Application of Dietary Phenolic Biomarkers in Epidemiology: Past, Present, and Future

ARTICLE *in* JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY · FEBRUARY 2012

Impact Factor: 2.91 · DOI: 10.1021/jf204742e · Source: PubMed

5 AUTHORS, INCLUDING:

Raul Zamora-Ros
University of Barcelona
71 PUBLICATIONS **1,074** CITATIONS
SEE PROFILE

Montserrat Rabassa
University of Barcelona
13 PUBLICATIONS **75** CITATIONS
SEE PROFILE

Rafael Llorach
University of Barcelona
81 PUBLICATIONS **2,613** CITATIONS
SEE PROFILE

Cristina Andres-Lacueva
University of Barcelona
177 PUBLICATIONS **5,313** CITATIONS
SEE PROFILE

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Review

pubs.acs.org/JAFC

Application of Dietary Phenolic Biomarkers in Epidemiology: Past, Present, and Future

Raul Zamora-Ros,[†] Montserrat Rabassa,[§] Rafael Llorach,[§] Carlos A. González,[†] and Cristina Andres-Lacueva*[,§]

[†]Unit of Nutrition, Environment and Cancer, Cancer Epidemiology Research Programme, Catalan Institute of Oncology, IDIBELL, Barcelona, Spain

[§]Nutrition and Food Science Department, XaRTA INSA, INGENIO-CONSOLIDER Program, FUN-C-Food CSD2007-063, Pharmacy School, University of Barcelona, Avinguda Joan XXIII sn, 08028 Barcelona, Spain

ABSTRACT: Phenolics are a large group of plant compounds that have been associated with protective health effects against several chronic diseases due to their potential antioxidant, anti-inflammatory, and anticarcinogenic properties. Consequently, in nutritional epidemiology it is essential to make an accurate assessment of phenolic exposure to evaluate their protective activities against human diseases. Self-reported questionnaires and biomarkers are the two main methods used for estimating dietary phenolics. Despite the limitations of self-reported methods, they are still an acceptable and useful measure. Meanwhile, nutritional biomarkers provide an alternative, more accurate measure, but they are expensive, and to date there are few validated biomarkers of phenolic intake. Nowadays, new analytical techniques, using both targeted and untargeted metabolomic approaches, play an important part in the rapid increase in the understanding of phenolic bioavailability and, consequently, have provided new potential biomarkers in small trials. In the near future, these dietary biomarkers should be tested in large epidemiological studies. Furthermore, the use of two independent measures—questionnaires and biomarkers—together provides a more thorough analysis of true phenolic exposure. Indeed, the challenge in the long term is to combine the information from biomarkers and self-reported questionnaires to clarify the relationship between dietary phenolics and disease.

KEYWORDS: *phenolics, biomarker, dietary assessment*

INTRODUCTION

Phenolics are a wide variety of chemical substances, ranging from simple phenolic acids to flavonoid polymers, which play a prominent role in the antioxidant network as constituents and/or modulators of enzyme activity.[1] Furthermore, in cell line and animal studies, phenolic compounds have been shown to provide a wide spectrum of potential health benefits, including anti-inflammatory, anticarcinogenic, antiobesity, antidiabetic, antiallergic, and hepato- and gastroprotective effects.[2,3] Recently, several epidemiological studies have focused on clarifying the relationship between phenolic consumption and disease occurrence in the human population.[4−7] Accurate and objective measures for evaluating dietary phenolic intake are needed for this. Self-reported questionnaires and nutritional biomarkers are the two main methods used for estimating dietary phenolic compounds.

Self-Reported Questionnaires. Dietary phenolic self-assessments have some substantial limitations that need to be considered. First, there are various methodological limitations regarding the kind of self-reported dietary collection methods used to estimate intake.[8−10] Furthermore, data from any dietary questionnaire can present both systematic and random measurement errors. For example, in food frequency questionnaires (FFQ), many details of dietary intake are usually not measured, and the quantification of intake is not as accurate as with recalls or records. Inaccuracies result from an incomplete listing of all possible foods and from errors in frequency and usual serving size estimations. Despite this, FFQs are the most common method used in large epidemiological studies, mainly because they are an easy, quick, and economical way to record the participants' habitual diet. To improve both the quantification and quality of the data gathered, the use of a previously validated FFQ is essential. In the field of phenolic compounds, however, only a few studies have used validated FFQs. These studies were usually evaluating isoflavone compounds, and the validation methods utilized were either 24 h recalls[11] or nutritional biomarkers.[12]

The second consideration is which food composition tables (FCT) are selected. The updated versions of the U.S. Department of Agriculture databases on flavonoids,[13] proanthocyanidins[14] and isoflavones,[15] and the Phenol-Explorer database released in 2009, which includes flavonoid and nonflavonoid data,[16] are the most complete and frequently used food composition databases on flavonoids/phenolics. However, these databases still have a large number of unknown values and a limited number of food items and do not provide composition data on cooked foods.

The third issue to consider is which phenolics are estimated. More than 8000 naturally occurring phenolics in plants have been characterized, although only a few of them are abundant

Special Issue: Food Bioactives and the Journal of Agricultural and Food Chemistry

Received: November 18, 2011
Revised: January 30, 2012
Accepted: February 1, 2012
Published: February 1, 2012

in common foods. The USDA FCTs contain only data on flavonoids (expressed as aglycones), whereas the Phenol-Explorer FCT provides data on more than 500 phenolic compounds expressed as such and as aglycones.[16] However, in a descriptive study using Phenol-Explorer, only 337 phenolics were consumed by a French cohort, including 258 phenolic compounds consumed by at least half of the cohort and 98 phenolics consumed in amounts of >1 mg/day.[17]

Despite the limitations outlined above, self-reported dietary questionnaires, particularly those that are previously validated, are still a useful methodology for estimating phenolic intake, especially in large epidemiological studies. In addition, new applications of information and communication technologies, such as multiple 24 h recalls using an Internet application, are being developed to reduce the limitations of traditionally self-reported dietary questionnaires.

Biomarkers. Nutritional biomarkers have become an alternative method for estimating dietary intake. The main reasons for using nutritional biomarkers instead of dietary questionnaires are (i) to provide a more objective and accurate measure of intake, decreasing the level of measurement error; (ii) to assess nutrients that have little or inadequate existing food composition data; and (iii) to achieve a closer measure of nutrient status, because they take into account the nutrient bioavailability and metabolism.[18] Traditionally, biomarkers are classified in three groups: (i) recovery biomarkers, which provide an estimation of absolute intake level over a fixed period of time (unfortunately, to date, only doubly labeled water, total urinary nitrogen, and potassium belong to this ideal biomarker group);[19,20] (ii) predictive biomarkers, which provide a high correlation with intake but a low overall recovery (currently, the urinary sucrose and fructose level as a biomarker for sugar consumption is the only predictive biomarker);[21] (iii) concentration biomarkers, which provide a measurement of the amount of this compound in tissues, taking into account intake, bioavailability, and physiological regulation of the compound level. These are widely used as a substitute or as complementary to dietary assessment.[19,20] Despite the potential advantages of biomarkers, there are only a few validated concentration markers of dietary phenolics, because it is very difficult to find markers that fulfill all of the following criteria: (i) to be specific; (ii) to have an adequate half-life; and (iii) to provide a good correlation between the biomarker and the intake.[22−24]

PAST OF PHENOLIC BIOMARKERS

Before a dietary phenolic, or its metabolite, can be used as a nutritional biomarker of phenolic, phenolic subgroup, food or food subgroup intake, certain information is required:[22] (a) a full understanding of the phenolic bioavailability in humans; (b) knowledge of the time−response curve between the phenolic intake and its presence in biofluids; (c) knowledge of the dose−response curve between the phenolic intake and its presence in biofluids; (d) an understanding of the interactions between the dietary, lifestyle, environmental, and genetic factors and phenolic bioavailability.[19] Therefore, to establish a nutritional biomarker, knowledge of its pharmacokinetic parameters is essential.

In this field, one of the biggest challenges to be faced in increasing our knowledge of phenolic bioavailability is the improvement of laboratory techniques for the analysis of phenolics in biofluids. The most common methods have been summarized in a number of reviews.[25−27] Briefly, first of all, samples can be subjected to acidic or enzymatic hydrolysis to break down the phenolic conjugates into their aglycones. Nowadays, hydrolysis is rare because it causes a lack of information about which metabolites and what proportion/amount of these metabolites occur in the biosample. The next step is a cleanup procedure, which is usually performed by a solid-phase extraction to remove any matrix interferences. Lastly, a separation and detection system is used; the most common is liquid or gas chromatography coupled to mass spectrometry. However, accurate quantification is still very limited as there are not many available standards for phenolic metabolites.

Over the past 15 years, several papers on phenolic bioavailability have been published. Previous studies were carried out on experimental animals, usually mice and rats, and with enormous oral doses. Subsequently, several studies were carried out on humans with, usually, high single oral doses.[22,28,29] As a result of these studies, knowledge on phenolic absorption, metabolism, distribution, and elimination has quickly improved. Nowadays, it is well-known that phenolic compounds are absorbed in the stomach (in small amounts) and in the small intestine or in the colon after the action of the microbiota. Following their absorption, they are almost totally metabolized in the gut or in the liver as glucuronides, sulfates, and methylates. Then, phenolic metabolites circulate in the bloodstream to the targeted organs. Finally, they are mainly excreted in urine as metabolites.

These pharmacokinetic studies provided essential information about the kinetic profile of phenolic metabolism and their appearance in biological specimens. The studies showed a high variability among phenolic compounds in the pharmacokinetic parameters including elimination half-life ($t_{1/2}$), time to reach $t_{1/2}$, maximum plasma concentration (C_{max}), time to reach C_{max} (t_{max}), and renal clearance or urinary recovery. t_{max} can differ greatly depending on the sugar moiety of the phenolic glycoside: for example, quercetin glucoside from onions had a t_{max} of 0.7 h, whereas quercetin aglycone from apples had a t_{max} of 2.5 h and quercetin-3-rutinoside had a t_{max} of 9.3 h.[30] Therefore, the plasma half-life of phenolics absorbed in the small intestine usually ranged between 1 and 12 h. However, if one considers metabolites from colonic microbiota (e.g., equol, a colonic metabolite of daidzein), $t_{1/2}$ increases to >2 days.[31] There are also large differences in urinary recoveries from 0.4% of anthocyanidin intake to >40% of isoflavone intake,[29] whereas the urinary $t_{1/2}$ ranged between 1.1 h (gallic acid) and 84 h (3-hydroxyphenylacetic acid) post ingestion.[29,32] Therefore, urinary phenolic metabolites could be a biomarker of recent intakes (3−4 days). Apart from the high variability among phenolic compounds, there is also a high variability within subjects (intraindividuality) and between subjects (interindividuality),[26] which hampers the use of phenolics as nutritional biomarkers.

Another important factor in the improvement of knowledge about phenolics is the increase in food composition data on most phenolic compounds. Over the past two decades, many new phenolics have been identified and quantified in foods. All of these composition values have recently been compiled into food composition databases on phenolic compounds.[13−16] These data are essential if we are to gain a full understanding of phenolic exposure.

Table 1. Plasma Biomarkers of Phenolics

phenolic	compound	source	dose ingested	duration	*N*	food survey	correlation (*r*)	*P*	ref
flavonoids	quercetin, kaempferol, isorhamnetin, apigenin, and luteolin		13.58, 14.97, 12.31, 4.23, and 8.08 mg/day	habitual diet	92	7 days register	0.33–0.51	<0.05	66
flavonols	quercetin, isorhamnetin, kaempferol, and myricetin	low flavonoid diet or on the same diet supplemented at one of two high-flavonols levels provided by supplements of tea daily and white onion fried in olive oil with and without tomato ketchup and herbs	1500 and 400 g (77.3 or 110.4 mg/day)	2 weeks	10	7 days register	0.75	0.001	67
flavonols and flavanones	quercetin		17.9 mg/day	habitual diet	52	7 days register	0.30	<0.05	68
	kaempferol		4.7 mg/day				0.46	<0.01	
	naringenin		12.1 mg/day				0.35	<0.05	
	hesperetin		17.4 mg/day				0.32	<0.05	
flavanones	hesperetin and naringenin	orange juice (0.5, 1 L)	22.5, 45 mg	single dose	5				69
flavanols	epigallocatechin-3-gallate	tea	500 or 1000 mg GTPs or placebo	3 months	124	weighted record	NA	<0.001	70
	epicatechin-3-gallate						NA	<0.001	
anthocyanidins		chokeberry juice (250 mL)	0.8 mg/kg/day	single dose	13				71
isoflavones	daidzein		1.2 mg/day	habitual diet	96	FFQ	0.37	<0.01	12
	genistein		0.9 mg/day	habitual diet			0.43	<0.01	
isoflavones	genistein and daidzein		4 doses	habitual diet	80	7 food diary and FFQ	0.53–0.80	<0.001	72
isoflavones	daidzein	soy nuts (10, 20, 40 g)	6.6, 13.2, 26.4 mg	single dose	10				73
isoflavones	daidzein	low, high-soy diet	0.54, 104 mg total isoflavones/day	10 weeks	76	7 day weighed record	NA	<0.001	74
phytoestrogens	daidzein and genistein	isoflavone supplement	35 mg/day	3 months	14	7 day weighed intake	0.92	<0.001	75
phenolics	anthocyanidins and phenolic acids	Concord grape juice (350 mL)		single dose	8				76
phenolic acids	caffeic acid	red wine (100, 200, 300 mL)	0.9, 1.8, 2.7 mg	single dose	5		0.84–0.99	<0.01	77
ellagitannins		pomegranate juice (240 mL)	500 mg	single dose	18				78

dx.doi.org/10.1021/jf204742e | *J. Agric. Food Chem.* 2012, 60, 6648–6657

Table 2. Phenolic Biomarkers of the Consumption of a Specific Food or Food Group

food	biomarker	sample	*N*	food survey	correlation (*r*)	*P*	ref
vegetables and fruit	total flavonoids	24 h urine	12	5 days dietary recall	0.86	<0.001	79
		morning urine			0.59	<0.001	
	total flavonoids	24 h urine	94	3 days dietary recall	0.35	<0.001	80
	total polyphenols	spot urine	60	FFQ	0.48	0.01	55
	naringenin	24 h urine	94	3 days dietary recall	0.30	0.004	80
	hesperetin				0.38	<0.001	
	tamarixetin				0.27	0.01	
	isorhamnetin				0.28	0.008	
vegetables	quercetin	24 h urine	94	3 days dietary recall	0.28	0.007	80
	enterolactone	24 h urine	53	2 days dietary recall	0.31	0.02	35
fruit	phloretin	24 h urine	94	3 days dietary recall	0.29	0.006	80
	kaempherol	spot urine	53	2 days dietary recall	0.30	0.03	35
	lignans	24 h urine	98	FFQ/2 days dietary recall	0.27	0.008	53
fruit juice	hesperetin	24 h urine	94	3 days dietary recall	0.32	0.002	80
	isohamnetin	spot urine	53	2 days dietary recall	0.30	0.03	35
	naringenin				0.44	0.001	
	hesperetin				0.39	0.004	
	gallic acid				0.33	0.02	
	4-*O*-methylgallic				0.37	0.006	
	naringenin	24 h urine			0.37	0.007	
apple	phloretin	spot urine	53	2 days dietary recall	0.60	<0.001	35
	m-coumaric acid	24 h urine			0.36	0.009	
	isohamnetin				0.31	0.02	
	kaempherol				0.45	<0.001	
	phloretin				0.35	0.01	
grape	naringenin	spot urine	53	2 days dietary recall	0.31	0.02	35
citric fruits	hesperetin	spot urine	53	2 days dietary recall	0.52	<0.001	35
	naringenin				0.56	<0.001	
	hesperetin	24 h urine			0.46	<0.001	
	naringenin				0.37	0.007	
coffee	chlorogenic acid	spot urine	53	2 days dietary recall	0.63	<0.001	35
	caffeic acid				0.29	0.03	
	isoferulic acid	24 h urine	344	FFQ	0.18−0.26	<0.001	81
tea	*m*-coumaric acid	spot urine	53	2 days dietary recall	0.44	0.001	35
	chlorogenic acid				0.31	0.03	
	gallic acid				0.45	<0.001	
	4-*O*-methyl gallic				0.54	<0.001	
	4-*O*-methylgallic	24 h urine	344	FFQ	0.50−0.57	<0.001	81
	epigallocatechin-3-gallate	Plasma	124	weighted record	NA	<0.001	70
	epicatechin-3-gallate				NA	<0.001	
virgin olive oil	tyrosol and hydrohytyrosol	24 h urine	12	weighted record	NA	<0.05	82
		24 h urine	12	weighted record	NA	<0.01	83
wine	gallic acid	spot urine	53	2 days dietary recall	0.45	<0.001	35
	4-*O*-methylgallic				0.37	<0.006	
	caffeic acid	24 h urine			0.38	0.005	
	gallic acid				0.70	<0.001	
	4-*O*-methylgallic				0.52	<0.001	
	resveratrol metabolites	Spot urine	52	FFQ	0.65	<0.001	34
			1000		0.90	<0.001	24
legumes	isoflavones	24 h urine	19	3 days dietary recall	0.67	<0.01	84

dx.doi.org/10.1021/jf204742e | *J. Agric. Food Chem.* 2012, 60, 6648−6657

Table 2. continued

food	biomarker	sample	N	food survey	correlation (r)	P	ref
	lignans				0.49	<0.05	
soy and soy products	isoflavones	24 h urine	60	FFQ	0.5	<0.001	85
	isoflavones, equol, and O-DMA	48 h urine	312	FFQ	0.30−0.60	<0.01	52
	isoflavones	24 h urine	98	FFQ/5 days dietary recall	0.39	<0.001	53
boiled soy	isoflavones	24 h urine	19	3 days dietary recall	0.76	<0.001	84
	lignans				0.85	<0.001	
soy products	isoflavones	24 h urine	19	3 days dietary recall	0.59	<0.01	84
soy protein	isoflavones	morning urine		3 days dietary recall	0.61	<0.001	86
soy and soy products	daidzein and genistein	plasma	80	FFQ/7 food diary	0.28−0.78	<0.05	72
integrals wheat and rye	alkylresorcinols	plasma	30	weighted 3 days dietary record	0.58	<0.001	51
rye bran flakes	alkylresorcinol metabolites	plasma 24 h urine	16	weighted record	NA	<0.001	87
cereal fiber	alkylresorcinol metabolites	24 h urine	56	3 days dietary recall	0.37−0.41	0.002−0.005	88
		plasma			0.26−0.41	0.001−0.052	
		plasma	56	5 days record	0.41−0.46	<0.002	88

PRESENT OF PHENOLIC BIOMARKERS

Over the past 5 years, several research groups have tried to establish phenolic metabolites as biomarkers of dietary phenolic exposure or biomarkers of phenolic-rich food intake. It has been possible to identify a number of biomarkers due to the increase in knowledge about phenolic pharmacokinetics, the increased food composition data on phenolic compounds now in existence, and, finally, the improvement in the methods used for analysis.

Pharmacokinetic studies, as previously described, have reported that phenolics mainly enter biofluids as metabolites. Moreover, their half-lives are between 1 and 12 h in plasma (short-term biomarkers) and between 1 and 5 days in urine (medium-term biomarkers), although there are no phenolic biomarkers of long-term intake (weeks or months), as with selenium in toenails.[33] Although most of these studies calculated the half-life using single doses of phenolics or phenolic-rich foods, in fact, the intake of phenolic compounds is usually chronic. Thus, phenolics could be accumulated in the body and slowly released into the bloodstream over a longer period of time. Therefore, after a chronic phenolic intake, plasma and urinary levels could be more useful biomarkers than after a single dose, particularly in epidemiological studies.

A comprehensive understanding of food composition data is also essential to accurately estimate phenolic exposure. In nutritional epidemiology, phenolics have been proposed as biomarkers of (a) the consumption of a phenolic or group of specific phenolics and (b) the consumption of a specific food or food group (phenolic-rich foods). In the first biomarker group, food composition data are essential to estimate phenolic intake and, therefore, validate the biomarkers. In the second case, it is essential to know if a specific phenolic compound occurs only in a single food or food subgroups; for example, some phenolics are related to the consumption of some phenolic-rich foods such as tea, coffee, wine, soybeans, or onions.[22]

The discovery and assessment of nutritional biomarkers greatly depend on appropriate analytical tools being used, and these should enable metabolites at very low levels in different biological samples to be detected, revealing slight changes in their concentration. New analytical methods and new statistical analyses have brought about two complementary approaches to searching for new biomarkers: (a) a targeted approach and (b) an untargeted or metabolomic approach.

Targeted Approach. In the targeted approach, you know which specific phenolic metabolites you are looking for. This approach is based on knowledge of the food composition. You know which phenolic is specific to/characteristic of the food or food group of interest, and you look for this phenolic or its metabolites in the biofluid. Furthermore, it is also useful when you want to know the intake of a specific phenolic or a phenolic subgroup, because you can look for its metabolites in the biofluid.

Until now, this has been the "classic" way to look for new nutritional biomarkers. A recent paper evaluated urinary phenolic metabolites as biomarkers of phenolic intake in humans.[23] This systematic review suggested that there are three kinds of phenolic biomarker groups: (a) biomarkers with a high urinary recovery (12−37%) and a high dose response (Pearson's correlation coefficients = 0.67−0.87), such as daidzein, genistein, glycitein, enterolactone, and hydroxytyrosol; (b) biomarkers with a very low urinary recovery (0.06−0.2%) and a high dose response (Pearson's correlation coefficients = 0.80−0.95), such as anthocyanidins; (c) biomarkers with a low urinary recovery (1−7%) and a weak dose response (Pearson's correlation coefficients = 0.21−0.52), such as hesperidin, naringenin, epicatechin, and quercetin. The last group is the most abundant. However, there have not been many clinical studies assessing the same phenolic biomarker (fewer than five studies for each phenolic compound), and most of these were carried out on only a few subjects (fewer

than 10 subjects for each study), with the exception of isoflavones.

Table 1 summarizes studies assessing phenolic metabolites in plasma as biomarkers of phenolic intake in humans. Plasma levels of isoflavones, lignans, and their metabolites were widely used as biomarkers of phytoestrogen intake in several clinical and epidemiological studies, although the correlation coefficients between both measures are usually weak (r = 0.3−0.5). Furthermore, some flavan-3-ol monomer metabolites were also evaluated as possible biomarkers of phenolics from tea or chocolate intake. Meanwhile, the remaining plasma biomarkers were tested in only small clinical studies with little to moderate success.

However, most phenolic metabolites were assessed as biomarkers of phenolic-rich food intake (Table 2). There are usually some phenolic biomarkers present in any food intake. For example, wine consumption was related to the urinary levels of resveratrol metabolites, gallic acid, 4-*O*-methylgallic acid, and caffeic acid.[23,34,35] Despite the relatively high number of phenolic biomarkers, only a few of them have been validated in different studies and populations and these by assessing a large number of subjects. The classic example is isoflavones and their metabolites as biomarkers of soy consumption. In addition, resveratrol metabolites, alkylresorcinols, and epigallocatechins are useful biomarkers of wine, whole cereals, and tea, respectively.

Untargeted or Metabolomic Approach. Metabolomics aims to provide a comprehensive picture of the chemical composition of a biological sample (metabolome).[36] The metabolome (i.e., human metabolome) is influenced by several factors, both intrinsic, such as gender, genotype, and age, and extrinsic, such as stress, physical activity, and diet.[37] Among these, diet has been highlighted as a particularly important factor to consider in metabolomic studies due to its impact on the biochemical modifications of different biofluid and tissue metabolomes.[38] Such dietary modifications are related to the exogenous metabolites from dietary food components or from the microbiota metabolism.[37] The part of the metabolome that is related to diet has been defined as the "nutrition metabolome" or "food metabolome".[37,39,40] Inside this particular portion of the metabolome would be all those metabolites from dietary phytochemicals. An important challenge for nutritional metabolomics is to develop new biomarkers or biomarker patterns that allow the intake of dietary components such as phenolics or phenolic-rich foods to be monitored.

In this context, Van Dorsten et al. applied metabolomics to study the differences between the consumption of black tea and green tea, finding that compounds derived from the colonic microbial metabolism of phenolics such as hippuric acid and 1,3-dihydroxyphenyl-2-*O*-sulfate were important markers of the consumption of both types of tea.[41] Walsh et al. investigated the influence of the acute consumption of phytochemicals in the human urinary metabolic profile.[42] The authors concluded that acute changes in the metabolomic profile occurring after the consumption of dietary phytochemicals could be based on the excretion of hippuric acid. When applying metabolomics for the study of the metabolism of phenolic compounds by the intestinal microbiota, Grün et al. identified 10 metabolites that significantly increased in concentration after the consumption of 800 mg of red wine and grape juice phenolics for 4 weeks.[43]

Llorach et al. applied metabolomics to explore urinary metabolome modifications after cocoa powder intake, an important source of procyanidin (polymers of (epi)catechin), in a randomized, crossover, and controlled trial with 10 volunteers.[44] An important part of the metabolome modifications was related to the phenolic metabolites, both human metabolites and, mainly, those produced by gut microbiota such as hydroxyphenylvalerolactones and hydroxyphenylvaleric acids. Another important source of procyanidins is almonds, mainly in the skin of these fruits. Llorach et al., applying metabolomics in an experiment with two groups (placebo and single dose of almond skin extract) identified (putatively) 34 metabolites (biomarkers) related to almond skin extract intake, including host and, in particular, phenolic microbiota derivative metabolites.[45]

Recently, Tulipani et al. applied metabolomics to study the urinary changes in volunteers with metabolic syndrome following 12 weeks of nut consumption.[46] The metabolomic approach revealed 20 potential markers of nut intake including serotonin and fatty acid metabolites, phase II, and microbial-derived phenolic metabolites. The authors concluded that the detection of urinary markers was related to phenolic gut microbial metabolism and phase II metabolism of nut phenolics, thereby reinforcing the need to fully elucidate the bioavailability of these compounds before future interpretations regarding the health effects of nut consumption. Again recently, van Duynhoven et al. reviewed the metabolic fate of phenolics in the human superorganism, highlighting the growing interest in applying metabolomics (among other approaches) to the study of the metabolic impact of phenolic consumption in humans.[47]

FUTURE OF PHENOLIC BIOMARKERS

So far, many biomarkers have been established in small clinical trials, especially in studies assessing a high single dose and, usually, using 24 h urine samples. In the near future, it would be advantageous to apply these biomarkers in epidemiological studies, with chronic nutritional doses, using the usual biofluids collected in epidemiological studies, such as plasma or morning urine.

Due to the improvement in the sensitivity and specificity of the analytical techniques employed, studies assessing phenolic biomarkers can reduce doses to nutritional levels, particularly with phenolic-rich foods. For example, the first studies with resveratrol were carried out with a single dose of 25 mg (around 0.357 mg/kg).[48,49] However, later studies reduced this amount to 5.4 mg (around 0.077 mg/kg), consumed in 250 mL of red wine.[50] Nowadays, most studies are already being carried out with doses at nutritional levels.

Another consideration is to provide biomarkers of chronic intakes, because phenolics and phenolic-rich foods are generally consumed almost daily. In the case of wine and resveratrol, after 28 days of dietary supplementation with 300 mL/day of sparkling wine (0.357 mg/day), 200 mL/day of white wine (0.398 mg/day), or 200 mL/day of red wine (2.56 mg/day), statistically significant urinary levels of resveratrol metabolites were found among all treatments (the three wine supplementations and the wash-out periods).[34] Another example is the ability of plasma alkylresorcinol biomarkers to discern between two treatments with refined cereals or integral cereals after an intervention of 6 weeks.[51]

The next step is the validation of these biomarkers in large clinical trials or small controlled epidemiological studies. In these kinds of studies a large number of participants are required, more than 50 subjects, to provide a wide range of

phenolic intakes for accurate assessment of the dose−response curves. To our knowledge, only the well-known isoflavones[52] and lignans,[53,54] and the more recent resveratrol[24] and total phenolics,[55,56] have been validated as biomarkers at an epidemiological level. In a validation study, the phenolic biomarker is compared to the classical gold standard. However, there are no available gold standards for phenolic or phenolic-rich food intakes, and for this reason, nutritional biomarkers are usually compared to self-reported questionnaires. Despite efforts to estimate the intake of phenolics accurately using the best dietary questionnaires, such as multiple 24 h recalls, 3 day records, and validated food frequency questionnaires, these still present some methodological limitations and contain measurement errors.[8−10] Therefore, a weak correlation between biomarker and dietary intake could be due to biological and analytical variabilities, dietary measurement errors, or the weak association between the two.

When the biomarker is validated, it is frequently converted to the gold standard and used to validate FFQs in large epidemiological studies. In the phenolic field, both isoflavone and lignan biomarkers have already been used.[12,57] However, one of the limitations of the use of phenolic biomarkers at epidemiological level is the biological specimen used. Most of the phenolic biomarkers have used 24 h urine samples, but this is not practical in large-scale studies.[18] Therefore, new biomarkers should be discovered in plasma or morning urine.

In the near future, more metabolomic studies at small/moderate epidemiological levels will be needed. Untargeted approaches can discover new biomarkers that can be validated and, later on, used in large epidemiological studies. One of the advantages of metabolomic studies is that any biological sample can be used. A combination of both an untargeted and targeted approach is necessary because an untargeted approach could produce new biomarkers (often producing qualitative concentrations) and a targeted approach allows us to obtain a "real" quantification of these biomarkers. Therefore, these biomarkers can be easily applied in epidemiological studies.

Biomarkers show great promise in providing a more accurate and objective measure of dietary intake; however, phenolic metabolites have a short half-life, and the analytical methods used are relatively expensive. Moreover, in cohort studies biological samples are usually collected only at baseline, even though the long-term dietary intake is to be evaluated. For these reasons, other strategies for quantifying nutrient exposures have recently been developed, using data from self-reported questionnaires together with nutritional biomarkers. Combining both methods can prove to be a powerful tool, because the errors associated with each are more likely to be reduced.[58] There are a couple of strategies to help deal with these variables, depending on whether data about the biomarker are available for the entire sample or only for a subsample.

First, the exposure is measured twice (via questionnaire and biomarker) in the whole population, but typically these two types of data are not used together because of collinearity problems. Therefore, statistical approaches for multiple measures of an exposure would be useful.[18] For example, the method of triads is a typical methodology used for assessing measurement errors and estimating real intake using combined data from FFQs, 24 h recalls, and biomarkers.[59,60] Another possibility is to use Howe's score,[61,62] in which participants are ranked from lowest to highest value for reported dietary intake and biomarker level. The two scores are then added and the quantiles created. To our knowledge, this has not yet been applied in the field of phenolic compounds.

Second, data from the questionnaires of all participants and biomarkers of only a subsample are made available. This situation is more common than the previous one because biomarkers are usually expensive. In this case, biomarkers are used to calibrate and/or validate nutrient consumption from self-reported questionnaires. FFQs are calibrated to correct for measurement bias in estimated measures of diet−disease association.[63] FFQs are typically validated/calibrated using a 24 h recall, but the errors in FFQs may be correlated with errors in 24 h recalls.[64] Therefore, the use of nutritional biomarkers becomes important because biomarker measurement errors are independent from the errors associated with self-reported data.[20] This is the best approach to take advantage of both questionnaires and biomarkers. Any combination of both methods provides a more effective analysis of the true exposure than one method used on its own.[18,65] In the near future, the challenge is to develop or adapt biostatistical methods to combine both kinds of data effectively.

CONCLUSIONS

Although self-reported questionnaires are not the most precise method for estimating dietary phenolic intake, they are still widely used in nutritional epidemiology. Nutritional phenolic biomarkers offer a more accurate exposure, but they are expensive and few are available. For these reasons, more accurate analytical methods for the identification and quantification of the phenolic compounds and new biomarkers of both phenolics and phenolic-rich foods are still needed. The untargeted and targeted approaches can provide new biomarkers that can fulfill the criteria to be established as validated ones. Moreover, it is also important to highlight the fact that two independent assessment methods (biomarkers and questionnaires) provide a more accurate estimation than only one. Therefore, the challenge is to combine the data from both estimations to increase our understanding of the association between diet and the risk of disease.

AUTHOR INFORMATION

Corresponding Author

*Phone: +34 934034840. Fax: +34 934035931. E-mail: candres@ub.edu.

Funding

This work was supported by the Spanish Ministry of Health (ISCIII RETICC RD06/0020) and the INGENIO−CONSOLIDER Program, AGL2009-13906-C02-01 and AGL2010-10084-E. R.Z.-R. is grateful to the Spanish postdoctoral program Sara Borrell from the Ministry of Science and Innovation and ISCIII (CD09/00133). R.Ll. thanks the Ramon y Cajal program from this Ministry and Fondo Social Europeo (FSE).

Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

FCT, Food Composition Table; FFQ, Food Frequency Questionnaires.

REFERENCES

(1) Beecher, G. R. Overview of dietary flavonoids: nomenclature, occurrence and intake. *J. Nutr.* **2003**, *133* (10), 3248S−3254S.

(2) Kampa, M.; Nifli, A. P.; Notas, G.; Castanas, E. Polyphenols and cancer cell growth. *Rev. Physiol. Biochem. Pharmacol.* **2007**, *159*, 79−113.
(3) Yao, L. H.; Jiang, Y. M.; Shi, J.; Tomas-Barberan, F. A.; Datta, N.; Singanusong, R.; Chen, S. S. Flavonoids in food and their health benefits. *Plant Foods Hum. Nutr.* **2004**, *59* (3), 113−122.
(4) Arts, I. C.; Hollman, P. C. Polyphenols and disease risk in epidemiologic studies. *Am. J. Clin. Nutr.* **2005**, *81* (1 Suppl.), 317S−325S.
(5) Knekt, P.; Kumpulainen, J.; Jarvinen, R.; Rissanen, H.; Heliovaara, M.; Reunanen, A.; Hakulinen, T.; Aromaa, A. Flavonoid intake and risk of chronic diseases. *Am. J. Clin. Nutr.* **2002**, *76* (3), 560−568.
(6) Hooper, L.; Kroon, P. A.; Rimm, E. B.; Cohn, J. S.; Harvey, I.; Le Cornu, K. A.; Ryder, J. J.; Hall, W. L.; Cassidy, A. Flavonoids, flavonoid-rich foods, and cardiovascular risk: a meta-analysis of randomized controlled trials. *Am. J. Clin. Nutr.* **2008**, *88* (1), 38−50.
(7) Wang, L.; Lee, I. M.; Zhang, S. M.; Blumberg, J. B.; Buring, J. E.; Sesso, H. D. Dietary intake of selected flavonols, flavones, and flavonoid-rich foods and risk of cancer in middle-aged and older women. *Am. J. Clin. Nutr.* **2009**, *89* (3), 905−912.
(8) Kelemen, L. E. Food frequency questionnaires: not irrelevant yet. *Cancer Epidemiol. Biomarkers Prev.* **2006**, *15* (5), 1054.
(9) Kristal, A. R.; Peters, U.; Potter, J. D. Is it time to abandon the food frequency questionnaire? *Cancer Epidemiol. Biomarkers Prev.* **2005**, *14* (12), 2826−2828.
(10) Tucker, K. L. Assessment of usual dietary intake in population studies of gene-diet interaction. *Nutr. Metab. Cardiovasc. Dis.* **2007**, *17* (2), 74−81.
(11) Chan, S. G.; Ho, S. C.; Kreiger, N.; Darlington, G.; Adlaf, E. M.; So, K. F.; Chong, P. Y. Validation of a food frequency questionnaire for assessing dietary soy isoflavone intake among midlife Chinese women in Hong Kong. *J. Nutr.* **2008**, *138* (3), 567−573.
(12) Frankenfeld, C. L.; Patterson, R. E.; Horner, N. K.; Neuhouser, M. L.; Skor, H. E.; Kalhorn, T. F.; Howald, W. N.; Lampe, J. W. Validation of a soy food-frequency questionnaire and evaluation of correlates of plasma isoflavone concentrations in postmenopausal women. *Am. J. Clin. Nutr.* **2003**, *77* (3), 674−680.
(13) U.S. Departament of Agriculture. *USDA Database for the Flavonoid Content of Selected Foods*, release 3.0 ed.; Beltsville, MD, 2011.
(14) U.S. Departament of Agriculture. *USDA Database for the Proanthocyanidin Content of Selected Foods*; Beltsville, MD, 2004.
(15) U.S. Departament of Agriculture. *USDA Database for the Isoflavone Content of Selected Foods*, release 2.0 ed.; Beltsville, MD, 2008.
(16) Neveu, V.; Perez-Jimenez, J.; Vos, F.; Crespy, V.; du Chaffaut, L.; Mennen, L.; Knox, C.; Eisner, R.; Cruz, J.; Wishart, D.; Scalbert, A. Phenol-Explorer: an online comprehensive database on polyphenol contents in foods. *Database (Oxford)* **2010**, *2010*, bap024.
(17) Perez-Jimenez, J.; Fezeu, L.; Touvier, M.; Arnault, N.; Manach, C.; Hercberg, S.; Galan, P.; Scalbert, A. Dietary intake of 337 polyphenols in French adults. *Am. J. Clin. Nutr.* **2011**, *93* (6), 1220−1228.
(18) Potischman, N. Biologic and methodologic issues for nutritional biomarkers. *J. Nutr.* **2003**, *133* (Suppl.3), 875S−880S.
(19) Jenab, M.; Slimani, N.; Bictash, M.; Ferrari, P.; Bingham, S. A. Biomarkers in nutritional epidemiology: applications, needs and new horizons. *Hum. Genet.* **2009**, *125* (5−6), 507−525.
(20) Kaaks, R. J. Biochemical markers as additional measurements in studies of the accuracy of dietary questionnaire measurements: conceptual issues. *Am. J. Clin. Nutr.* **1997**, *65* (4 Suppl.), 1232S−1239S.
(21) Tasevska, N.; Runswick, S. A.; McTaggart, A.; Bingham, S. A. Urinary sucrose and fructose as biomarkers for sugar consumption. *Cancer Epidemiol. Biomarkers Prev.* **2005**, *14* (5), 1287−1294.
(22) Spencer, J. P.; bd El Mohsen, M. M.; Minihane, A. M.; Mathers, J. C. Biomarkers of the intake of dietary polyphenols: strengths, limitations and application in nutrition research. *Br. J. Nutr.* **2008**, *99* (1), 12−22.
(23) Perez-Jimenez, J.; Hubert, J.; Hooper, L.; Cassidy, A.; Manach, C.; Williamson, G.; Scalbert, A. Urinary metabolites as biomarkers of polyphenol intake in humans: a systematic review. *Am. J. Clin. Nutr.* **2010**, *92* (4), 801−809.
(24) Zamora-Ros, R.; Urpi-Sarda, M.; Lamuela-Raventos, R. M.; Estruch, R.; Martinez-Gonzalez, M. A.; Bullo, M.; Aros, F.; Cherubini, A.; Andres-Lacueva, C. Resveratrol metabolites in urine as a biomarker of wine intake in free-living subjects: The PREDIMED Study. *Free Radical Biol. Med.* **2009**, *46* (12), 1562−1566.
(25) Day, A. J.; Williamson, G. Biomarkers for exposure to dietary flavonoids: a review of the current evidence for identification of quercetin glycosides in plasma. *Br. J. Nutr.* **2001**, *86* (Suppl. 1), S105−S110.
(26) Linseisen, J.; Rohrmann, S. Biomarkers of dietary intake of flavonoids and phenolic acids for studying diet-cancer relationship in humans. *Eur. J. Nutr.* **2008**, *47* (Suppl. 2), 60−68.
(27) Merken, H. M.; Beecher, G. R. Measurement of food flavonoids by high-performance liquid chromatography: a review. *J. Agric. Food Chem.* **2000**, *48* (3), 577−599.
(28) Manach, C.; Donovan, J. L. Pharmacokinetics and metabolism of dietary flavonoids in humans. *Free Radical Res.* **2004**, *38* (8), 771−785.
(29) Manach, C.; Williamson, G.; Morand, C.; Scalbert, A.; Remesy, C. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *Am. J. Clin. Nutr.* **2005**, *81* (1), 230S−242S.
(30) Hollman, P. C.; van Trijp, J. M.; Buysman, M. N.; van der Gaag, M. S.; Mengelers, M. J.; de Vries, J. H.; Katan, M. B. Relative bioavailability of the antioxidant flavonoid quercetin from various foods in man. *FEBS Lett.* **1997**, *418* (1−2), 152−156.
(31) Lu, L. J.; Grady, J. J.; Marshall, M. V.; Ramanujam, V. M.; Anderson, K. E. Altered time course of urinary daidzein and genistein excretion during chronic soya diet in healthy male subjects. *Nutr. Cancer* **1995**, *24* (3), 311−323.
(32) Rechner, A. R.; Kuhnle, G.; Bremner, P.; Hubbard, G. P.; Moore, K. P.; Rice-Evans, C. A. The metabolic fate of dietary polyphenols in humans. *Free Radical Biol. Med.* **2002**, *33* (2), 220−235.
(33) Marshall, J. R. Methodologic and statistical considerations regarding use of biomarkers of nutritional exposure in epidemiology. *J. Nutr.* **2003**, *133* (Suppl. 3), 881S−887S.
(34) Zamora-Ros, R.; Urpi-Sarda, M.; Lamuela-Raventos, R. M.; Estruch, R.; Vazquez-Agell, M.; Serrano-Martinez, M.; Jaeger, W.; Andres-Lacueva, C. Diagnostic performance of urinary resveratrol metabolites as a biomarker of moderate wine consumption. *Clin. Chem.* **2006**, *52* (7), 1373−1380.
(35) Mennen, L. I.; Sapinho, D.; Ito, H.; Bertrais, S.; Galan, P.; Hercberg, S.; Scalbert, A. Urinary flavonoids and phenolic acids as biomarkers of intake for polyphenol-rich foods. *Br. J. Nutr.* **2006**, *96* (1), 191−198.
(36) Dettmer, K.; Aronov, P. A.; Hammock, B. D. Mass spectrometry-based metabolomics. *Mass Spectrom. Rev.* **2007**, *26* (1), 51−78.
(37) Gibney, M. J.; Walsh, M.; Brennan, L.; Roche, H. M.; German, B.; van Ommen, B. Metabolomics in human nutrition: opportunities and challenges. *Am. J. Clin. Nutr.* **2005**, *82* (3), 497−503.
(38) Gu, H.; Chen, H.; Pan, Z.; Jackson, A. U.; Talaty, N.; Xi, B.; Kissinger, C.; Duda, C.; Mann, D.; Raftery, D.; Cooks, R. G. Monitoring diet effects via biofluids and their implications for metabolomics studies. *Anal. Chem.* **2007**, *79* (1), 89−97.
(39) Wishart, D. Metabolomics: applications to food science and nutrition research. *Trends Food Sci. Technol.* **2008**, *19* (9), 482−493.
(40) Manach, C.; Hubert, J.; Llorach, R.; Scalbert, A. The complex links between dietary phytochemicals and human health deciphered by metabolomics. *Mol. Nutr. Food Res.* **2009**, *53* (10), 1303−1315.
(41) Van Dorsten, F. A.; Daykin, C. A.; Mulder, T. P.; Van Duynhoven, J. P. Metabonomics approach to determine metabolic

differences between green tea and black tea consumption. *J. Agric. Food Chem.* **2006**, *54* (18), 6929−6938.
(42) Walsh, M. C.; Brennan, L.; Pujos-Guillot, E.; Sebedio, J. L.; Scalbert, A.; Fagan, A.; Higgins, D. G.; Gibney, M. J. Influence of acute phytochemical intake on human urinary metabolomic profiles. *Am. J. Clin. Nutr.* **2007**, *86* (6), 1687−1693.
(43) Grun, C. H.; Van Dorsten, F. A.; Jacobs, D. M.; Le, B. M.; van Velzen, E. J.; Bingham, M. O.; Janssen, H. G.; Van Duynhoven, J. P. GC-MS methods for metabolic profiling of microbial fermentation products of dietary polyphenols in human and in vitro intervention studies. *J Chromatogr., B: Anal. Technol. Biomed. Life Sci.* **2008**, *871* (2), 212−219.
(44) Llorach, R.; Urpi-Sarda, M.; Jauregui, O.; Monagas, M.; Andres-Lacueva, C. An LC-MS-based metabolomics approach for exploring urinary metabolome modifications after cocoa consumption. *J. Proteome Res.* **2009**, *8* (11), 5060−5068.
(45) Llorach, R.; Garrido, I.; Monagas, M.; Urpi-Sarda, M.; Tulipani, S.; Bartolome, B.; Andres-Lacueva, C. Metabolomics study of human urinary metabolome modifications after intake of almond (*Prunus dulcis* (Mill.) D.A. Webb) skin polyphenols. *J. Proteome Res.* **2010**, *9* (11), 5859−5867.
(46) Tulipani, S.; Llorach, R.; Jauregui, O.; Lopez-Uriarte, P.; Garcia-Aloy, M.; Bullo, M.; Salas-Salvado, J.; Andres-Lacueva, C. Metabolomics unveils urinary changes in subjects with metabolic syndrome following 12-week nut consumption. *J. Proteome Res.* **2011**, *10* (11), 5047−5058.
(47) van, D. J.; Vaughan, E. E.; Jacobs, D. M.; Kemperman, R. A.; van Velzen, E. J.; Gross, G.; Roger, L. C.; Possemiers, S.; Smilde, A. K.; Dore, J.; Westerhuis, J. A.; Van de Wiele, T. Metabolic fate of polyphenols in the human superorganism. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108* (Suppl. 1), 4531−4538.
(48) Soleas, G. J.; Yan, J.; Goldberg, D. M. Measurement of trans-resveratrol, (+)-catechin, and quercetin in rat and human blood and urine by gas chromatography with mass selective detection. *Methods Enzymol.* **2001**, *335*, 130−145.
(49) Walle, T.; Hsieh, F.; DeLegge, M. H.; Oatis, J. E.; Walle, U. K. High absorption but very low bioavailability of oral resveratrol in humans. *Drug Metab. Dispos.* **2004**, *32* (12), 1377−1382.
(50) Urpi-Sarda, M.; Zamora-Ros, R.; Lamuela-Raventos, R. M.; Cherubini, A.; Jauregui, O.; de la Torre, R.; Covas, M.; Estruch, R.; Jaeger, W.; Andres-Lacueva, C. HPLC-tandem mass spectrometric method to characterize resveratrol metabolism in humans. *Clin. Chem.* **2007**, *53* (2), 292−299.
(51) Landberg, R.; Kamal-Eldin, A.; Andersson, A.; Vessby, B.; Aman, P. Alkylresorcinols as biomarkers of whole-grain wheat and rye intake: plasma concentration and intake estimated from dietary records. *Am. J. Clin. Nutr.* **2008**, *87* (4), 832−838.
(52) Atkinson, C.; Skor, H. E.; Fitzgibbons, E. D.; Scholes, D.; Chen, C.; Wahala, K.; Schwartz, S. M.; Lampe, J. W. Overnight urinary isoflavone excretion in a population of women living in the United States, and its relationship to isoflavone intake. *Cancer Epidemiol. Biomarkers Prev.* **2002**, *11* (3), 253−260.
(53) Lampe, J. W.; Gustafson, D. R.; Hutchins, A. M.; Martini, M. C.; Li, S.; Wahala, K.; Grandits, G. A.; Potter, J. D.; Slavin, J. L. Urinary isoflavonoid and lignan excretion on a Western diet: relation to soy, vegetable, and fruit intake. *Cancer Epidemiol. Biomarkers Prev.* **1999**, *8* (8), 699−707.
(54) Lampe, J. W. Isoflavonoid and lignan phytoestrogens as dietary biomarkers. *J. Nutr.* **2003**, *133* (Suppl.3), 956S−964S.
(55) Medina-Remon, A.; Barrionuevo-Gonzalez, A.; Zamora-Ros, R.; Andres-Lacueva, C.; Estruch, R.; Martinez-Gonzalez, M. A.; Diez-Espino, J.; Lamuela-Raventos, R. M. Rapid Folin-Ciocalteu method using microtiter 96-well plate cartridges for solid phase extraction to assess urinary total phenolic compounds, as a biomarker of total polyphenols intake. *Anal. Chim. Acta* **2009**, *634* (1), 54−60.
(56) Zamora-Ros, R.; Rabassa, M.; Cherubini, A.; Urpí-Sardà, M.; Llorach, R.; Bandinelli, S.; Ferrucci, L.; Andres-Lacueva, C. Comparison of 24-h volume and creatinine-corrected total urinarry polyphenol as a biomarker of total dietary polyphenols in the Invecchiare in Chianti study. *Anal. Chim. Acta* **2011**, *704* (1−2), 110−115.
(57) Heald, C. L.; Bolton-Smith, C.; Ritchie, M. R.; Morton, M. S.; Alexander, F. E. Phyto-oestrogen intake in Scottish men: use of serum to validate a self-administered food-frequency questionnaire in older men. *Eur. J. Clin. Nutr.* **2006**, *60* (1), 129−135.
(58) Potischman, N.; Herrero, R.; Brinton, L. A.; Reeves, W. C.; Stacewicz-Sapuntzakis, M.; Jones, C. J.; Brenes, M. M.; Tenorio, F.; de Britton, R. C.; Gaitan, E. A case-control study of nutrient status and invasive cervical cancer. II. Serologic indicators. *Am. J. Epidemiol.* **1991**, *134* (11), 1347−1355.
(59) Kabagambe, E. K.; Baylin, A.; Allan, D. A.; Siles, X.; Spiegelman, D.; Campos, H. Application of the method of triads to evaluate the performance of food frequency questionnaires and biomarkers as indicators of long-term dietary intake. *Am. J. Epidemiol.* **2001**, *154* (12), 1126−1135.
(60) Rosner, B.; Michels, K. B.; Chen, Y. H.; Day, N. E. Measurement error correction for nutritional exposures with correlated measurement error: use of the method of triads in a longitudinal setting. *Stat. Med.* **2008**, *27* (18), 3466−3489.
(61) Freedman, L. S.; Kipnis, V.; Schatzkin, A.; Tasevska, N.; Potischman, N. Can we use biomarkers in combination with self-reports to strengthen the analysis of nutritional epidemiologic studies? *Epidemiol. Perspect. Innov.* **2010**, *7* (1), 2.
(62) Freedman, L. S.; Tasevska, N.; Kipnis, V.; Schatzkin, A.; Mares, J.; Tinker, L.; Potischman, N. Gains in statistical power from using a dietary biomarker in combination with self-reported intake to strengthen the analysis of a diet-disease association: an example from CAREDS. *Am. J. Epidemiol.* **2010**, *172* (7), 836−842.
(63) Kaaks, R.; Riboli, E. Validation and calibration of dietary intake measurements in the EPIC project: methodological considerations. European Prospective Investigation into Cancer and Nutrition. *Int. J. Epidemiol.* **1997**, *26* (Suppl.1), S15−S25.
(64) Kipnis, V.; Midthune, D.; Freedman, L. S.; Bingham, S.; Schatzkin, A.; Subar, A.; Carroll, R. J. Empirical evidence of correlated biases in dietary assessment instruments and its implications. *Am. J. Epidemiol.* **2001**, *153* (4), 394−403.
(65) Marshall, J. R.; Graham, S. Use of dual responses to increase validity of case-control studies. *J. Chronic Dis.* **1984**, *37* (2), 125−136.
(66) Cao, J.; Zhang, Y.; Chen, W.; Zhao, X. The relationship between fasting plasma concentrations of selected flavonoids and their ordinary dietary intake. *Br. J. Nutr.* **2010**, *103* (2), 249−255.
(67) Noroozi, M.; Burns, J.; Crozier, A.; Kelly, I. E.; Lean, M. E. J. Prediction of dietary flavonol consumption from fasting plasma concentration or urinary excretion. *Eur. J. Clin. Nutr.* **2000**, *54* (2), 143−149.
(68) Radtke, J.; Linseisen, J.; Wolfram, G. Fasting plasma concentrations of selected flavonoids as markers of their ordinary dietary intake. *Eur. J. Nutr.* **2002**, *41* (5), 203−209.
(69) Manach, C.; Morand, C.; Gil-Izquierdo, A.; Bouteloup-Demange, C.; Remesy, C. Bioavailability in humans of the flavanones hesperidin and narirutin after the ingestion of two doses of orange juice. *Eur. J. Clin. Nutr.* **2003**, *57* (2), 235−242.
(70) Wang, J. S.; Luo, H.; Wang, P.; Tang, L.; Yu, J.; Huang, T.; Cox, S.; Gao, W. Validation of green tea polyphenol biomarkers in a phase II human intervention trial. *Food Chem. Toxicol.* **2008**, *46* (1), 232−240.
(71) Wiczkowski, W.; Romaszko, E.; Piskula, M. K. Bioavailability of cyanidin glycosides from natural chokeberry (*Aronia melanocarpa*) juice with dietary-relevant dose of anthocyanins in humans. *J. Agric. Food Chem.* **2010**, *58* (23), 12130−12136.
(72) Verkasalo, P. K.; Appleby, P. N.; Allen, N. E.; Davey, G.; Adlercreutz, H.; Key, T. J. Soya intake and plasma concentrations of daidzein and genistein: validity of dietary assessment among eighty British women (Oxford arm of the European Prospective Investigation into Cancer and Nutrition). *Br. J. Nutr.* **2001**, *86* (3), 415−421.
(73) Setchell, K. D.; Brown, N. M.; Desai, P. B.; Zimmer-Nechimias, L.; Wolfe, B.; Jakate, A. S.; Creutzinger, V.; Heubi, J. E. Bioavailability, disposition, and dose-response effects of soy isoflavones when

consumed by healthy women at physiologically typical dietary intakes. *J. Nutr.* **2003**, *133* (4), 1027−1035.
(74) Wiseman, H.; Casey, K.; Bowey, E. A.; Duffy, R.; Davies, M.; Rowland, I. R.; Lloyd, A. S.; Murray, A.; Thompson, R.; Clarke, D. B. Influence of 10 wk of soy consumption on plasma concentrations and excretion of isoflavonoids and on gut microflora metabolism in healthy adults. *Am. J. Clin. Nutr.* **2004**, *80* (3), 692−699.
(75) Ritchie, M. R.; Morton, M. S.; Deighton, N.; Blake, A.; Cummings, J. H. Plasma and urinary phyto-oestrogens as biomarkers of intake: validation by duplicate diet analysis. *Br. J. Nutr.* **2004**, *91* (3), 447−457.
(76) Stalmach, A.; Edwards, C. A.; Wightman, J. D.; Crozier, A. Identification of (poly)phenolic compounds in concord grape juice and their metabolites in human plasma and urine after juice consumption. *J. Agric. Food Chem.* **2011**, *59* (17), 9512−9522.
(77) Simonetti, P.; Gardana, C.; Pietta, P. Plasma levels of caffeic acid and antioxidant status after red wine intake. *J. Agric. Food Chem.* **2001**, *49* (12), 5964−5968.
(78) Seeram, N. P.; Henning, S. M.; Zhang, Y.; Suchard, M.; Li, Z.; Heber, D. Pomegranate juice ellagitannin metabolites are present in human plasma and some persist in urine for up to 48 h. *J. Nutr.* **2006**, *136* (10), 2481−2485.
(79) Krogholm, K. S.; Haraldsdottir, J.; Knuthsen, P.; Rasmussen, S. E. Urinary total flavonoid excretion but not 4-pyridoxic acid or potassium can be used as a biomarker for the intake of fruits and vegetables. *J. Nutr.* **2004**, *134* (2), 445−451.
(80) Nielsen, S. E.; Freese, R.; Kleemola, P.; Mutanen, M. Flavonoids in human urine as biomarkers for intake of fruits and vegetables. *Cancer Epidemiol. Biomarkers Prev.* **2002**, *11* (5), 459−466.
(81) Hodgson, J. M.; Chan, S. Y.; Puddey, I. B.; Devine, A.; Wattanapenpaiboon, N.; Wahlqvist, M. L.; Lukito, W.; Burke, V.; Ward, N. C.; Prince, R. L.; Croft, K. D. Phenolic acid metabolites as biomarkers for tea- and coffee-derived polyphenol exposure in human subjects. *Br. J. Nutr.* **2004**, *91* (2), 301−306.
(82) Miro-Casas, E.; Covas, M. I.; Farre, M.; Fito, M.; Ortuno, J.; Weinbrenner, T.; Roset, P.; de la Torre, R. Hydroxytyrosol disposition in humans. *Clin. Chem.* **2003**, *49* (6 Part 1), 945−952.
(83) Weinbrenner, T.; Fito, M.; Farre, A. M.; Saez, G. T.; Rijken, P.; Tormos, C.; Coolen, S.; De La Torre, R.; Covas, M. I. Bioavailability of phenolic compounds from olive oil and oxidative/antioxidant status at postprandial state in healthy humans. *Drugs Exp. Clin. Res.* **2004**, *30* (5−6), 207−212.
(84) Adlercreutz, H.; Honjo, H.; Higashi, A.; Fotsis, T.; Hamalainen, E.; Hasegawa, T.; Okada, H. Urinary-excretion of lignans and isoflavonoid phytoestrogens in Japanese men and women consuming a traditional Japanese diet. *Am. J. Clin. Nutr.* **1991**, *54* (6), 1093−1100.
(85) Chen, Z.; Zheng, W.; Custer, L. J.; Dai, Q.; Shu, X. O.; Jin, F.; Franke, A. A. Usual dietary consumption of soy foods and its correlation with the excretion rate of isoflavonoids in overnight urine samples among Chinese women in Shanghai. *Nutr. Cancer* **1999**, *33* (1), 82−87.
(86) Maskarinec, G.; Singh, S.; Meng, L.; Franke, A. A. Dietary soy intake and urinary isoflavone excretion among women from a multiethnic population. *Cancer Epidemiol. Biomarkers Prev.* **1998**, *7* (7), 613−619.
(87) Landberg, R.; Aman, P.; Friberg, L. E.; Vessby, B.; Adlercreutz, H.; Kamal-Eldin, A. Dose response of whole-grain biomarkers: alkylresorcinols in human plasma and their metabolites in urine in relation to intake. *Am. J. Clin. Nutr.* **2009**, *89* (1), 290−296.
(88) Aubertin-Leheudre, M.; Koskela, A.; Marjamaa, A.; Adlercreutz, H. Plasma alkylresorcinols and urinary alkylresorcinol metabolites as biomarkers of cereal fiber intake in Finnish women. *Cancer Epidemiol. Biomarkers Prev.* **2008**, *17* (9), 2244−2248.