are metabolized, but their ability to accumulate within specific target tissues needs to be further investigated. Polyphenols and their derivatives are eliminated chiefly in urine and bile. Polyphenols are secreted via the biliary route into the duodenum, where they are subjected to the action of bacterial enzymes, especially β-glucuronidase, in the distal segments of the intestine, after which they may be reabsorbed. This enterohepatic recycling may lead to a longer presence of polyphenols within the body.

Intestinal absorption and metabolism

Much about the intestinal mechanisms of the gastrointestinal absorption of polyphenols remains unknown. Most polyphenols are probably too hydrophilic to penetrate the gut wall by passive diffusion, but the membrane carriers that could be involved in polyphenol absorption have not been identified. To date, the unique active transport mechanism that has been described is a Na^+-dependent saturable transport mechanism involved in cinnamic and ferulic acid absorption in the rat jejunum (84).

In foods, all flavonoids except flavanols are found in glycosylated forms, and glycosylation influences absorption. The fate of glycosides in the stomach is not clear. Experiments using surgically treated rats in which absorption was restricted to the stomach showed that absorption at the gastric level is possible for some flavonoids, such as quercetin and daidzein, but not for their glycosides (85, 86). Most of the glycosides probably resist acid hydrolysis in the stomach and thus arrive intact in the duodenum (87). Only aglycones and some glucosides can be absorbed in the small intestine, whereas polyphenols linked to a rhamnose moiety must reach the colon and be hydrolyzed by rhamnosidases of the microflora before absorption (88, 89). The same probably applies to polyphenols linked to arabinose or xylose, although this question has not been specifically studied. Because absorption occurs less readily in the colon than in the small intestine because of a smaller exchange area and a lower density of transport systems, as a general rule, glycosides with rhamnose are absorbed less rapidly and less efficiently than are aglycones and glucosides. This has been clearly shown in humans for quercetin glycosides: maximum absorption occurs 0.5–0.7 h after ingestion of quercetin 4'-glucoside and 6–9 h after ingestion of the same quantity of rutin (quercetin-3β-rutinoside). The bioavailability of rutin is only 15–20% of that of quercetin 4'-glucoside (90, 91). Similarly, absorption of quercetin is more rapid and efficient after ingestion of onions, which are rich in glucosides, than after ingestion of apples containing both glucosides and various other glycosides (92). In the case of quercetin glucosides, absorption occurs in the small intestine, and the efficiency of absorption is higher than that for the aglycone itself (93, 94). The underlying mechanism by which glucosylation facilitates quercetin absorption has been partly elucidated. Hollman et al suggested that glucosides could be transported into enterocytes by the sodium-dependent glucose transporter SGLT1 (93). They could then be hydrolyzed inside the cells by a cytosolic β-glucosidase (95). Another pathway involves the lactase phloridzine hydrolase, a glucosidase of the brush border membrane of the small intestine that catalyzes extracellular hydrolysis of some glucosides, which is followed by diffusion of the aglycone across the brush border (96). Both enzymes are probably involved, but their relative contribution for the various glucosides remains to be clarified. Quercetin 3-glucoside, which is not a substrate for cytosolic β-glucosidase, is certainly absorbed after hydrolysis by lactase phloridzine hydrolase, at least in rats, whereas hydrolysis of quercetin 4'-glucoside seems to involve both pathways (97, 98). In humans, whatever the mechanism of deglucosylation, the kinetics of plasma concentrations are similar after ingestion of quercetin 3-glucoside or quercetin 4'-glucoside (99). Isoflavone glycosides present in soya products can also be deglycosylated by β-glucosidases from the human small intestine (95, 96). However, the effect of glucosylation on absorption is less clear for isoflavones than for quercetin. Aglycones present in fermented soya products were shown to be better absorbed than were the glucosides ingested from soybeans (100). However, a dose or matrix effect may explain the difference in absorption observed in this first study. Setchell et al (101) showed that when pure daidzein, genistein, or their corresponding 7-glucosides were administered orally to healthy volunteers, a tendency toward greater bioavailability was observed with the glucosides, as measured from the area under the curve of the plasma concentrations: 2.94, 4.54, 4.52, and 4.95 μg $\cdot$ h/mL for daidzein, genistein, daidzin, and genistin, respectively. However, in another human study, peak plasma concentrations were markedly higher after aglycone ingestion than after glucoside ingestion, and this effect was observed with low or high single doses and after long-term intakes (102). In addition, hydrolysis of isoflavone glycosides into aglycones in a soy drink did not change the bioavailability of the isoflavones in humans (103). No data are available for other polyphenols in humans, but note that in rats, no enhancement of

absorption was observed with glucosylation of naringenin and phlorizin (104, 105). Furthermore, diglucosylation of the lignan secoisolariciresinol decreases its absorption (106).

Glycosylation does not influence the nature of the circulating metabolites. Intact glycosides of quercetin, daidzein, and genistein were not recovered in plasma or urine after ingestion as pure compounds or from complex food (107–110). For flavanones, only trace amounts of glycosides have been detected in human urine, corresponding to 0.02% of the administered dose of naringin (111). But a very high dose (500 mg) of the pure compound was administered in this study, and some metabolic processes may have been saturated by this nonnutritional intake. Anthocyanins constitute an exception, because intact glycosides are the major circulating forms. The explanation for this may lie in the instability of these molecules in the aglycone form or in a specific mechanism of absorption or metabolism for anthocyanins. Passamonti et al (112) have proposed that glycosides of anthocyanins may be transported by bilitranslocase at the gastric level, bacause they have been shown to be good ligands for this carrier. They could also be directly converted into glucuronides by a UDP glucose dehydrogenase as suggested by Wu et al (113).

Proanthocyanidins differ from most other plant polyphenols because of their polymeric nature and high molecular weight. This particular feature should limit their absorption through the gut barrier, and oligomers larger than trimers are unlikely to be absorbed in the small intestine in their native forms. In vitro experiments using single layers of Caco-2 cells as a model of absorption in the small intestine showed that only the dimers and trimers of flavanols are able to cross the intestinal epithelium (114). Procyanidin B2 is very poorly absorbed in rats, whereas procyanidin B3 is not absorbed (115, 116). The possibility that procyanidin oligomers are hydrolyzed to mixtures of flavanol monomers and dimers in acidic conditions was suggested by Spencer et al from in vitro experiments (117). However, purified procyanidin dimer B3, as well as grapeseed proanthocyanidins having a higher degree of polymerization, are not degraded to more readily absorbable monomers in rats (116). The stability of proanthocyanidins was investigated in humans by regular analysis of gastric juice sampled with a gastric probe after ingestion of a proanthocyanidin-rich cocoa beverage (118). This study confirmed that proanthocyanidins are not degraded in the acidic conditions of the stomach in vivo. A minor absorption of some procyanidin dimers seems possible in humans. The procyanidin dimer B2 was detected in the plasma of volunteers after ingestion of a cocoa beverage; however, the maximal plasma concentration that was reached 2 h after ingestion was much lower than that reached after a roughly equivalent intake of epicatechin (0.04 compared with 6.0 μmol/L) (119). Proanthocyanidins, which are among the most abundant dietary polyphenols, are very poorly absorbed and may exert only local activity in the gastrointestinal tract or activity mediated by phenolic acids produced through microbial degradation. Their local action may nevertheless be important because the intestine is particularly exposed to oxidizing agents and may be affected by inflammation and numerous diseases such as cancer (120). Polyphenol concentrations in the colon can reach several hundred micromoles per liter (83), and together with a few carotenoids, polyphenols constitute the only dietary antioxidants present in the colon, because vitamins C and E are absorbed in the upper segments of the intestine.

Despite the scarcity of studies performed on the bioavailability of hydroxycinnamic acids, when ingested in the free form, these compounds are rapidly absorbed from the small intestine and are conjugated and, in particular, glucuronidated in the same way that flavonoids are (54, 121). However these compounds are naturally esterified in plant products, and this impairs their absorption. Human tissues (intestinal mucosa, liver) and biological fluids (plasma, gastric juice, duodenal fluid) do not possess esterases capable of hydrolyzing chlorogenic acid to release caffeic acid (122–124). This has also been observed in rats (125, 126). Only the colonic microflora would be capable of carrying out this hydrolysis, and some of the bacterial strains involved have been identified (127). Consequently, as observed for flavonoid glycosides that must be hydrolyzed by the microflora, the efficiency of absorption of phenolic acids is markedly reduced when they are present in the esterified form rather than in the free form (123, 125, 128). In patients who have undergone colonic ablation, caffeic acid was much better absorbed than was chlorogenic acid: 11% and 0.3% of the ingested doses were excreted in urine, respectively (123). Similarly, when chlorogenic acid was given by gavage to rats, no intact compound was detected in plasma in the following 6 h, and the maximum concentrations of metabolites obtained after administration of caffeic acid in the same conditions were 100-fold those of the metabolites (various glucuronidated or sulfated derivatives of caffeic and ferulic acids) obtained after chlorogenic acid administration (125). Surprisingly, the plasma concentrations were maximal only 30 min after gavage, which may seem inconsistent with hydrolysis of chlorogenic acid in the cecum. The same observation was made in a human study. When volunteers ingested coffee containing high amounts of esterified phenolic acids but no free caffeic acid, the peak plasma concentration of caffeic acid was observed only 1 h after ingestion of the coffee (129). In this study, the alkaline hydrolysis of coffee showed that chlorogenic acid represented only 30% of the bound caffeic acid. Thus, a possible explanation is that other forms of caffeic acid present in coffee may have been hydrolyzed in the upper part of the gut. Furthermore, the modes of administration used in both studies, ie, direct stomach intubation in the rat study and ingestion of coffee alone by fasted volunteers in the second study, might allow a rapid transit to the colon and explain the rapid kinetics of appearance of plasma metabolites. However, these 2 studies raise doubt about the total inability of the tissues to hydrolyze esterified phenolic acids.

In addition to being esterified to simple acids, hydroxycinnamic acids may be bound to polysaccharides in plant cell walls; the main example of this is esterification of ferulic acid to arabinoxylans in the outer husks of cereals. Although free ferulic acid is reported to be rapidly and efficiently absorbed (up to 25%) from tomatoes in humans (130), its absorption after ingestion of cereals is expected to be much lower because of this esterification. Ferulic acid metabolites recovered in the urine of rats represent only 3% of the ingested dose when ferulic acid is provided as wheat bran, whereas the metabolites represent 50% of the dose when ferulic acid is provided as a pure compound (131). Another study showed that feruloyl esterases are present throughout the entire gastrointestinal tract, particularly in the intestinal mucosa, and that some of the ester bonds between ferulic acid and polysaccharides in cell walls may thus be hydrolyzed in the small intestine (126). However, the role of feruloyl esterases seems to be very limited, and absorption occurs mainly in the colon after hydrolysis by enzymes of bacterial origin. Xylanases degrade the

parietal polymers to small, soluble feruloyl oligosaccharides, and then esterases release free ferulic acid. Note that diferulic acids from cereal brans have been shown to be absorbed in rats (132).

The effects of the food matrix on the bioavailability of polyphenols have not been examined in much detail. Direct interactions between polyphenols and some components of food, such as binding to proteins and polysaccharides, can occur, and these interactions may affect absorption. Furthermore, more indirect effects of the diet on various parameters of gut physiology (pH, intestinal fermentations, biliary excretion, transit time, etc) may have consequences on the absorption of polyphenols. Enzymes and carriers involved in polyphenol absorption and metabolism may also be induced or inhibited by the presence of some micronutrients or xenobiotics. Interactions with milk proteins were considered first because Serafini et al (133) reported that addition of milk to black tea abolished the increase in antioxidant potential that was observed when tea was consumed without milk. However, subsequent studies showed that addition of milk to black or green tea had no effect on the bioavailability of catechins, quercetin, or kaempferol in humans (134, 135). Some investigators have speculated that the presence of alcohol in red wine could improve the intestinal absorption of polyphenols by increasing their solubility. Ethanol was shown to enhance the absorption of quercetin in rats, but only when present at a concentration too high to be attained in the diet (>30%, by vol) (136). In humans, plasma concentrations of catechin metabolites were similar after consumption of red wine or dealcoholized red wine (137). Yet, 20% more catechin metabolites were excreted in urine after red wine intake than after dealcoholized red wine intake, which indicates a possible role of ethanol in enhancing the rate of polyphenol elimination, perhaps by a diuretic effect (138). On the other hand, tartaric acid, which is a major organic acid in wine, was shown to enhance the absorption of catechin in rats (139).

Existing data do not suggest a marked effect of the various diet components on polyphenol bioavailability. The absorption of quercetin, catechin, and resveratrol in humans was recently shown to be broadly equivalent when these polyphenols were administered in 3 different matrices: white wine, grape juice, and vegetable juice (140). According to Hendrich (141), neither the background diet or type of soy food nor the presence of 40 g wheat fiber significantly alters the apparent absorption of isoflavones. However, more studies are needed, especially on dietary fiber. Dietary fiber is generally associated with polyphenols in plant foods and stimulates intestinal fermentation, which could influence the production of particular microbial metabolites. Administration of polyphenols without a food matrix could markedly affect their bioavailability. With regard to flavonols, much higher plasma concentrations were achieved when quercetin glucosides were administered to fasted volunteers in the form of a water-alcohol solution (up to 5 μmol/L) (99) than when an equivalent quantity was ingested with foods such as onions, apples, or a complex meal (0.3–0.75 nmol/L) (92, 107). This suggests that the consumption of any food may limit polyphenol absorption and that high plasma concentrations would be obtained only if supplements were taken separately from meals.

The role of the colonic microflora

Polyphenols that are not absorbed in the small intestine reach the colon. The microflora hydrolyzes glycosides into aglycones and extensively metabolizes the aglycones into various aromatic acids (8, 142). Aglycones are split by the opening of the heterocycle at different points depending on their chemical structure: flavonols mainly produce hydroxyphenylacetic acids, flavones and flavanones mainly produce hydroxyphenylpropionic acids, and flavanols mainly produce phenylvalerolactones and hydroxyphenylpropionic acids. These acids are further metabolized to derivatives of benzoic acid. The microbial metabolites are absorbed and conjugated with glycine, glucuronic acid, or sulfate. The cleavage and metabolic pathways are well established in animals, and the influence of chemical structure on degradation is known. For example, the absence of a free hydroxyl in position 5, 7, or 4' protects the compound from cleavage (143). However, data are still limited in humans, so it is possible that new microbial metabolites will be identified. Interindividual variations and the influence of the microflora composition and of the usual diet on microbial metabolite production have to be evaluated. Recent studies have shown that plasma concentrations and urinary excretion of microbial metabolites in humans can be higher than those of tissular metabolites, especially for polyphenols such as wine polyphenols that are not easily absorbed (128, 144, 145). Thus, the identification and quantification of microbial metabolites constitute an important field of research. Some microbial metabolites may have a physiologic effect; for example, hydroxyphenylacetic acids have been suggested to inhibit platelet aggregation (146). Besides, among the wide array of aromatic acids with low molecular weight, some may be used as biomarkers for polyphenol intake. An association between polyphenol intake and the amount of excreted hippuric acid was found after consumption of black tea or a crude extract from *Equisenum arvense* (147, 148). However, hippuric acid is not a degradation product of catechin and can be derived from sources other than polyphenols, such as quinic acid and the aromatic amino acids; thus, it is not a suitable biomarker of polyphenol intake (144). 3-Hydroxyhippuric acid may be a more valid biomarker (124).

Specific active metabolites are produced by the colonic microflora. This is the case with lignans from linseed, which are metabolized to enterolactone and enterodiol, which have agonistic or antagonistic effects on estrogens (149, 150). Similarly, equol produced from soya daidzein appears to have phytoestrogenic properties equivalent to or even greater than those of the original isoflavone (151, 152). There is a great interindividual variability in the capacity to produce equol. Only 30-40% of the occidental people excrete significant quantities of equol after consumption of isoflavones, and these persons are called “equol producers” (152, 153). The corresponding percentage among Asian populations is unknown, but a recent study suggested that the percentage in Japanese men could be as high as 60% (154). The ability or inability of persons to produce equol seems to remain the same for at least several years (152, 155). The composition of the intestinal flora plays a major role. Inoculation of germ-free rats with human flora from equol producers confers on these rats the capacity to produce this metabolite, whereas colonization with flora from non–equol producers leaves the rats incapable of producing equol (156). Equol is not recovered in plasma from infants who are fed soy-based formulas, which suggests that the bacteria responsible for its production are not developed in the first months of life (157). Three strains of bacteria are reportedly able to convert pure daidzein to equol in vitro: *Streptococcus intermedius* ssp., *Ruminococcus productus* spp., and *Bacteroides ovatus* spp. (158). The possibility of con-

verting nonproducers to producers by food must be investigated. Equol producers tend to consume less fat and more carbohydrates as percentages of energy than do non–equol producers (159, 160). Consumption of dietary fiber has been suspected to affect equol production by favoring the growth of certain bacterial species. However, supplementation with 16 g wheat bran/d did not increase equol production in young women (159). In mice, equol production increased with the addition of fructooligosaccharides in the diet (161). But this result needs to be confirmed in humans because of obvious interspecies differences, which are shown by the fact that rats are constitutive equol producers. The effect of adaptation of the intestinal flora to the consumption of isoflavones is not clear. Lu et al (162) observed an increase in equol production after 1 mo of isoflavone consumption. Some non-equol-producing women even acquired the ability to produce equol after consuming soymilk for 2 wk (153). But Lampe et al (163) did not observe any effect on equol production of a 1-mo adaptation in comparison with a 4-d supplementation. A more comprehensive knowledge of the factors that may influence equol production is all the more essential because Setchell et al (152) convincingly proposed that equol producers might gain more benefits from soya consumption than would nonproducers.

Conjugation and nature of metabolites

Once absorbed, polyphenols are subjected to 3 main types of conjugation: methylation, sulfation, and glucuronidation. Catechol-*O*-methyl transferase catalyzes the transfer of a methyl group from *S*-adenosyl-L-methionine to polyphenols having an *o*-diphenolic (catechol) moiety. Such a reaction is well known for quercetin, catechin, caffeic acid, and luteolin, and Wu et al (113) recently showed for the first time the methylation of cyanidin to peonidin in humans. The methylation generally occurs predominantly in the 3' position of the polyphenol, but a minor proportion of 4'-*O*-methylated product is also formed. Note that a substantial amount of 4'-methylepigallocatechin was detected in human plasma after ingestion of tea (164, 165). Catechol-*O*-methyl transferase is present in a wide range of tissues. Its activity is highest in the liver and the kidneys (166, 167) although significant methylation was reported for catechin in the small intestine of rats (168). Sulfotransferases catalyze the transfer of a sulfate moiety from 3'-phosphoadenosine-5'-phosphosulfate to a hydroxyl group on various substrates (steroids, bile acids, polyphenols, etc). Neither the isoforms that are specifically involved in the conjugation of polyphenols nor the positions of sulfation for the various polyphenols have yet been clearly identified, but sulfation clearly occurs mainly in the liver (166, 169). UDP-glucuronosyltransferases are membrane-bound enzymes that are located in the endoplasmic reticulum in many tissues and that catalyze the transfer of a glucuronic acid from UDP-glucuronic acid to steroids, bile acids, polyphenols, and thousands of dietary constituents and xenobiotics. The presence of glucuronidated metabolites in the mesenteric or portal blood after perfusion of polyphenols in the small intestine of rats shows that glucuronidation of polyphenols first occurs in the enterocytes before further conjugation in the liver (170–172). This is probably the case in humans as well, because in humans the in vitro glucuronidation of quercetin and luteolin by microsomes from the intestine is markedly higher than that by microsomes from the liver (173). About 15 isoforms of UDP-glucuronosyltransferases have been identified in humans, and these isoforms have broad and overlapping substrate specificities and different tissue distribution (174). The subfamily of UDP-glucuronosyltransferases called UGT1A that is localized in the intestine probably plays a major role in the first-pass metabolism of polyphenols. These isoenzymes have a wide polymorphic expression pattern that could result in a high interindividual variability in polyphenol glucuronidation. The active isoenzymes of the 1A class seem to differ according to the polyphenol considered (173, 175). In vitro glucuronidation of quercetin, luteolin, or isorhamnetin by rat or human microsomes in the intestine and the liver showed that, even if the nature of the glucuronides formed is constant, the proportion of the various metabolites varies widely depending on the species and organ (173, 176, 177). The highest rate of conjugation is observed at the 7-position, and the 5-position does not appear to be a site for glucuronidation. For most flavonoids, a significant proportion of the glucuronides that are formed in the intestinal mucosa are secreted back to the gut lumen, which reduces net absorption (178, 179). The transporter multiresistant protein 2 (MRP2) or the *P*-glycoprotein may be involved in this efflux (180, 181). The proportion of glucuronides that are secreted toward the mucosal side depends markedly on the structure of the polyphenol (0–52% of the initial dose) (182). Intestinal excretion of glucuronides does not occur with catechin and ferulic acid, which indicates that this is not a mechanism of elimination for all polyphenols (131, 168, 182).

The metabolic fate in the liver of the conjugates that are produced in the intestine is not yet clear. After penetration into HepG2 cells, quercetin 7-glucuronide and quercetin 3-*O*-glucuronide undergo 2 types of metabolism: methylation of the catechol and deglucuronidation followed by 3'-sulfation (183). However, in the same conditions, quercetin 4'-glucuronide is not metabolized. This could result from a lower rate of penetration into the cells or a lower affinity of the metabolizing enzymes for this substrate. A complex set of conjugating enzymes and carrier systems is probably involved in the regulation of uptake and the production and release of the various polyphenol metabolites by the hepatocytes, as shown for other conjugates (184, 185). The activity of these enzymes and carrier systems may depend on the nature of the polyphenol and may be influenced by genetic polymorphisms that lead to important interindividual differences in the capacity to metabolize polyphenols.

The relative importance of the 3 types of conjugation (methylation, sulfation, and glucuronidation) appears to vary according to the nature of the substrate and the dose ingested. Sulfation is generally a higher-affinity, lower-capacity pathway than is glucuronidation, so that when the ingested dose increases, a shift from sulfation toward glucuronidation occurs (186). The balance between sulfation and glucuronidation of polyphenols also seems to be affected by species, sex, and food deprivation (187). Moreover, inhibition of methylation by a specific inhibitor shifts metabolism of quercetin glucuronides toward sulfation in HepG2 cells (183). Regardless of the respective contributions of methylation, sulfation, and glucuronidation, in general, the capacity for conjugation is high. The concentration of free aglycone is usually very low in plasma after the intake of a nutritional dose, except for tea catechins (up to 77% for epigallocatechin gallate) (164). Saturation of the conjugation processes has been observed in rats administered high doses and rats given an acute supply of polyphenols by gavage (166, 170). Competitive inhibition of conjugation could also occur in the presence of various polyphenols and xenobiotics in the intestine, but it has never been studied.

In these conditions, significant amounts of free aglycones could circulate in blood, probably with biological effects different from those of conjugated metabolites.

Identification of circulating metabolites has been undertaken for only a few polyphenols. This identification must include not only the nature and number of the conjugating groups but also the positions of these groups on the polyphenol structure because these positions can affect the biological properties of the conjugates (176). After consumption of onions containing glucosides of quercetin, the major circulating compounds in human plasma were identified as quercetin 3-*O*-glucuronide, 3'-*O*-methylquercetin 3-*O*-glucuronide, and quercetin 3'-*O*-sulfate (188). However, analysis by liquid chromatography–tandem mass spectrometry of human plasma samples obtained in very similar conditions did not confirm the presence of sulfated quercetin (109). For other polyphenols, only scarce data on the proportions of the various types of conjugates and the percentages of free forms in plasma are available (101, 164, 175, 189–192). The main circulating compounds are generally glucuronides.

Plasma transport and partitioning into lipid structures

Polyphenol metabolites are not free in the blood. In vitro incubation of quercetin in normal human plasma showed that quercetin is extensively bound to plasma proteins (99% for concentrations up to 15 μmol/L), whereas binding to VLDL is not significant ($<$0.5%) (193). Metabolites of quercetin are also extensively bound to plasma proteins in the plasma of rats fed a quercetin-enriched diet (88). Albumin is the primary protein responsible for the binding. The affinity of polyphenols for albumin varies according to their chemical structure. Kaempferol and isorhamnetin, which differ from quercetin in the nature of the B-ring substitution, have an affinity for human serum albumin that is similar to that of quercetin (194). In contrast, substitution of 3-OH markedly weakens binding to albumin, as shown for rutin and isoquercitrin, the 3-*O*-glycosides of quercetin (195). The effect of sulfation and glucuronidation is unknown, but it probably depends highly on the position of substitution. Hydroxycinnamic acids, especially ferulic and coumaric acids, have a low affinity for bovine serum albumin but may have a different affinity for albumin of human origin (196). No data are available for the other polyphenols. It must be kept in mind that although the intrinsic affinity of circulating polyphenol conjugates for albumin may be much weaker than that of quercetin itself, the physiologic concentration of serum albumin ($\approx$0.6 mmol/L) is probably large enough to allow their extensive binding. The degree of binding to albumin may have consequences for the rate of clearance of metabolites and for their delivery to cells and tissues. The conventional view is that cellular uptake is proportional to the unbound concentration of metabolites. Yet, variations in local pH at specific sites may induce conformational changes in albumin, which lead to dissociation of the ligand-albumin complex. Conformational changes in albumin have been shown to be induced by nonspecific interactions with various membranes (197). Whether such changes could facilitate the cellular uptake of ligands such as polyphenol metabolites is unclear. However, incubations of quercetin in human whole blood or in suspensions of erythrocytes in the absence of plasma proteins suggests that binding to albumin could considerably decrease the association of quercetin with these cells (193). The effect of albumin binding on the biological activity of polyphenols is unclear. Does the bound ligand have some biological activity, or does the polyphenol have to be in the free form to be active? Dangles et al (195) showed that the catechol moiety of albumin-bound quercetin remains accessible to oxidizing agents such as periodate. If this key structural element of quercetin is also accessible to free radicals, this suggests that quercetin could exert its antioxidant activity even when it is bound to albumin. However, the biological properties of polyphenols are certainly not limited only to their antioxidant capacity; thus, binding to albumin may have a considerable effect.

The partitioning of polyphenols and their metabolites between aqueous and lipid phases is largely in favor of the aqueous phase because of their hydrophilicity and binding to albumin. However, in some lipophilic membrane models, some polyphenols penetrate the membrane to various extents (198–202). Quercetin showed the deepest interaction, probably because of its ability to assume a planar conformation (203). At physiologic pH, most polyphenols interact with the polar head groups of phospholipids at the membrane surface via the formation of hydrogen bonds that involve the hydroxyl groups of the polyphenols (204). A high number of hydroxyl groups on the polyphenol structure and an increase in pH that leads to deprotonation of the hydroxyl groups would thus enhance interactions between the polyphenols and the membrane surface. This adsorption of polyphenols probably limits the access of aqueous oxidants to the membrane surface and their initial attack on that surface.

LDL is made up of lipophilic structures that, once oxidized, participate in the etiology of atherosclerosis. Many studies have shown that various polyphenols have the ability to protect LDL from oxidation. However, a very small proportion of plasma polyphenols are in fact associated with the LDL fraction after consumption of nutritional doses of these compounds (205, 206). They are associated with lipoproteins only by ionic interactions with charged residues on the surface of the particules. The low integration of polyphenols into LDL has been confirmed by in vitro incubation experiments (207, 208). Protection probably occurs at the interface between lipophilic and hydrophilic phases. However, a recent study in which [^{3}H]genistein was incubated in human plasma showed that genistein and its lipophilic derivatives were incorporated into HDL and, to a lesser extent, into LDL (209). These lipophilic derivatives could result from enzymatic esterification with fatty acids in plasma, as reported for estrogens, but further characterization of these derivatives is needed.

Plasma concentrations

Plasma concentrations reached after polyphenol consumption vary highly according to the nature of the polyphenol and the food source. They are on the order of 0.3–0.75 μmol/L after consumption of 80–100 mg quercetin equivalent administered in the form of apples, onions, or meals rich in plant products (90, 92, 107). When ingested in the form of green tea (0.1–0.7 μmol/L for an intake of 90–150 mg), cocoa (0.25–0.7 μmol/L for an intake of 70–165 mg) (210–213), or red wine (0.09 μmol/L for an intake of 35 mg) (137), catechin and epicatechin are as effectively absorbed as is quercetin. The maximum concentrations of hesperetin metabolites determined in plasma 5–7 h after consumption of orange juice were 1.3–2.2 μmol/L for an intake of 130–220 mg (189, 214). Naringenin in grapefruit juice appears to be absorbed even better: a peak plasma concentration of 6 μmol/L is obtained after ingestion of 200 mg. In contrast,

plasma concentrations of anthocyanins are very low: peak concentrations, which occur between 30 min and 2 h after consumption, are on the order of a few tens of nanomoles per liter for an intake of ≈110–200 mg anthocyanins (215–217). Similarly, the intake of ≈25 mg secoisolariciresinol diglucoside in the form of linseed produces only a slight increase (≈30 nmol/L) in plasma lignan concentrations, and this increase occurs gradually between 9 and 24 h (218). Isoflavones are certainly the best absorbed flavonoids: plasma concentrations of 1.4–4 μmol/L are obtained between 6 and 8 h in adults who consume relatively low quantities of soya derivatives supplying ≈50 mg isoflavones (219–221). This raises the question of the harmlessness of soymilk, which is consumed in large quantities by infants who are allergic to cow milk. In 4-mo-old infants, isoflavone intake can reach 50 mg/d, which, when expressed relative to body weight, is 5–10-fold the dose shown to exert a physiologic effect on the hormonal regulation of women's menstrual cycles (222). Plasma concentrations of genistein and daidzein in these infants can reach several micromoles per liter (223).

Tissue uptake

Determination of the actual bioavailability of polyphenol metabolites in tissues may be much more important than is knowledge of their plasma concentrations. Data are still very scarce, even in animals.

When single doses of radiolabeled polyphenols (quercetin, epigallocatechin gallate, quercetin 4'-glucoside, resveratrol) are given to rats or mice killed 1–6 h later, radioactivity is mainly recovered in blood and in tissues of the digestive system, such as the stomach, intestine, and liver (224–227). However, polyphenols have been detected by HPLC analysis in a wide range of tissues in mice and rats, including brain (228, 229), endothelial cells (230), heart, kidney, spleen, pancreas, prostate, uterus, ovary, mammary gland, testes, bladder, bone, and skin (225, 231–233). The concentrations obtained in these tissues ranged from 30 to 3000 ng aglycone equivalents/g tissue depending on the dose administered and the tissue considered. The time of tissue sampling may be of great importance because we have no idea of the kinetics of penetration and elimination of polyphenols in the tissues.

It is still difficult to say whether some polyphenols accumulate in specific target organs. A few studies seem to indicate that some cells may readily incorporate polyphenols by specific mechanisms. The endothelium is likely to be one of the primary sites of flavonoid action. Schramm et al (234) showed that a rapid, energy-dependent transport system is active in aortic endothelial cells for the uptake of morin. This system may also transport other hydroxylated phenolic compounds. Microautoradiography of mice tissues after administration of radiolabeled epigallocatechin gallate or resveratrol indicated that radioactivity is unequally incorporated into the cells of organs (225, 227). Regional selectivity has also been observed in the prostate and the brain. After 9 wk of feeding isoflavone aglycones to rats, accumulation of isoflavones in the dorsolateral zone of the prostate was shown to be 4-fold that in the ventral zone of this organ (235). Similarly, after 28 d of oral administration of tangeretin, a polymethoxylated flavone from *Citrus*, to rats, tangeretin concentrations in the brain were 6-fold those in other tissues (heart, lung, liver, kidney), and distribution of tangeretin was unequal in the various zones of the brain: concentrations in the hypothalamus, hippocampus, and striatum were 10-fold those in the brainstem and cerebellum (228).

The nature of the tissular metabolites may be different from that of blood metabolites because of the specific uptake or elimination of some of the tissular metabolites or because of intracellular metabolism. Youdim et al (236) showed that the uptake of flavanone glucuronides by rat and mouse brain endothelial cultured cells is much lower than that of their corresponding aglycones. In rats fed a genistein-supplemented diet, the fraction of genistein present in the aglycone form was much more important in several tissues than in blood. It accounted for >50% of the total genistein metabolites in mammary gland (237), uterus, and ovary and 100% in the brain (231) and prostate (238), whereas it represented only 8% of the total plasma metabolites.

Only 2 studies reported data on polyphenol concentrations in human tissues. The first study measured phytoestrogens in human prostate tissue. Surprisingly, the study showed significantly lower prostatic concentrations of genistein in men with benign prostatic hyperplasia than in those with a normal prostate, whereas plasma genistein concentrations were higher in men with benign prostatic hyperplasia (239). In addition, concentrations of enterodiol and enterolactone were higher in prostatic tissue than in plasma, whereas the opposite was true for daidzein, genistein, and equol. In the other study, equol concentrations in women who ingested isoflavones were found to be higher in breast tissue than in serum, whereas genistein and daidzein were more concentrated in serum than in breast tissue. Note that very high equol concentrations have been obtained in breast tissue, and these concentrations are equivalent to 6 μmol/L for an intake of ≈110 mg of its precursor, daidzein (240). These initial studies show that plasma concentrations are not directly correlated with concentrations in target tissues and that the distribution between blood and tissues differs between the various polyphenols. This raises the question of whether plasma concentrations are accurate biomarkers of exposure.

Elimination

Metabolites of polyphenols may follow 2 pathways of excretion, ie, via the biliary or the urinary route. Large, extensively conjugated metabolites are more likely to be eliminated in the bile, whereas small conjugates such as monosulfates are preferentially excreted in urine. In laboratory animals, the relative magnitude of urinary and biliary excretion varies from one polyphenol to another (182). Biliary excretion seems to be a major pathway for the elimination of genistein, epigallocatechin gallate, and eriodictyol (170, 241). Biliary excretion of polyphenols in humans may differ greatly from that in rats because of the existence of the gall bladder in humans; however, this has never been examined. Intestinal bacteria possess β-glucuronidases that are able to release free aglycones from conjugated metabolites secreted in bile. Aglycones can be reabsorbed, which results in enterohepatic cycling. Pharmacokinetic studies in rats have shown a second maximum plasma concentration ≈7 h after genistein administration, which is consistent with enterohepatic circulation (233). A second plasma peak was also observed in some human volunteers 10–12 h after ingestion of hesperetin from orange juice or of isoflavones from soya (101, 189, 221).

Urinary excretion has often been determined in human studies. The total amount of metabolites excreted in urine is roughly correlated with maximum plasma concentrations. It is quite high

for flavanones from citrus fruit (4–30% of intake), especially for naringenin from grapefruit juice (111, 189, 214, 242, 243), and is even higher for isoflavones: the percentages excreted are 16–66% for daidzein and 10–24% for genistein (103, 192, 219, 221, 244). It may appear surprising that plasma concentrations of genistein are generally higher than those of daidzein despite the higher urinary excretion of daidzein, but this can be explained by the efficient biliary excretion of genistein. Urinary excretion percentages may be very low for other polyphenols, such as anthocyanins (0.005–0.1% of intake) (113, 216, 217, 245, 246), although Lapidot et al (247) reported elevated percentages of anthocyanin excretion (up to 5%) after red wine consumption. Low values could be indicative of pronounced biliary excretion or extensive metabolism. Certain metabolites of anthocyanins may still be unidentified as a result of analytic difficulties with these unstable compounds. Felgines et al (248) reported that the major metabolites of pelargonidin 3-glucoside that are recovered in human urine after strawberry intake are glucurono- and sulfoconjugates of pelargonidin that are extensively degraded by simple freezing of the urine samples. Urinary excretion of flavonols accounts for 0.3–1.4% of the ingested dose for quercetin and its glycosides (90, 92, 93) but reaches 3.6% when purified glucosides are given in hydroalcoholic solution to fasted volunteers (99). Urinary recovery is 0.5–6% for some tea catechins (210, 249), 2–10% for red wine catechin (138), and up to 30% for cocoa epicatechin (190). For caffeic and ferulic acids, relative urinary excretion ranges from 5.9% to 27% (124, 130, 250)

The exact half-lives of polyphenols in plasma have rarely been calculated with great precision but are on the order of 2 h for compounds such as anthocyanins (217) and 2–3 h for flavanols (17, 164, 251, 252), except for epigallocatechin gallate, which is eliminated more slowly probably because of higher biliary excretion or greater complexing with plasma proteins, as described for galloylated compounds (253, 254). The half-lives of isoflavones and quercetin are on the order of 4–8 (25, 244, 255) and 11–28 (90, 92) h, respectively. This suggests that maintenance of high plasma concentrations of flavonoid metabolites could be achieved with regular and frequent consumption of plant products. For instance, consumption of onions 3 times/d favors accumulation of quercetin in plasma (256). For compounds, such as tea catechins, with rapid absorption and a short half-life, repeated intakes must be very close together in time to obtain an accumulation of metabolites in plasma (257); otherwise, plasma concentrations regularly fluctuate after repeated ingestions, and no final accumulation occurs (205).

Biological effects of polyphenol metabolites

The biological activities of polyphenols have often been evaluated in vitro on pure enzymes, cultured cells, or isolated tissues by using polyphenol aglycones or some glycosides that are present in food. Very little is known about the biological properties of the conjugated derivatives present in plasma or tissues because of the lack of precise identification and commercial standards. However, reflection on the antioxidative activity of polyphenols suggests that the metabolism of polyphenols may have a considerable effect. For example, the hydrophobicity of polyphenols is intermediate between that of vitamin C (highly hydrophilic) and that of vitamin E (highly hydrophobic). Polyphenols are thus expected to act at water-lipid interfaces and may be involved in oxidation regeneration pathways with vitamins C and E. Glucuronidation and sulfation render polyphenols more hydrophilic and can affect their site of action and their interactions with other antioxidants. Furthermore, their intrinsic reductive capacity may be changed. The antioxidant effect of conjugated derivatives of quercetin on copper ion–induced LDL oxidation in vitro is about one-half that of the aglycone and is dependent on the binding site of the glucuronic acid (107, 176, 258). Cren-Olive et al (259) also reported that the capacity of 3'-*O*-methylcatechin and 4'-*O*-methylcatechin to protect LDL from in vitro oxidation is lower than that of catechin. However, an increase in the antioxidant capacity of plasma was observed after the consumption of various polyphenol-rich foods, which indirectly shows that at least some of the polyphenol metabolites retain antioxidant activity (212, 260–264). Conjugation might enhance certain specific biological activities, as shown for some xenobiotics (265). Koga and Meydani (266) showed that plasma metabolites of catechin have an inhibitory effect on monocyte adhesion to interleukin 1β–stimulated human aortic endothelial cells, whereas catechin and metabolites of quercetin had no effect. In another in vitro study, quercetin 3-*O*-glucuronide prevented vascular smooth muscle cell hypertrophy by angiotensin II (267). However, conjugation seems instead to decrease the specific activities of polyphenols. The affinities for estrogenic receptors of the aglycones of daidzein and genistein are 10- and 40-fold, respectively, those of the respective glucuronides, but the glucuronides still show weak estrogenic activity at physiologic concentrations (268). Spencer et al (269) showed the inability of 5- and 7-*O*-glucuronides of epicatechin to protect fibroblasts and neuronal cells from oxidative stress in vitro, whereas epicatechin and methylepicatechin were protective. Nevertheless, it is still difficult to draw any conclusions from the few existing studies regarding the effects of the type and position of conjugation on the various potential activities of polyphenols.

Polyphenol metabolites could also exert biological activities after deconjugation at the cellular level. This possibility has been shown for sulfates and glucuronides of endogenous estrogens (270, 271). Quercetin glucuronides were hydrolyzed by cell-free extracts of human neutrophils, liver, and small intestine (272). However, the possibility of hydrolysis of flavonoid glucuronides inside cells has not been studied. We have seen above that the proportion of free aglycone in some tissues may be higher than that in blood, especially in the case of genistein in rats. This may be explained by specific uptake of the aglycone or intracellular deconjugation. This last hypothesis implies that anionic conjugates could be transported across plasma membranes via carrier systems, as shown for other glucuronides (184, 273). Furthermore, β-glucuronidase is located in the lumen of the endoplasmic reticulum in various organs and would also have to be reached by polyphenol glucuronides. Note that carrier-mediated bidirectional transport across the membrane of the endoplasmic reticulum in rat hepatocytes has been described for other glucuronides, such as estrogen glucuronides (274). Inside the endoplasmic reticulum, UDP-glucuronosyltransferases are present along with β-glucuronidase. The respective K_m values of these enzymes toward flavonoids and their glucuronides seem to be in favor of glucuronidation rather than deglucuronidation at physiologic pH values (176, 272). These results are not consistent enough to give a clear view of what occurs inside the cells, and additional studies are certainly needed. β-Glucuronidase is also present in the lysosomes of various cells, from which it can be released in some particular situations such as oxidative stress. Its activity increases in some physiopathologic states, such as inflammation,

cancer, and AIDS (275, 276). Luteolin 7-*O*-glucuronide is hydrolyzed into aglycone by lysosomal β-glucuronidase released from neutrophils after induction of inflammation and in the plasma of rats treated with lipopolysaccharide (277). In situ deconjugation of polyphenol metabolites might occur only in particular places, such as at inflammation sites or in tumors. Situations that decrease the pH would favor deglucuronidation because the activity of β-glucuronidase is optimal at pH 4–5 and is reduced 9-fold at neutral pH (275).

Polyphenols may also have an indirect effect on health because they are metabolized by the same pathways as various xenobiotics or endogenous hormones. Flavonoids such as quercetin and fisetin are better substrates for catechol-*O*-methyl transferase than are its endogenous substrates, catecholamines and catechol estrogens. Deregulation of the *O*-methylation metabolism of neurotransmitters and hormones in humans is an important risk factor for the development of some neurodegenerative diseases, cardiovascular diseases, and hormone-dependent cancers (278). Thus, if confirmed in humans, the potential competitive inhibition of the catechol-*O*-methyl transferase–catalyzed *O*-methylation of endogenous catecholamines and catechol estrogens by polyphenols with catechol groups may have a beneficial effect on these pathologies. Some polyphenols, such as quercetin and daidzein sulfoconjugates, are also efficient inhibitors of sulfotransferases (279–282) and thus may have an effect on the function of thyroid hormones, steroids, and catecholamines (283). Whether UDP-glucuronosyltransferase is induced or inhibited by polyphenols needs further investigation (284–286). Interactions with drug transporters should also be considered (287). Isoflavones were shown to interact with transporters such as P-glycoprotein and canalicular multispecific organic anion transporter (288). Furthermore, some flavonoids could act as cytochrome P450 inhibitors and enhance drug bioavailability. Increased concentrations of many drugs have been shown with coadministrated grapefruit juice, and the effect was attributed in part to inhibition of the intestinal cytochrome P450 isoform 3A4 by naringenin (289). These data suggest that polyphenols could affect the bioavailability of many carcinogens, other toxic chemicals, and therapeutic drugs by affecting the activities of various enzymes involved in their own metabolism.

CONCLUSION

The many analytic studies of polyphenols in foods that have been conducted to date provide a good indication of polyphenol distribution. Fruit and beverages such as tea, red wine, and coffee constitute the principal sources of polyphenols, but vegetables, leguminous plants, and cereals are also good sources. Polyphenol concentrations in foods vary according to numerous genetic, environmental, and technologic factors, some of which may be controlled to optimize the polyphenol content of foods. The main tasks ahead are identifying the plant varieties that are the richest in the polyphenols of interest, improving growing methods, and limiting losses during the course of industrial processing and domestic cooking.

The health effects of polyphenols depend on both their respective intakes and their bioavailability, which can vary greatly. Although very abundant in our diet, proanthocyanidins are either very poorly absorbed or not absorbed at all, and their action is thus restricted to the intestine. The same appears to be true for anthocyanins, unless some of their metabolites are not yet identified but are well absorbed. Intakes of monomeric flavonols, flavones, and flavanols are relatively low, and plasma concentrations rarely exceed 1 μmol/L because of limited absorption and rapid elimination. Flavanones and isoflavones are the flavonoids with the best bioavailability profiles, and plasma concentrations may reach 5 μmol/L. However, the distribution of these substances is restricted to citrus fruit and soya. Finally, hydroxycinnamic acids are found in a wide variety of foods, often at high concentrations, but esterification decreases their intestinal absorption. As a general rule, the metabolites of polyphenols are rapidly eliminated from plasma, which indicates that consumption of plant products on a daily basis is necessary to maintain high concentrations of metabolites in the blood.

Recent studies have greatly increased our knowledge of the plasma concentrations and urinary excretion of polyphenol metabolites in humans. However, values for these variables do not seem to be well correlated with concentrations measured in tissues. Available data, essentially those obtained from animal studies, indicate that some polyphenol metabolites may accumulate in certain target tissues rather than just equilibrate between blood and tissues. The metabolites present may differ between tissues and plasma, and the nature of these metabolites needs to be further elucidated. More animal studies are needed to investigate intracellular metabolism and the accumulation of polyphenol metabolites in specific organs. However, some important differences may exist between animals and humans in some metabolic processes, especially the conjugation process.

The notion of bioavailability integrates several variables, such as intestinal absorption, excretion of glucuronides toward the intestinal lumen, metabolism by the microflora, intestinal and hepatic metabolism, plasma kinetics, the nature of circulating metabolites, binding to albumin, cellular uptake, intracellular metabolism, accumulation in tissues, and biliary and urinary excretion. The difficulty lies in integrating all the information and relating the variables to health effects at the organ level. These tasks are made all the more difficult because the relative weight of each variable may depend on the polyphenol considered. Some polyphenols may be less efficiently absorbed than are others but nevertheless reach equivalent plasma concentrations because of lower secretion toward the intestinal lumen and lower metabolism and elimination.

Better knowledge of bioavailability is essential for investigating the health effects of polyphenols, whatever the approach used. The fact that aglycones are not important metabolites in blood because of extensive intestinal and hepatic conjugation has thus far been largely ignored, and many in vitro studies on the mechanisms of action of polyphenols continue to concentrate on aglycones or glycosides rather than on the identified metabolites, often at concentrations that cannot realistically be attained in the body. It is thus essential to confirm the effects observed with aglycones through studies using physiologic concentrations of the metabolites actually found in the body. In addition, the activities of microbial metabolites must be examined in further studies to determine active structures, available concentrations, and potential modulation of the capacity of the microflora to produce such metabolites. Clinical studies will be of great help in investigating the health effect of polyphenols, provided that markers of effects that are reliable and related to the prevention of diseases are available. Better knowledge of some variables of polyphenol bioavailability, such as the kinetics of absorption, accumulation, and elimination, will facilitate the design of such

studies. Besides, more precise data on the nature of the circulating metabolites and on metabolism by the microflora can now be used for interpretations. For example, taking into account whether subjects are equol producers or non–equol producers seems particularly judicious in evaluating the health effects of soya isoflavone consumption.

Research on polyphenol bioavailability must finally allow us to correlate polyphenol intakes with one or several accurate measures of bioavailability (such as concentrations of key bioactive metabolites in plasma and tissues) and with potential health effects in epidemiologic studies. Knowledge of these correlations must be attained despite the difficulties linked to the high diversity of polyphenols, their different bioavailabilities, and the high interindividual variability observed in some metabolic processes, especially those in which the microflora is involved.

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