Measuring exposure to the polyphenol metabolome in observational epidemiologic studies: current tools and applications and their limits[1–3]

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ABSTRACT

Much experimental evidence supports a protective role of dietary polyphenols against chronic diseases such as cardiovascular diseases, diabetes, and cancer. However, results from observational epidemiologic studies are still limited and are often inconsistent. This is largely explained by the difficulties encountered in the estimation of exposure to the polyphenol metabolome, which is composed of ~500 polyphenols distributed across a wide variety of foods and characterized by diverse biological properties. Exposure to the polyphenol metabolome in epidemiologic studies can be assessed by the use of detailed dietary questionnaires or the measurement of biomarkers of polyphenol intake. The questionnaire approach has been greatly facilitated by the use of new databases on polyphenol composition but is limited by bias as a result of self-reporting. The use of polyphenol biomarkers holds much promise for objective estimation of polyphenol exposure in future metabolome-wide association studies. These approaches are reviewed and their advantages and limitations discussed by using examples of epidemiologic studies on polyphenols and cancer. The current improvement in these techniques, along with greater emphasis on the intake of individual polyphenols rather than polyphenols considered collectively, will help unravel the role of these major food bioactive constituents in disease prevention. *Am J Clin Nutr* 2014;100:11–26.

INTRODUCTION

Polyphenols are plant secondary metabolites that are present in a diverse range of foods and beverages such as tea, coffee, wine, fruit, vegetables, whole-grain cereals, and cocoa. Their antioxidant properties have raised considerable interest and a large number of clinical, preclinical, and epidemiologic studies have suggested a possible role in the prevention of chronic diseases such as cardiovascular diseases, diabetes, cancers, osteoporosis, and neurodegenerative diseases (1–3). The strongest evidence of health-protective effects is for cardiovascular diseases (4–7). In contrast, epidemiologic evidence that polyphenol intake protects against cancer is still limited and inconsistent (8–11), although many experimental studies in animal and cell culture models and a few human interventions have shown that polyphenols may exert anticarcinogenic effects (12, 13). More epidemiologic studies are required to further explore associations between polyphenol intake and the risk of cancers and other diseases.

Large-scale observational epidemiologic studies investigating the relation between polyphenol intake and health rely on the accurate estimation of intake by participants, but measurement of intake is challenging because of the large number of compounds present in foods, their distribution across a wide range of foods, and the many factors that may affect their contents in foods such as plant variety, season of harvest, or food processing and cooking (14). The most common dietary assessment methods use food-frequency questionnaires (FFQs)[4], 24-h dietary recalls (24-HDRs), and food diaries (15). These methods rely on both the participants' ability to report their own food intake and the availability of reliable data on the polyphenol contents of foods, which often results in biases and measurement errors. More refined techniques for dietary assessment of polyphenol intake are therefore required. The use of innovative technologies and methodologies for the dietary assessment of polyphenol intake such as the collection of multiple 24-HDRs and food records and interactive computer- and camera-based technologies may facilitate this process (16, 17). Alternatively, biomarkers that reflect the intake of individual or groups of polyphenols may be measured. Recent developments in analytic techniques and in metabolomics should allow the measurement of large sets of polyphenols in blood or urine as indicators of exposure to the polyphenol metabolome, which is defined as the whole set of polyphenols or polyphenol metabolites present in foods or in human biospecimens (18).

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[4] Abbreviations used: FFQ, food-frequency questionnaire; ICC, intraclass correlation; LOD, limit of detection; 24-HDR, 24-hour dietary recall.

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The aim of this article is to review and critically evaluate the techniques available for measuring exposure to the polyphenol metabolome. The advantages and limits of methods based on dietary assessment and biomarkers are successively discussed. Results of observational epidemiologic studies on polyphenols and cancer obtained by either of the 2 approaches are compared. With >500 polyphenols known in foods, the data described in this article also suggest some promising approaches for characterizing the complex relations between exposure to highly diverse families of bioactive constituents in foods and the associated disease risk.

THE POLYPHENOL METABOLOME

Dietary polyphenols form a large family of >500 different molecules with highly diverse structures and are divided into 4 main classes: flavonoids, phenolic acids, lignans, and stilbenes (14). Flavonoids are themselves distributed into several subclasses: anthocyanidins, flavonols, flavanones, flavones, flavanols or flavan-3-ols (including monomers, proanthocyanidin oligomers and polymers and the oxidized theaflavins and thearubigins), isoflavones, chalcones, dihydrochalcones, and dihydroflavonols. To add to this diversity, polyphenols in foods are not usually found in the free (aglycone) form but are usually bound to sugars, in the case of flavonoids, or esterified to polyols, in the case of phenolic acids (19).

Food sources

Polyphenols can be found in all plants and foods of plant origin (19). Some, such as quercetin and (+)-catechin, occur in a wide range of foods, whereas others are characteristic of single foods, such as theaflavins in tea or phloretin in apples. Major differences in polyphenol profile can be found between members of the same botanical family. For example, both garlic and onions belong to *Alliaceae*, but only onions are a major dietary source of quercetin (14). Polyphenol profiles in individual foods also vary as a result of variety, geographical area, state of maturity at harvest, and food processing and cooking. A range of samples must therefore be analyzed to obtain representative polyphenol content values. Polyphenols present in one variety of a plant food may also be absent from another because of variation of the expression of some biosynthetic enzymes. For example, red onions contain anthocyanins that give them their typical color, whereas white onions do not (14).

Some dietary sources are particularly rich in polyphenols, such as spices, cocoa powder, berries, and nuts, whose polyphenol contents range from 200 to 15,000 mg/100 g (20). Other foods such as tea, red wine, coffee, some fruit and vegetables, legumes, and cereals, although less rich in polyphenols, are more widely consumed and still constitute major sources (20–22).

Bioavailability

Polyphenols are usually absorbed in the small intestine or in the colon. They are almost totally metabolized in the gut mucosa and the liver and conjugated to glucuronide, sulfate, and/or methyl groups. Polyphenols that reach the colon are extensively transformed by the microbiota; and their main products, phenolic acids, are themselves absorbed and found in the systemic circulation. Finally, these metabolites are largely excreted in urine and the bile, usually within 24–48 h (19, 23).

The chemical structures of polyphenols greatly influence gut absorption and metabolic fate in the body. The recovery in urine of intact polyphenols can be as low as <0.01% for anthocyanins or as high as 43% for some isoflavones (23). Glycosylation of flavonoids and esterification of phenolic acids are key factors affecting their absorption from the gut. The type of glycosylation also influences bioavailability. Glucosides of quercetin, as found in onions, are efficiently absorbed in the small intestine, whereas rhamnoglucosides of quercetin are poorly absorbed until they reach the colon where they are deglycosylated by the microbiota and finally absorbed as quercetin aglycone (23–25). Esterification also limits the bioavailability of phenolic acids when compared with their free form (26, 27). These few examples show that it is essential to take into account the fine chemical structures of polyphenols to understand their role in the prevention of diseases through epidemiologic studies.

MEASURING POLYPHENOL INTAKE THROUGH DIETARY ASSESSMENT

The most common method of estimating polyphenol intake in epidemiologic studies is to use dietary questionnaires, such as FFQs, 24-HDRs, and food diaries, to record all food consumption over a prescribed time period. Food-composition tables built from databases are then used to estimate intake per individual. The estimation of intake is complex, because many foods contribute to polyphenol intake; for example, 232 foods contributed to the intake of 337 polyphenols in the French SU.VI.MAX (Supplementation en Vitamines et Mineraux Antioxydants) cohort (21). It is therefore important to collect accurate data on the intake of all polyphenol-containing foods together with accurate content values for all polyphenols in these foods.

Databases on polyphenol content in foods and polyphenol intake measurement

Food-composition tables for polyphenols are built with the use of data from large polyphenol databases containing extensive food-composition data extracted from the scientific literature (28, 29). Analytic methods used to obtain these data vary, and the quality of the analyses must be carefully evaluated before data are accepted for inclusion in these databases and exploited to build food-composition tables. HPLC is sensitive and specific and is the method most commonly used to quantify individual polyphenols. A hydrolysis step is sometimes necessary to convert glycosides to their aglycones to simplify the analysis of complex extracts when standards for individual glycosides are not available. Alternatively, total polyphenols may be measured by using the Folin-Ciocalteu colorimetric assay, which provides crude estimates of polyphenol contents in foods. However, the assay is susceptible to interference by other nonphenolic constituents that may be present such as ascorbic acid, sugars, and other reducing agents (20) and different analytic methods should then be used. Where possible, content data for individual polyphenols should be preferred given their variable bioavailabilities and bioactivities.

The first database on polyphenol contents in foods was developed by the USDA in the early 2000s and has been periodically updated since. It provides data on the contents of 38 of the most important flavonoids in foods, expressed as aglycones (28, 30, 31). In 2009, Phenol-Explorer, a comprehensive Web database on the content in foods of all known polyphenols, was released. It contains data on 502 polyphenols from all classes (flavonoids, phenolic acids, lignans, and stilbenes) and thus

differs from the USDA database by its more comprehensive coverage of dietary polyphenols (29). The content in food of all known aglycones and their glycosides or esters are described. This detailed information on all individual polyphenol compounds is important because glycosylation and esterification strongly influence gut absorption and bioavailability of polyphenols (*see* previous subsections).

The USDA and Phenol-Explorer databases have recently been exploited in France and Finland to provide the most comprehensive data on polyphenol exposure. A mean total polyphenol intake of ~850 mg/d (polyphenols expressed as aglycone equivalents) was reported in both studies (21, 22). Phenolic acids accounted for the highest proportion of all dietary polyphenols (50–75% of total intake) followed by flavonoids (25–50%). Intake of polyphenols from other classes was very limited (<30 mg/d). Mean flavonoid intake was also compared in different European countries and ranged from 161 to 428 mg/d (expressed as aglycone equivalents) (32). Intake was most influenced by the consumption of coffee and tea, which were the most frequently consumed polyphenol-containing foodstuffs. Polyphenol intake was also shown to be associated with age, sex, socioeconomic status, and ethnicity, all factors known to affect food choices (32, 33).

Limitations of polyphenol intake estimations

Despite advances in dietary data collection techniques that have decreased the frequency of systematic and random measurement errors (15, 34), dietary questionnaires are subject to bias as a result of self-reporting. Participants may report perceived acceptable rather than actual food intakes or just report foods inaccurately. Data from FFQs only concern the foods most commonly consumed by the target population and may not be detailed enough to reliably estimate the intake of highly diverse polyphenol-containing foods. More precise polyphenol intake measurements are obtained when using 24-HDRs or food diaries, but only short-term intake is measured unless repeated measurements are carried out during the year to provide more long-term intake estimates as required in epidemiologic studies (16, 17).

Databases on polyphenol content in foods also have their limitations. Polyphenol content in a given food can vary widely according to plant species, time of year, year of harvest, and extent of processing. The exact nature of the foods and beverages consumed and their mode of preparation are not always fully documented in dietary records. Red and white wines differ greatly in their polyphenol content but their intake is often not distinguished in dietary records. Polyphenol concentrations in coffee are also quite different between an espresso and a cup of filtered coffee (35), and the brewing method used by individuals is often not available. In addition, the reliability of a particular polyphenol content value increases with the number of samples analyzed to produce a representative mean content value (29). Polyphenol content values may be missing for some foods, and missing data cannot be easily extrapolated because polyphenol profiles may vary considerably between similar foods. For example, citrus fruit are the main sources of flavanones, but each citrus fruit provides different profiles of flavanones. Oranges are rich in hesperetin and naringenin, grapefruit in naringenin, lemons in eriodictyol and hesperetin, and limes in hesperetin (28, 29). Furthermore, polyphenol contents change with processing, such as after cooking, storing, jam-making, canning, and freezing. For example, flavonoid losses of 30–75% have been reported after different culinary treatments (36, 37). New information on polyphenol retention factors after cooking and processing has been recently incorporated into the Phenol-Explorer database (38). This will further improve the coverage and accuracy of polyphenol food-composition data. Last, some polyphenols are commonly used as additives in food formulation (39). They can serve as natural or synthetic phenolic pigments (eg, elderberry and grape skin extracts rich in anthocyanins) or preservatives (eg, rosemary extracts rich in phenolic acids). Their contribution to polyphenol intake is not known.

Another limitation of polyphenol intake measurements is the lack of accurate data on the consumption of polyphenols from dietary supplements. Many herbal or plant extract supplements that are rich in polyphenols have been marketed worldwide for >20 y. They may contain much greater quantities of polyphenols than are possible to ingest naturally from foods. In general, dietary supplements have not been considered in polyphenol databases and food-composition tables, and few resources exist on the identity and composition of polyphenol supplements given the wide and unregulated product market (40–42). The estimation of the polyphenol content of dietary supplements is also problematic. Dietary supplements are regulated as foods (eg, by the European Food Safety Agency in Europe and the Food and Drug Administration in the United States), and polyphenol contents are often not indicated on the label. Polyphenol content in supplements varies widely, as has been shown for soy isoflavone products commonly consumed as alternatives to hormone replacement therapy, and the dose indicated on the supplement label was often found to be unreliable (43, 44). Overall, this lack of data may result in the underestimation of intake for some specific phenolic compounds or among particular populations such as those taking polyphenol-rich supplements.

MEASURING POLYPHENOL EXPOSURE THROUGH BIOMARKERS

Polyphenol biomarkers could replace or complement traditional dietary assessment as a means of reducing self-reporting inaccuracies and improve the reliability of exposure measurements (45). Biomarkers in the field of nutrition can be defined as any compound measurable in biological specimens that is an indicator of intake, exposure, or status of some food or nutrient (46). Being independent of the errors associated with dietary questionnaires, their use provides more objective estimates of exposure that can be used to validate measurements of dietary intake. Unfortunately, such a validation has rarely been performed for polyphenols. This may raise doubts about the reliability of some epidemiologic data on polyphenols and diseases. Biomarkers may be particularly useful when reliable food content values are missing (eg, no difference is generally made in questionnaires between different types of coffee varying widely in their dilution) or when a polyphenol compound is distributed in a large diversity of foods (eg, quercetin present in tea, onions, and various other fruit and vegetables), making the evaluation of its intake particularly difficult and prone to errors.

Most of the epidemiologic studies that have used polyphenol biomarkers concern flavonols, isoflavones, and lignans, particularly for cancer studies as presented in **Table 1**. These 3 classes of polyphenols account for only a minor fraction of all

TABLE 1
Epidemiologic studies on the association between polyphenol biomarkers and cancer[1]

Cancer site and polyphenol class	Biomarker	Specimen	Type of study	Country	Population	Cases	Sex	Age	Follow-up	Type of variable	Association	P^2	Ref
					n	*n*	*% females*	*y*	*y*				
Breast													
Flavanols	EC, ECG, EGC, EGCG	Plasma	Nested CC	Japan	432	144	100	40–69	10.6	Tertiles	—	NS	(47)
Flavanols	EC, EGC, metabolites	Urine	Nested CC	China	1054	353	100	40–70	7	Tertiles	—	NS	(48)
Flavanones	Hesperetin and naringenin	Urine	CC	China	500	250	100	25–64		Tertiles	—	NS	(49)
Flavonols	Quercetin, kaempferol	Urine	Nested CC	China	1054	353	100	40–70	7	Tertiles	—	NS	(48)
Isoflavonoids	GEN	Plasma	Nested CC	Japan	432	144	100	40–69	10.6	Quartiles	0.34 (0.16, 0.74)[3]	0.02	(50)
Isoflavonoids	DAI	Plasma	Nested CC	Japan	432	144	100	40–69	10.6	Quartiles	—	NS	(50)
Isoflavonoids	GEN	Plasma	Nested CC	Netherlands	766	383	100	35–70	6.5	Tertiles	0.68 (0.47, 0.98)	0.07	(51)
Isoflavonoids	Equol	Urine	Nested CC	UK	333	114	100	45–75	8	Log_2	1.34 (1.06, 1.70)	0.013	(52)
Isoflavonoids	DAI	Serum	Nested CC	UK	284	97	100	45–75	8	Log_2	1.22 (1.01, 1.48)	0.044	(52)
Isoflavonoids	GEN	Serum	Nested CC	UK	284	97	100	45–75	8	Log_2	—	NS	(52)
Isoflavonoids	Equol	Serum	Nested CC	UK	284	97	100	45–75	8	Log_2	1.46 (1.05, 2.02)	0.024	(52)
Isoflavonoids	GLY	Urine	CC	China	120	60	100	25–64	—	Tertiles	0.41 (0.15, 1.11)	0.06	(53)
Isoflavonoids	Total isoflavones, DAI, GEN, equol, O-DMA	Urine	CC	China	120	60	100	25–64	—	Tertiles	—	NS	(53)
Isoflavonoids	Total isoflavones (DAI, DH-DAI, GEN, DH-GEN, GLY, equol, O-DMA)	Urine	CC	China	334	117	100	25–64	—	Tertiles	0.46 (0.22, 0.95)	0.04	(54)
Isoflavonoids and lignans	ENL and GEN	Urine	Nested CC	Netherlands	356	88	100	50–64	9	Tertiles	—	NS	(55)
Isoflavonoids	Total isoflavones (DAI, DH-DAI, GEN, DH-GEN, GLY, O-DMA)	Urine	CC	China	500	250	100	25–64	—	Tertiles	0.62 (0.39, 0.99)	0.04	(49)
Isoflavonoids	Equol	Urine	CC	Australia	288	144	100	30–84	—	Quartiles	0.27 (0.10, 0.69)	0.009	(56)
Isoflavonoids	Total isoflavones (DAI, GEN, GLY, equol, O-DMA)	Urine	Nested CC	UK	1189	237	100	45–75	9.5	Log_2	1.08 (1.00, 1.16)	0.055	(57)
Isoflavonoids	Total isoflavones (DAI, GEN, GLY, equol, O-DMA)	Serum	Nested CC	UK	1064	213	100	45–75	9.5	Log_2	—	NS	(57)
Isoflavonoids and lignans	DAI, GLY, O-DMA, equol, END, ENL	Plasma	Nested CC	Netherlands	766	383	100	35–70	6.5	Tertiles	—	NS	(51)
Isoflavonoids and lignans	DAI, GEN, GLY, O-DMA, END, ENL	Urine	Nested CC	UK	333	114	100	45–75	8	Log_2	—	NS	(52)
Isoflavonoids and lignans	GLY, END, ENL	Serum	Nested CC	UK	284	97	100	45–75	8	Log_2	—	NS	(52)
Isoflavonoids and lignans	DAI, END, matairesinol	Urine	CC	Australia	288	144	100	30–84	—	Quartiles	—	NS	(56)
Lignans	Total lignans (END, ENL)	Urine	CC	China	334	117	100	25–64	—	Tertiles	—	NS	(56)
Lignans	Total lignans (END, ENL)	Urine	CC	China	500	250	100	25–64	—	Tertiles	0.40 (0.24, 0.64)	<0.001	(49)
Lignans	Total lignans (END, ENL)	Urine	Nested CC	UK	1189	237	100	45–75	9.5	Log_2		NS	(57)
Lignans	Total lignans (END, ENL)	Serum	Nested CC	UK	1064	213	100	45–75	9.5	Log_2	—	NS	(57)
Lignans	ENL	Plasma	Nested CC	Sweden	740	248	100	25–64	10	Quintiles		NS	(58)
Lignans	ENL	Plasma	CC	Finland	402	194	100	25–75	—	Quintiles	0.38 (0.18, 0.77)	0.03	(59)
Lignans	ENL	Urine	CC	Australia	288	144	100	30–84	—	Quartiles	0.36 (0.15, 0.86)	0.013	(56)

(Continued)

TABLE 1 (*Continued*)

Cancer site and polyphenol class	Biomarker	Specimen	Type of study	Country	Population	Cases	Sex	Age	Follow-up	Type of variable	Association	P^2	Ref
Breast (cont'd)													
Polyphenols (total)	Phenols	Urine	CC	China	120	60	100	25–64	—	Tertiles	—	NS	(52)
Prostate													
Isoflavonoids	GEN, DAI, equol	Serum	Nested CC	Japan	191	40	0	>40	9	Tertiles	—	NS	(60)
Isoflavonoids	DAI	Urine	Nested CC	USA	653	249	0	45–75	1.9	Quintiles	0.55 (0.31, 0.98)	0.03	(61)
Isoflavonoids	GEN, equol	Urine	Nested CC	USA	653	249	0	45–75	1.9	Quintiles		NS	(61)
Isoflavonoids	GEN	Plasma	Nested CC	Europe	1992	950	0	43–76	4.2	Quintiles	0.74 (0.54, 1.00)	0.05	(62)
Isoflavonoids	DAI, equol	Plasma	Nested CC	Europe	1992	950	0	43–76	4.2	Quintiles	—	NS	(62)
Isoflavonoids	Total isoflavones, DAI, GEN, equol	Plasma	CC	Scotland	454	249	0	50–74	—	Quartiles	—	NS	(63)
Isoflavonoids	DAI, GEN, GLY, equol	Plasma	Nested CC	Japan	603	201	0	40–69	12.8	Tertiles	—	NS	(64)
Isoflavonoids and lignans	DAI, GEN, GLY, equol, O-DMA, END, ENL	Plasma	Nested CC	UK	1006	191	0	45–75	9	Log_2	—	NS	(65)
Isoflavonoids and lignans	DAI, GEN, GLY, equol, O-DMA, END, ENL	Urine	Nested CC	UK	817	152	0	45–75	9	Log_2	—	NS	(65)
Lignans	ENL	Urine	Nested CC	USA	653	249	0	45–75	1.9	Quintiles	—	NS	(61)
Lignans	END, ENL	Plasma	Nested CC	Europe	1992	950	0	43–76	4.2	Quintiles	—	NS	(62)
Lignans	ENL	Plasma	CC	Scotland	454	249	0	50–74	—	Quartiles	0.40 (0.22, 0.71)	0.002	(63)
Lignans	ENL	Plasma	Nested CC	Finland, Sweden, Norway	3344	794	0	25–64	14.2	Quartiles	—	NS	(66)
Uterine fibroids													
Isoflavonoids	Total isoflavones (DAI, GEN, equol, O-DMA)	Urine	CC	USA	340	168	100	20–75	—	Quartiles	—	NS	(67)
Lignans	Total lignans (END, ENL)	Urine	CC	USA	343	170	100	20–75	—	Quartiles	0.47 (0.23, 0.98)	0.07	(67)
Endometrium													
Alkylresorcinols	Alkylresorcinols (17:0, 19:0, 21:0, 23:0, 25:0)	Plasma	Case-cohort	Denmark	329	177	100	50–64	11	Quartiles	—	NS	(68)
Esophagus													
Flavanols	EGC + 4′-MeEGC + EC + metabolites	Urine	Nested CC	China	251	42	0	45–64	12	Quartiles	—	NS	(69)
Stomach													
Flavanols	EC	Plasma	Nested CC	Japan	662	331	0	40–69	14	Tertiles	2.06 (1.23, 3.45)	0.003	(70)
Flavanols	ECG	Plasma	Nested CC	Japan	326	163	100	40–69	14	Tertiles	0.25 (0.08, 0.73)	0.02	(70)
Flavanols	ECG	Plasma	Nested CC	Japan	662	331	0	40–69	14	Tertiles	—	NS	(70)
Flavanols	EC	Plasma	Nested CC	Japan	326	163	100	40–69	14	Tertiles	—	NS	(70)
Flavanols	ECG, EGC	Plasma	Nested CC	Japan	988	494	33	40–69	14	Tertiles	—	NS	(70)
Flavanols	EGC + 4′-MeEGC + EC + metabolites	Urine	Nested CC	China	753	190	0	45–64	12	Quartiles	—	NS	(70)
Colorectum													
Isoflavonoids and lignans	DAI, GEN, GLY, equol, O-DMA, END, ENL	Plasma	Nested CC	UK	1091	214	43	45–75	9	Log_2	—	NS	(65)
Isoflavonoids and lignans	DAI, GEN, GLY, equol, O-DMA, END, ENL	Urine	Nested CC	UK	832	146	43	45–75	9	Log_2	—	NS	(65)

(Continued)

TABLE 1 (*Continued*)

Cancer site and polyphenol class	Biomarker	Specimen	Type of study	Country	Population	Cases	Sex	Age	Follow-up	Type of variable	Association	P^2	Ref
Colon													
Flavanols	EGC + 4′-MeEGC	Urine	Nested CC	China	498	83	0	45–64	16	Quartiles	0.42 (0.68, 0.94)	0.007	(71)
Flavanols	EC + metabolites	Urine	Nested CC	China	498	83	0	45–64	16	Quartiles	—	NS	(71)
Rectum													
Flavanols	EGC + 4′-MeEGC + EC + metabolites	Urine	Nested CC	China	474	79	0	45–64	16	Quartiles	—	NS	(71)
Lung													
Isoflavonoids	GEN	Plasma	Nested CC	Japan	318	106	100	40–69	13.5	Quintiles	0.31 (0.12, 0.86)	0.085	(72)
Isoflavonoids	Total isoflavones, DAI, GLY, equol	Plasma	Nested CC	Japan	318	106	100	40–69	13.5	Quintiles	—	NS	(72)

[1] CC, case-control; DAI, daidzein; DH-DAI, dihydrodaidzein; DH-GEN, dihydrogenistein; EC, epicatechin; ECG, epicatechin gallate; EGC, epigallocatechin; EGCG, epigallocatechin gallate; END, enterodiol; ENL, enterolactone; GEN, genistein; GLY, glycitein; MeEGC, methylepigallocatechin gallate; O-DMA, *O*-desmethylangolensin; Ref, reference; —, no significant association.

[2] P is P-trend when the association was measured in quantiles and value when association was measured continuously.

[3] OR; 95% CI in parentheses (all such values).

polyphenols regularly consumed (~50 mg of a total of 1193 mg consumed per day in a French cohort) (21). The same polyphenol biomarkers have also been measured in cross-sectional studies aiming to validate tools and methods for measurement of polyphenol intake (**Table 2**). Notably, the applicability of polyphenol biomarkers in epidemiologic studies relies on their ability to reflect the dose ingested, reliability over time, and availability of appropriate analytic methods for their estimation in biospecimens.

Analytic techniques for biomarker measurement

Polyphenols are commonly measured in human biofluids after enzymatic deconjugation with glucuronidases and sulfatase, and the released aglycones are analyzed by chromatography with mass, fluorescent, or electrochemical detection (95). Polyphenols are found in low concentrations (from nmol to μmol/L ranges) in both plasma and urine (23), and analytic methods must be sensitive enough to allow reliable and reproducible quantitation. In a European cohort study, plasma concentrations of the ubiquitous enterolactone were below the limit of detection (LOD; 0.4 nmol/L) in 31% of subjects (96). Similarly, in a Chinese study, plasma concentrations of quercetin, kaempferol, isorhamnetin, apigenin, and luteolin were below the LOD for 20%, 39%, 22%, 27%, and 35% of subjects, respectively (75). There is thus a need to improve the sensitivity of analytic methods for polyphenols found in low concentrations in plasma.

LODs depend on analytic techniques and on the nature of the polyphenol biomarker. LODs commonly reported for isoflavones and lignans in plasma or urine were 10, 0.4, and 0.3 nmol/L when using HPLC coupled to diode array detection, gas chromatography–mass spectrometry, and liquid chromatography–tandem mass spectrometry techniques, respectively (84, 88, 96, 97, 98). LODs were reported to be similar for other polyphenols: for example, 0.3 nmol/L for resveratrol in urine with the use of liquid chromatography–tandem mass spectrometry (99) or 2.3 nmol/L for some flavonoids in plasma with the use of HPLC coupled to diode array detection (75). Mass spectrometry techniques are evolving rapidly and should constitute the best option for the analysis of a large number of polyphenol biomarkers in a single analytic run with high sensitivity. Labeled internal standards should be used to reduce technical variability (100–102). However, none of the methods presented in Table 2, often based on mass spectrometry, used such labeled standards because of the high cost to synthesize them. There is a need to make these standards available at lower prices for use in large epidemiologic studies.

Polyphenol metabolism and selection of polyphenol biomarkers

Choosing an appropriate polyphenol biomarker requires a good understanding of metabolism. In particular, it is essential to clearly identify all possible precursors of each biomarker, particularly for metabolites that may have several precursors. Our knowledge of polyphenol metabolism has increased enormously over the past 20 y (19, 103). More than 350 polyphenol metabolites have been described and compiled in the Phenol-Explorer database, and it is now possible to quickly identify all known precursors of a particular metabolite or all metabolites formed from a parent polyphenol (104, 105). The parent compounds, as present in the diet

TABLE 2
Validation studies for polyphenol intake measurement showing correlations between polyphenol concentrations in plasma or urine with habitual polyphenol intake in various populations[1]

Polyphenol ingested	Biomarker[2]	Biofluid	No. of subjects	Country	Dietary assessment	*r*	*P*	Ref
Flavonols								
Kaempferol	Kaempferol	Plasma (F)	48	Germany	7-d DR	0.46	<0.01	(73)
Quercetin	Quercetin	Plasma (F)	48	Germany	7-d DR	0.3	<0.05	(73)
Kaempferol	Kaempferol	Plasma (F)	92	China	FFQ	0.52	0.001	(74)
Quercetin	Quercetin	Plasma (F)	92	China	FFQ	0.46	0.001	(74)
Isorhamnetin	Isorhamnetin	Plasma (F)	92	China	FFQ	0.37	0.026	(74)
Quercetin	Quercetin	Plasma (F)	92	China	7-d DR	0.51	<0.05	(75)
Quercetin, kaempferol, isorhamnetin	Quercetin, kaempferol, isorhamnetin	Plasma (F)	92	China	7-d DR	0.48	<0.05	(75)
Kaempferol	Kaempferol	Plasma (F)	92	China	7-d DR	0.44	<0.05	(75)
Isorhamnetin	Isorhamnetin	Plasma (F)	92	China	7-d DR	0.33	<0.05	(75)
Flavones								
Apigenin	Apigenin	Plasma (F)	92	China	FFQ	0.52	0.002	(74)
Luteolin	Luteolin	Plasma (F)	92	China	FFQ	0.5	0.012	(74)
Apigenin, luteolin	Apigenin, luteolin	Plasma (F)	92	China	7-d DR	0.46	<0.05	(75)
Luteolin	Luteolin	Plasma (F)	92	China	7-d DR	0.44	<0.05	(75)
Apigenin	Apigenin	Plasma (F)	92	China	7-d DR	0.42	<0.05	(75)
Flavanones								
Naringenin	Naringenin	Plasma (F)	48	Germany	7-d DR	0.35	<0.05	(73)
Hesperetin	Hesperetin	Plasma (F)	48	Germany	7-d DR	0.32	<0.05	(73)
Isoflavones								
Daidzein	Daidzein, equol, O-DMA, dihydrodaidzein	Urine (spot)	24	Korea	3 × 3-d DR	0.72	<0.001	(76)
Genistein	Genistein, dihydrogenistein	Urine (spot)	24	Korea	3 × 3-d DR	0.64	<0.01	(76)
Glycitein	Glycitein	Urine (spot)	24	Korea	3 × 3-d DR	0.57	<0.01	(76)
Genistein	Genistein	Urine (24-h)	27	USA	FFQ	0.54		(77)
Daidzein	Daidzein, equol, O-DMA	Urine (24-h)	27	USA	FFQ	0.49		(77)
Genistein	Genistein	Plasma (NF)	80	UK	7-d DR	0.8	<0.001	(78)
Daidzein	Daidzein	Plasma (NF)	80	UK	7-d DR	0.79	<0.001	(78)
Daidzein, genistein, glycitein, formononetin, biochanin A	Daidzein, genistein, equol, O-DMA	Urine (spot)	2908	USA	24-h DR	0.48	<0.001	(79)
Daidzein	Daidzein, equol, O-DMA	Urine (spot)	2908	USA	24-h DR	0.46	<0.001	(79)
Genistein	Genistein	Urine (spot)	2908	USA	24-h DR	0.45	<0.001	(79)
Genistein	Genistein	Urine (24-h)	105	USA	FFQ	0.31	<0.01	(80)
Daidzein, genistein	Daidzein, genistein, equol, O-DMA	Urine (24-h)	105	USA	FFQ	0.29	<0.01	(80)
Daidzein	Daidzein, equol, O-DMA	Urine (24-h)	105	USA	FFQ	0.28	<0.01	(80)
Daidzein	Daidzein	Urine (24-h)	93	Japan	14 × 24-h DR	0.43		(81)
Genistein	Genistein	Plasma (F)	196	Japan	14 × 24-h DR	0.42		(81)
Daidzein	Daidzein	Plasma (F)	196	Japan	14 × 24-h DR	0.39		(81)
Genistein	Genistein	Urine (24-h)	93	Japan	14 × 24-h DR	0.38		(81)
Daidzein	Daidzein	Urine (24-h)	69	USA	FFQ	0.49	<0.001	(82)
Genistein	Genistein	Urine (24-h)	69	USA	FFQ	0.3	0.035	(82)
Daidzein	Daidzein, equol, O-DMA, dihydrodaidzein	Urine (24-h)	195	USA	FFQ	0.55		(83)
Genistein, daidzein, biochanin A, formononetin	Genistein, daidzein, equol, O-DMA, dihydrodaidzein	Urine (24-h)	195	USA	FFQ	0.5		(83)
Genistein	Genistein	Urine (24-h)	195	USA	FFQ	0.45		(83)

(Continued)

TABLE 2 (*Continued*)

Polyphenol ingested	Biomarker[2]	Biofluid	No. of subjects	Country	Dietary assessment	*r*	*P*	Ref
Daidzein, genistein	Daidzein, genistein, equol	Plasma (NF)	203	Scotland	FFQ	0.27	<0.001	(84)
Genistein	Genistein	Plasma (NF)	203	Scotland	FFQ	0.26	<0.001	(84)
Daidzein	Daidzein, equol	Plasma (NF)	203	Scotland	FFQ	0.24	0.001	(84)
Genistein	Genistein	Plasma (F)	96	USA	FFQ	0.38	<0.001	(85)
Daidzein	Daidzein	Plasma (F)	96	USA	FFQ	0.35	<0.001	(85)
Genistein	Genistein	Plasma (F)	77	USA	FFQ	0.46		(86)
Daidzein	Daidzein	Plasma (F)	77	USA	FFQ	0.45		(86)
Daidzein, genistein, glycitein	Daidzein, genistein, glycitein, equol, O-DMA	Urine (spot)	60	China	FFQ	0.54	<0.001	(87)
Lignans								
LARI, PINO, SECO, MAT	Enterolactone	Plasma (NF)	637	Netherlands	FFQ	0.18	<0.001	(88)
LARI, PINO, SECO, MAT	Enterodiol	Plasma (NF)	637	Netherlands	FFQ	0.09	<0.05	(88)
SECO, MAT	Enterodiol, enterolactone	Urine (24-h)	195	USA	FFQ	0.16		(83)
Stilbenes								
Resveratrol + piceid	Resveratrol metabolites (glucuronides and sulfates)	Urine (spot)	1000	Spain	FFQ	0.89	<0.001	(89)
Alkylresorcinols								
Total alkylresorcinols	Total alkylresorcinols	Plasma (F)	30	Sweden	3-d DR	0.33–0.40	<0.001	(90)
Alkylresorcinols (17:0–25:0)	Alkylresorcinols (17:0–25:0)	Plasma (F)	51	Sweden	2 × 3-d weighed DR	0.48–0.65	<0.001	(91)
Total polyphenols	Total polyphenols	Urine (spot)	60	Spain	FFQ	0.257	0.04	(92)
	Total polyphenols	Urine (spot)	612	Spain	FFQ	0.179	<0.001	(93)
	Total polyphenols	Urine (24-h)	928	Italy	FFQ	0.211	<0.001	(94)

[1] DR, dietary recall; F, fasting; FFQ, food-frequency questionnaire; LARI, lariciresinol; MAT, matairesinol; NF, nonfasting; O-DMA, *O*-desmethylangolensin; PINO, pinoresinol; Ref, reference; SECO, secoisolariciresinol.

[2] Biomarkers were measured after deconjugation of glucuronides and sulfate esters with glucuronidases and sulfatases, respectively.

(generally measured in biofluids after enzymatic deconjugation of their glucuronides and sulfate esters), are the most direct indicators of exposure to the polyphenol ingested.

Polyphenol metabolites can also be measured in biofluids and have sometimes been used as biomarkers of exposure (106, 107). However, interindividual variability in their bioavailability may limit their reliability as biomarkers. Genetic factors such as polymorphisms of the xenobiotic-metabolizing enzymes may contribute to this interindividual variation (108). The gut microbiome also differs between individuals, and this may also result in interindividual variations in the concentrations of polyphenol microbial metabolites. For example, the microbial metabolites equol, *O*-desmethylangolensin, dihydrodaidzein formed from daidzein, and dihydrogenistein from genistein were less strongly correlated with polyphenol intake than their parent compounds (109). Therefore, they will be poorer indicators of exposure to their parent compounds.

Correlation of polyphenol biomarkers with polyphenol intake

Some polyphenol biomarkers reflect very specifically the intake of plant foods, such as resveratrol for intake of wine (89) and alkylresorcinol metabolites for intake of whole-grain cereals (110). A number of cross-sectional studies have shown that concentrations of these biomarkers in plasma or urine often correlate well with intakes of polyphenol-rich foods or of individual polyphenols consumed the previous day (111). A linear response to the dose ingested has generally been observed in a large number of small acute-intervention studies, with high correlation coefficients (0.7–0.9) (109). The strong correlations seen in intervention studies when compared with observational studies are mainly explained by a controlled or more accurate measurement of polyphenol intake, the more homogeneous population, the collection of the biospecimens straight after polyphenol intake, and the better handling and shorter storage time of biospecimens.

Polyphenol biomarkers have been measured both in urine (spot and 24-h) and plasma (fasting and nonfasting). Polyphenols are absorbed and excreted relatively quickly after ingestion, reaching maximum concentrations in plasma after as little as 0.5 h (flavanols) and as long as 9 h (isoflavones, flavonols) depending on the nature of the compounds and the food source (23). Their elimination half-lives also vary from 1 h (flavanols) to 28 h (flavonols) (23). These variations in concentrations in plasma resulting from rapid absorption and elimination might be less prominent in urine, because urine samples integrate polyphenol elimination over a few hours. However, in a Japanese study, both plasma and 24-h urine isoflavone concentrations correlated similarly with intake (81), which may be explained by the relatively long half-life of isoflavones and the frequency of consumption of their food sources. Overall, no clear difference in the correlations with intake can be observed between urine and plasma for the different biomarkers (Table 2).

For isoflavones, the strongest correlations between intake and biomarker concentrations (0.57–0.72) were observed in Korean and Chinese populations in whom the consumption of soy products, rich in isoflavones, is substantially higher (32–46 mg/d) (112) than that of Western populations. In the Western populations studied, the consumption of soy products is much less frequent and the average consumption of isoflavones did not usually exceed 2 mg/d (113). Correlations between intake and biomarker concentrations were lower and varied between 0.24 and 0.54, with the exception of one study conducted in the United Kingdom for which high (mean isoflavone intake: 49 mg/d) and low soy consumers were selected (78).

Plasma and urine concentrations of the 2 mammalian lignans, enterolactone and enterodiol, which are formed in the gut by the microbiota, did not reflect lignan intake ($r = 0.10$–0.20) (83, 88). This is attributed to the limited understanding of their dietary origin. In these studies, the intakes of 2 to 4 dietary lignans were measured. These lignans are present in trace amounts in a range of foods and their concentrations may be insufficient to explain the high concentrations of mammalian lignans in biofluids; other precursors, such as lignin polymers which are most abundant in whole-grain cereal products, may actually be the main precursors of the mammalian lignans (114).

Biomarkers of flavonol and flavone consumption have been measured in plasma. Correlation with intake varied from 0.30 to 0.52 (Table 2). Similarly, correlations between alkylresorcinol concentrations in plasma and alkylresorcinol intake varied between 0.33 and 0.65 depending on the study and the nature of the polyphenol within each class. The correlation coefficient between resveratrol metabolites in spot urine and resveratrol intake was particularly high ($r = 0.89$) (89). This finding could be attributable to the limited dietary distribution of resveratrol, whose principal contributor was wine (98%) in Spanish populations (115).

Last, total polyphenols measured with the Folin-Ciocalteu colorimetric assay in urine were poorly correlated with total polyphenol intake measured by the same assay ($r = 0.18$–0.26). These low values are explained by the lack of specificity of the colorimetric assay used for these measurements and the well-known occurrence of interfering substances in both foods and urine, such as ascorbic acid, sugars, thiols, and other reducing agents (92).

Biomarker reliability

Biomarker reliability over time is another key issue to consider in epidemiology. In most prospective cohort studies, biospecimens are collected at a single time point. It is therefore essential to check that measurements made at this time point reflect usual exposure. Reliable biomarkers should be subject to little intraindividual variability relative to interindividual variability. Reliability is often expressed as the intraclass correlation (ICC) coefficient, defined as interindividual variance over total variance (intra- plus interindividual variance). Ideally, this ICC value should be close to 1. However, it rarely reaches this value because of host factors, such as variations in intestinal transit time, microbiota, and expression of metabolic enzymes and transporters that may influence polyphenol absorption and metabolism; interactions of polyphenols with other dietary factors in the gut; or the irregular consumption of the individual's dietary sources.

ICC values are usually measured on repeated biospecimens collected at different time intervals in a set of individuals. They have rarely been estimated for polyphenol biomarkers (**Table 3**). Low ICC values were observed for isoflavones (<0.1) in an American cohort because of the low frequency of their consumption (118). ICC values of ~ 0.6 were measured for other flavonoids, phenolic acids, and lignans. Values were similar for biospecimens collected 4 d or 4 wk apart. In one study in which alkylresorcinols were measured in fasting and nonfasting plasma samples, the ICC coefficient was found to be lower for nonfasting plasma (91). This is

TABLE 3
Summary of reliability studies on biomarkers of polyphenol consumption[1]

Biomarker[2]	Biofluid	No. of subjects	Country	Period of sample collection	No. of samples	ICC coefficient[3]	Ref
Flavanols							
Gallocatechin	Plasma (fasting)	7	Germany	4 wk	3	0.60	(116)
Quercetin	Plasma (fasting)	7	Germany	4 wk	3	0.79	(116)
Quercetin	Urine (24-h)	154	France	4 d	3–4	0.61	(117)
Kaempferol	Plasma (fasting)	7	Germany	4 wk	3	0.78	(116)
Kaempferol	Urine (24-h)	154	France	4 d	3–4	0.54	(117)
Isorhamnetin	Plasma (fasting)	7	Germany	4 wk	3	0.68	(116)
Isorhamnetin	Urine (24-h)	154	France	4 d	3–4	0.59	(117)
Flavones							
Luteolin	Plasma (fasting)	7	Germany	4 wk	3	0.67	(116)
Flavanones							
Hesperetin	Plasma (fasting)	7	Germany	4 wk	3	0.65	(116)
Hesperetin	Urine (24-h)	154	France	4 d	3–4	0.57	(117)
Naringenin	Urine (24-h)	154	France	4 d	3–4	0.58	(117)
Isoflavones							
Daidzein	Plasma (nonfasting)	40	USA	2–3 y	2	0.00	(118)
Daidzein	Urine (24-h)	45	USA	2–3 y	2	0.00	(118)
Genistein	Plasma (nonfasting)	40	USA	2–3 y	2	0.03	(118)
Genistein	Urine (24-h)	45	USA	2–3 y	2	0.02	(118)
Equol	Plasma (nonfasting)	40	USA	2–3 y	2	0.00	(118)
Equol	Urine (24-h)	45	USA	2–3 y	2	0.09	(118)
Lignans							
Enterolactone	Plasma (nonfasting)	40	USA	2–3 y	2	0.44	(118)
Enterolactone	Urine (24-h)	45	USA	2–3 y	2	0.52	(118)
Enterolactone	Plasma (fasting)	7	Germany	4 wk	3	0.70	(116)
Enterolactone	Urine (24-h)	154	France	4 d	3–4	0.65	(117)
Enterodiol	Urine (24-h)	154	France	4 d	3–4	0.57	(117)
Alkylresorcinols							
Total alkylresorcinols	Plasma (fasting)	18	Sweden	3 d	3	0.60	(91)
Total alkylresorcinols	Plasma (nonfasting)	18	Sweden	3 d	3	0.18	(91)
Phenolic acids							
Caffeic acid	Plasma (fasting)	7	Germany	4 wk	3	0.61	(116)
Caffeic acid	Urine (24-h)	154	France	4 d	3–4	0.58	(117)
Chlorogenic acid	Urine (24-h)	154	France	4 d	3–4	0.64	(117)
Ferulic acid	Plasma (fasting)	7	Germany	4 wk	3	0.76	(116)
p-Coumaric acid	Plasma (fasting)	7	Germany	4 wk	3	0.67	(116)
m-Coumaric acid	Urine (24-h)	154	France	4 d	3–4	0.54	(117)
Gallic acid	Urine (24-h)	154	France	4 d	3–4	0.59	(117)
4-*O*-Methylgallic acid	Urine (24-h)	154	France	4 d	3–4	0.48	(117)
Ellagic acid	Plasma (fasting)	7	Germany	4 wk	3	0.73	(116)
Dihydrochalcones							
Phloretin	Urine (24-h)	154	France	4 d	3–4	0.48	(117)

[1] ICC, intraclass correlation; Ref, reference.
[2] Biomarkers were measured after deconjugation of glucuronides and sulfate esters with glucuronidases and sulfatases, respectively.
[3] ICC coefficients describe the reliability of biomarkers and are defined as the proportion of variance between and within individuals.

likely explained by the high intraindividual variability resulting from the different time intervals that elapsed between consumption of the sources of polyphenols during the meal and biospecimen collection. Similarly, a relatively low ICC value was observed for enterolactone when measured in nonfasting plasma samples (118). ICC coefficients measured in these few studies suggest that plasma or urine samples can equally be used to measure polyphenol biomarkers in epidemiologic studies and fasting samples should also be recommended when available.

Limitations of polyphenol biomarker measurements

The data discussed above show the potential benefits of using biomarkers to improve the assessment of polyphenol exposures. However, polyphenol biomarkers also have a number of limitations that need to be addressed. The first is the lack of available methods combining high sensitivity and coverage to quantify the many polyphenols present in human biospecimens. Tagging polyphenols with an isotope-labeled reagent and quantification of the labeled polyphenols by mass spectrometry constitute a promising approach to both increase the sensitivity of detection and to alleviate the need for synthesizing costly labeled polyphenol standards (119, 120).

The rapid absorption and elimination of polyphenols may also limit the use of polyphenol biomarkers in observational epidemiology. Polyphenols differ from other nutritional biomarkers such as carotenoids or lipids that are partly stored in fatty tissues and which show for this reason more stable concentrations in

blood (121). However, a number of polyphenol biomarkers show ICC values that range between 0.50 and 0.79 (Table 3), which were considered "good" to "excellent" in a previous study on 86 biomarkers measured in samples from the Nurses' Health Study and comparable to ICC values of other nutritional biomarkers commonly measured in epidemiology (118). In agreement with these relatively high ICC values, polyphenol biomarkers were not only correlated with acute polyphenol intake but also with habitual polyphenol intake as estimated with FFQs (Table 2). Nevertheless, the reliability of polyphenol biomarkers also depends on the nature of the polyphenol and on the population in whom it is applied. Isoflavone biomarkers can be used reliably in Asian populations who regularly consume soy products, whereas they are too unstable (ICC <0.1; Table 3) in Western populations.

The availability of biological samples is another factor to consider. Polyphenol biomarkers have been measured in both plasma and urine. Urine samples are collected less often in large cohort studies, but they offer some advantages, notably higher polyphenol concentrations when compared with plasma and a more straightforward sample processing before analysis. Unlike blood, urine must be normalized to urine volume or creatinine to take into account variations in dilution (122). The measurement of polyphenol biomarkers also requires appropriate equipment, analytic skills, and resources. Projects based on the use of polyphenol biomarkers are necessarily resource-dependent, which limits the number of samples that can be analyzed in a particular study. For these reasons, the use of polyphenol biomarkers so far has been limited to (nested) case-control studies with a number of subjects not exceeding 2000 (Table 1).

Last, when correlating many polyphenol biomarkers with the risk of chronic diseases, a set of statistical inferences are being made simultaneously. This results in a problem well known by the epidemiologists as multiple comparison testing. Several statistical techniques have been developed to counter this problem. The Bonferroni test is considered the simplest and most conservative method to control the family-wise error rate. The false discovery rate is also commonly used, because it is less stringent than family error rate procedures. Despite these statistical techniques, it is highly recommended to retest the hypotheses in another independent study and verify that the results are not a result of chance (123). Another approach is to limit the redundancy of variables corresponding to highly correlated polyphenols that cooccur in a same food source. Principal components analysis can then be used to reduce the number of polyphenol variables in the data set to a smaller number of uncorrelated factors (124).

MEASUREMENT OF POLYPHENOL EXPOSURE IN OBSERVATIONAL EPIDEMIOLOGIC STUDIES ON CANCER

Polyphenol exposure has been assessed in numerous observational epidemiologic studies by using either food-composition tables or biomarkers to evaluate the possible role of polyphenols in the prevention of chronic diseases. Because of limitations in the analytic instrumentation and databases used to estimate polyphenol exposure, most research conducted so far has been focused on a limited number of polyphenol variables. These specific approaches largely failed to consider the polyphenol family in all of its complexity. Cancer epidemiologic studies are reviewed here to critically evaluate the utilization of these tools and to make recommendations for future studies.

Polyphenol intake assessment in observational epidemiologic studies on cancer

Most observational studies on polyphenols and cancer risk have reported polyphenol intake based on polyphenol food-composition tables and dietary questionnaires (Supplemental Table 1 under "Supplemental data" in the online issue). Polyphenol food-composition tables were first developed for phytoestrogens (mostly isoflavones and lignans) because of their putative effects on hormone-dependent cancers. Meta-analyses could be conducted only on dietary intake of isoflavones and lignans and the risk of breast (125, 126) or prostate (127) cancer because of the lack of sufficient data from individual epidemiologic studies on other polyphenols and other cancer sites, except for flavonoid intake and the risk of lung (10) and breast (128) cancer. These observational epidemiologic studies on phytoestrogens were extended to other specific classes of flavonoids, particularly flavonols (129) and flavanols (130), after additional food-composition data became available, and later to all main classes of flavonoids (8, 9) after the release of the first flavonoid database from the USDA. Because of the relatively recent publication of Phenol-Explorer, the most detailed database on polyphenol contents of foods, this database was still little used to investigate the link between polyphenol intake and risk of cancers (131–134).

In most observational epidemiologic studies published to date, polyphenols were considered grouped into classes rather than as individual compounds because of the large complexity of the various classes of polyphenols. This reduces the number of variables considered in association studies, but it also presents 2 major drawbacks. First, differences in bioactivities of individual polyphenols within each particular class are masked. Second, it makes it difficult to compare studies in which polyphenol classes rather than individual polyphenols are considered, because no data on the detailed composition of the classes are usually given. For example, a very low intake of total anthocyanidins was measured in the Iowa Women's Health Study (0.1 mg/d) (135). This value is surprisingly low in comparison to intakes observed in French, Finnish, or Spanish cohorts (35, 47, and 19 mg/d, respectively) (21, 22, 136) and raises questions about the reliability of the polyphenol intake measurements in this study and the plausibility of the inverse association found between anthocyanidin intake and cardiovascular disease mortality. Therefore, for future epidemiologic studies, the publication of polyphenol food-composition tables or the study of individual polyphenols rather than total polyphenols in different polyphenol classes is recommended to facilitate comparisons of results obtained in different study populations.

Polyphenol biomarkers in observational epidemiologic studies on cancer

Cancer-risk associations have also been assessed in observational studies with the use of polyphenol biomarkers (Table 1), although less frequently than those studies based on intake measurements (Supplemental Table 1 under "Supplemental data" in the online issue). Similar to dietary studies, most polyphenol biomarker research has focused on phytoestrogens and their possible protection against sex hormone–related cancers. Few biomarker studies have been carried out on other classes of polyphenols, such as flavanols and flavanones.

The biomarker-based approach presents the advantage of taking into account interindividual variations in bioavailability and

interactions with other dietary compounds (45). However, the limited number of biomarker-based observational studies conducted so far limits comparison with studies based on intake measurements. Both types of studies suggest protective effects of isoflavones against breast cancer in Asian populations. In contrast, among men, no associations between isoflavones and the risk of prostate cancer could be observed in studies that used biomarkers (60, 64), whereas a meta-analysis on isoflavone intake and prostate cancer in Asian populations showed a reduced risk of cancer in individuals with a high dietary intake of soy isoflavones (127). The limited number of biomarker-based studies in diverse populations with very different lifestyles or an insufficient reliability of isoflavone biomarkers over time may explain these discrepancies between results obtained by the 2 different approaches.

Similarly, lignan exposure has been inconsistently associated with postmenopausal breast cancer risk, both in studies based on lignan intake assessment (125, 132) and in studies based on lignan biomarkers (125, 137). The limited knowledge of the dietary precursors of mammalian lignans (114) makes the comparison between both types of studies difficult and suggests that current food-composition tables for the few lignans often present in trace amounts in foods are insufficient to assess exposure to mammalian lignans. Further study of lignins and other potential precursors of mammalian lignans is warranted.

These 2 examples, isoflavones and lignans, show the inconsistencies of observational studies that are based on either biomarkers or intake measurements and the possible bias and systematic errors in the estimation of polyphenol exposures. It will be essential for future work on polyphenol epidemiology to validate polyphenol intake measurements with biomarkers and to better assess the reliability of polyphenol biomarkers. Most biomarker-based studies used a single measurement of polyphenol exposure, and repeated measures might be needed to increase the reliability of long-term exposure measurements.

The use of a larger variety of biomarkers will also be essential in future epidemiologic research. More than 300 polyphenol metabolites have been described in various clinical and experimental studies (103), of which any one could represent a potential polyphenol biomarker to be used in some metabolome-wide association studies. The recent and rapid development of metabolomics brings new opportunities to discover novel polyphenol biomarkers and to develop studies on the polyphenol metabolome in which a large number of polyphenol biomarkers could be simultaneously measured (18, 138–140). Such studies would offer great promise in identifying the phenolic compounds most significant for health. They will also be needed to further validate polyphenol food-composition tables and polyphenol intake measurements in dietary intervention and observational studies.

CONCLUSIONS

Most studies investigating the links between polyphenol exposure and risk of chronic diseases have relied on the estimation of polyphenol intake from dietary questionnaires. The USDA flavonoid databases, and more recently the Phenol-Explorer database, have provided new opportunities to establish links between diseases and intakes of various polyphenols. A limitation of the studies published so far is that they most often measured consumption of total polyphenols or total polyphenols in each class, instead of individual polyphenols, masking their large diversity in terms of structure, physicochemical properties, and biological effects. More studies on individual polyphenols should be conducted in the future. The polyphenol databases now available should allow us to estimate the wide variety of compounds consumed within the diet and help in identifying the components of the polyphenol metabolome that play a major role in the maintenance of health.

Biomarkers are a promising alternative to traditional dietary assessment methods and may reduce biases associated with self-reporting. They may also better reflect exposure of target tissues to polyphenols than intake measurements, which do not take into account interindividual variations in bioavailability. Their application to polyphenol epidemiology has so far been essentially limited to phytoestrogens. The main barrier to the successful use of biomarkers as dietary assessors is the lack of comprehensive and validated analytic methods for their measurement in population studies. These methods should be highly sensitive and specific to be compatible with the low concentrations commonly found in plasma and urine samples. The reliability of these biomarkers over time should also be carefully assessed to ensure that they reflect habitual exposure, particularly for polyphenols and their food sources, which may not be regularly consumed.

There is greater interest than ever in improving and refining the estimation of intake of and exposure to the many nutrients and bioactive compounds regularly consumed within the diet. The development of databases for other food bioactive components and of analytic techniques for their measurement in human biospecimens should greatly contribute to clarify their effects on health and diseases. Progress recently made on the highly complex polyphenol family, and more particularly the development of comprehensive databases on polyphenol content in foods and their metabolism, could be extended to other classes of food bioactive constituents, such as terpenoids, alkaloids, glucosinolates, or fatty acids, to develop a broad information system on dietary constituents, their chemical structures, occurrence and concentrations in foods, biological properties, and effects on health (29). This should contribute to the development of metabolome-wide association studies and to the further integration of nutrition and food science into the "omics" era. This is a major challenge for nutritionists of the 21st century, which, if properly addressed, may radically change our understanding of the relations between diet and health.

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