Flavonoids and Heart Health: Proceedings of the ILSI North America Flavonoids Workshop, May 31–June 1, 2005, Washington, DC[1–4]

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Abstract

This article provides an overview of current research on flavonoids as presented during a workshop entitled, ''Flavonoids and Heart Health,'' held by the ILSI North America Project Committee on Flavonoids in Washington, DC, May 31 and June 1, 2005. Because a thorough knowledge and understanding about the science of flavonoids and their effects on health will aid in establishing dietary recommendations for bioactive components such as flavonoids, a systematic review of the science of select flavonoid classes (i.e., flavonols, flavones, flavanones, isoflavones, flavan-3-ols, anthocyanins, and proanthocyanidins) was presented. The objectives of the workshop were to 1) present and discuss current research on flavonoid intake and the relation between flavonoids and heart health; 2) develop information that could lead to expert consensus on the state-of-the-science of dietary intake of flavonoids on heart health; and 3) summarize and prioritize the research needed to establish the relations between specific flavonoids and heart health. Presentations included the basics of the biology of flavonoids, including the types and distribution in foods, analytical methodologies used to determine the amounts in foods, the bioavailability, the consumption patterns and potential biomarkers of intake, risk assessment and safety evaluation, structure/function claims, and the proposed mechanism(s) of the relation between certain flavonoids and heart health endpoints. Data presented support the concept that certain flavonoids in the diet can be associated with significant health benefits, including heart health. Research gaps were identified to help advance the science.   J. Nutr. 137: 718S–737S, 2007.

Over the past decade, the Food and Nutrition Board of the Institute of Medicine has convened several Dietary Reference Intake (DRI)[20] panels to review the state-of-the-science on various macro- and micronutrients and to provide recommendations

on the amounts of these dietary constituents that an individual should consume daily. This approach provides a set of 4 nutrient-based reference values, including the Estimated Average Requirement (EAR), the Recommended Dietary Allowance (RDA), the Adequate Intakes, and the Tolerable Upper Intake Level.

Flavonoids represent a large class of polyphenolic components that occur in most plant foods; a significant number of flavonoids are purported to have beneficial health effects. The DRI approach has been considered useful for assessing certain bioactive nonnutrient dietary constituents such as dietary fiber, and it may be so for others as well. The first steps in developing dietary reference standards for intakes of these other constituents such as flavonoids include chemically defining the composition of the class of nutrients in foods people consume; determining population intakes of these foods; and determining the health outcomes in populations that consume larger, vs. smaller, amounts of foods with high content of the compounds.

The North American Branch of the International Life Sciences Institute (ILSI North America) Project Committee on Flavonoids held a workshop entitled, "Flavonoids and Heart Health," May 31 and June 1, 2005 in Washington, DC. The objectives of the workshop were to *1*) present and discuss the most current research on flavonoid intake and the relation between flavonoids and heart health; *2*) develop information that could lead to expert consensus on the state-of-the-science on dietary intake of flavonoids on heart health; and *3*) summarize and prioritize the research needed to establish the relationships between specific flavonoids and heart health. Representatives from industry, academia, the governments of the United States and Canada, and scientific associations attended.

The purpose of this article is to summarize the state-of-the-science on flavonoids presented at the workshop and to identify key research gaps that must be addressed to gain consensus on the science that is needed to make dietary recommendations for bioactive components such as flavonoids.

Flavonoid basics

Plants contain a large and heterogeneous group of biologically active compounds, which includes a subgroup of phytochemicals known as phenolic compounds (see **Fig. 1**). Phenolics are secondary metabolites synthesized by plants that are ubiquitous throughout the plant kingdom. Phenolics are present in significant amounts in many commonly consumed fruits, vegetables, grains, herbal products, and beverages. Over 8000 phenolic compounds have been isolated from different natural products, including flavonoids, phenolic acids, coumarins, and tannins. Each group is further divided into subgroups on the basis of chemical structure.

Flavonoids are a family of phenolic compounds with strong antioxidant activity present in fruits, vegetables, and other plant foods. More than 5000 distinct flavonoids have been identified

in plants, and several hundreds are known to occur in commonly consumed foods.

Chemistry, structure, and classification. Flavonoids comprise a large family of compounds synthesized by plants that have a common basic chemical structure shown in **Figure 2**. Structurally, flavonoids consist of 2 aromatic rings (A and B rings) linked by a 3-carbon chain that forms an oxygenated heterocyclic ring (C ring). Differences in the generic structure of the heterocyclic C ring, as well as the oxidation state and functional groups of the C ring, classify flavonoids as flavonols, flavones, flavanones, flavan-3-ols (flavans), and anthocyanins. The isoflavones are characterized by attachment of the B-ring at the 2-position instead of the 3-position. The proanthocyanidins are oligomers of flavan-3-ols. Anthocyanidins are distinguished from other flavonoids as a separate class by virtue of their ability to form flavylium cations (1). The subclasses of flavonoids, their chemical structures, and the prominent food flavonoids within each subclass are listed in **Table 1** (2).

The hydroxyl functional groups found on all 3 rings are potential sites for links to carbohydrates. Flavonoids that are bound to 1 or more sugar molecules are known as flavonoid glycosides, whereas those that are not bound to a sugar molecule are called aglycones. With the exception of flavan-3-ols, flavonoids occur in plants and most foods as glycosides. The structural complexity of flavonoids is further increased with the linking of acetyl and malonyl groups to the sugar conjugates. The combination of flavonoid structures, sugars, and acylation contribute to their complexity and the large number of individual molecules (>5000) that have been identified (2).

Types and distribution of flavonoids in foods. Flavonols are the most widespread flavonoids in foods, and the most prominent flavonols in food are quercetin and kaempferol. They are generally present at relatively low concentrations of ~15 to 30 mg/kg fresh weight. The richest food sources of flavonols are onions, curly kale, leeks, broccoli, apples, and blueberries (3–5). Red wine and tea can also contain a significant amount of flavonols.

Flavones are much less common than flavonols in fruits and vegetables. The prominent flavones in food are luteolin and apigenin. Parsley and celery are the primary food sources.

Flavanones are present in high concentrations in citrus fruits. The main aglycones in citrus are naringenin, hesperetin, and eriodictyol. Orange juice contains between 200 and 600 mg hesperidin/L and 15 to 85 mg narirutin/L. However, the whole fruit can contain up to 5 times as many flavanones as a glass of orange juice because of the presence of the albedo, the white spongy portion of the fruit, which has high flavanone content (5,6).

Isoflavones are flavonoids with structural similarities to estrogens. Soy and soybean-derived products are the main sources of isoflavones in the diet. The 3 soybean isoflavones are genistein (4′,5,7-trihydroxyisoflavone), daidzein (4′,7-dihydroxyisoflavone), and glycitein (7,4′-dihydroxy-6-methoxyisoflavone). Typically, more genistein exists in soybeans and soyfoods than daidzein, and glycitein comprises <10% of the total isoflavone content (7). Isoflavones are naturally present in the soybean primarily in their β-glycoside form (genistein, daidzein, and glycitein).

Flavan-3-ols are present in many fruits such as grape products (e.g., wine and juice), teas (green, black, and oolong), cocoa, and chocolate. They are either monomers (epicatechin and catechin) or oligomers (e.g., proanthocyanidins). Polymers are also sometimes called condensed tannins and are responsible for the astringency of various fruits or fruit-derived products. Catechin and epicatechin are the main flavan-3-ols in fruits and cocoa,
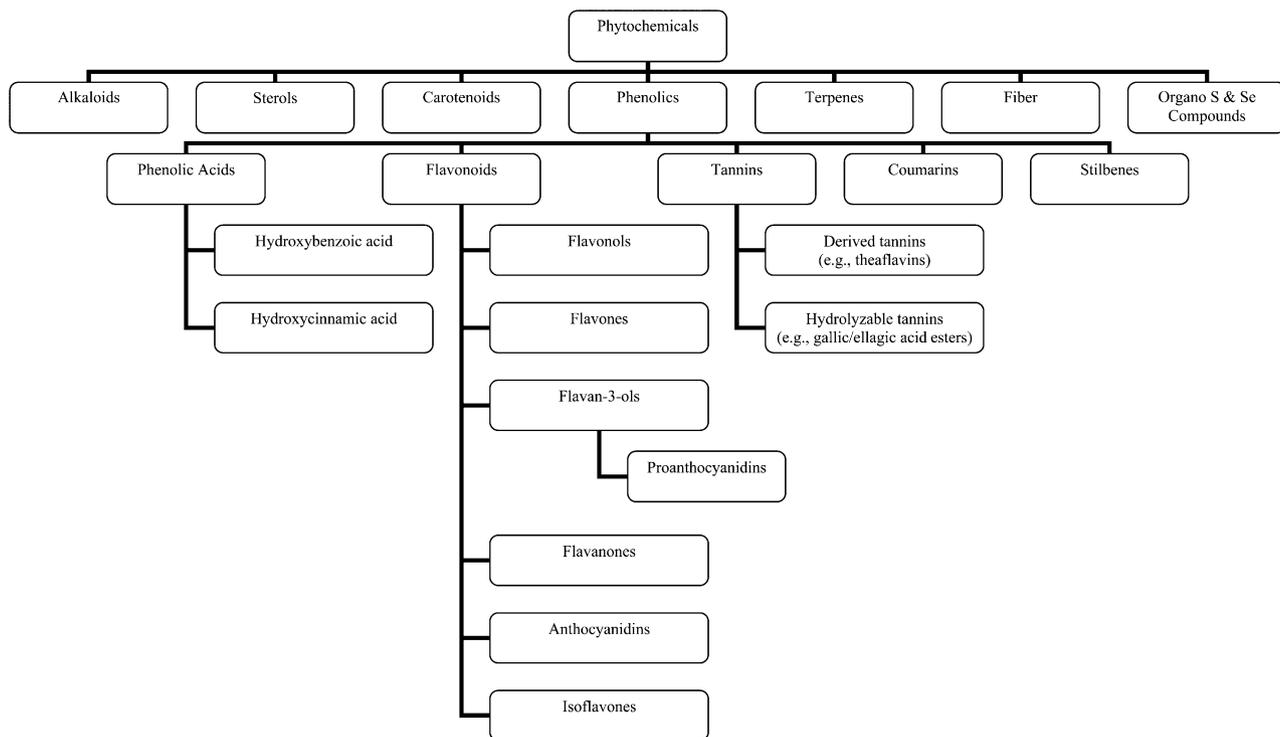
Figure 1 Classification of phytochemicals. Adapted from Liu (288).

whereas epicatechin gallate (ECG), gallocatechin, epigallocatechin (EGC), and epigallocatechin gallate (EGCG) are found in certain seeds of leguminous plants, in grapes, and in tea (8).

Anthocyanins are water-soluble pigments that are responsible for most of the red, blue, and purple colors of fruits, vegetables, and other plant tissues and products. They are most abundant in berries and grapes and are also present in red wine, certain varieties of cereals, and some leafy and root vegetables such as cabbage, beans, onions, and radishes. Cyanidin is the most common anthocyanidin in foods. Black grapes can contain up to 600 mg anthocyanins/100 g, and berries up to 500 mg/100 g (9).

Analytical methodology. The levels of flavonoids in fruits and vegetables are influenced by genotype (cultivar or variety), agronomic practices (irrigation, fertilization, and pest management), climatic and regional conditions (temperature, light, moisture, and ultraviolet radiation), maturity at harvest, postharvest handling, and storage conditions. These variations cause significant challenges for accurately assessing intake of the flavonoids and determining their potential health benefits.

The development of accurate and reliable analytical methods is a key first step for quantification of flavonoids in foods. A large number of analytical methods have been reported for the flavonoids (10). Methods vary in their specificity design; e.g.,

some can measure numerous flavonoids in a single food, whereas others detect just a few flavonoids in many foods. Most methods use extraction using aqueous methanol, ethanol, dimethyl sulfoxide, acetone, or acetonitrile.

Analytical separation is usually accomplished using HPLC with a C18 reverse-phase column, although some derivatization methods have been reported and normal-phase columns have been used for the separation of polymerized flavan-3-ols (11). Ultraviolet/visible (UV/Vis) spectrophotometry using a diode array detector (DAD) and MS are the methods used most frequently. However, neither the DAD nor the MS provides sufficient information for the conclusive identification of individual flavonoids without authentic standards. The lack of authentic standards and/or their prohibitive expense still constitute major obstacles for accurate and comprehensive analysis of most flavonoids (10).

Authentic standards are available for many of the aglycones. Therefore, retention times, UV/Vis spectra, absorption coefficients, molecular ions, and fragmentation patterns are well characterized for the aglycones. Glycosylation alters the absorption coefficient and shifts the wavelength of maximum absorbance. Quantification of the glycosylated flavonoids is only approximate without authentic standards. Hydrolysis of the glycosylated forms to the aglycones is at times employed to allow quantification using aglycone calibration standards. In addition, without authentic standards, conclusive identification of the compounds may require further analytical steps such as tandem mass spectrometry (MS/MS) and NMR.

Simultaneous analysis of multiple flavonoids has been accomplished by the methods of Merken and Beecher (12–14). Merken and Beecher (12) developed a HPLC system for the separation and quantification of 17 flavonoids and their aglycones, representing all 5 subclasses. These flavonoids represent those that are thought to be most prominent in foods commonly consumed. This method was applied to determine the flavonoids (as aglycones) in 59 fresh fruits, vegetables, and nuts by the
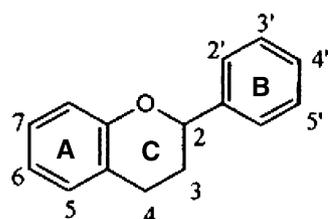

Figure 2 Basic structure of a flavonoid.

TABLE 1 Flavonoid subclasses and their chemical structures, names of prominent food flavonoids, and common food sources

Flavonoid subclass	Chemical structure	Prominent food flavonoids	Common food sources
Flavonols		Isorhamnetin, kaempferol, myricetin, quercetin	Onions, curly kale, leeks, broccoli, blueberries, cherry tomatoes, red wine, tea
Flavones		Apigenin, luteolin	Green leafy spices (e.g., parsley)
Flavanones		Eriodictyol, hesperetin, naringenin	Citrus fruits
Isoflavones		Daidzein, genistein, glycitein	Soybeans, soy foods, legumes
Flavan-3-ols		(+)-Catechin, (+)-gallocatechin, (−)-epicatechin, (−)-epigallocatechin, (−)-epicatechin 3-gallate, (−)-epigallocatechin 3-gallate	Teas, red grapes, red wines, cocoa, chocolate, apricots
Anthocyanidins		Cyanidin, delphinidin, malvidin, pelargonidin, peonidin, petunidin	Red, purple, and blueberries; black grapes, leafy and root vegetables

USDA and the Produce for Better Health Foundation (PBHF). (Results from these analyses will be incorporated into the second release of the USDA flavonoid database.) Sakakibara et al. (14) have developed a method for simultaneously determining a large number of polyphenols in vegetables, fruits, and teas using HPLC and a photodiode array to construct a library of retention times, spectra of aglycones, and respective calibration curves for 100 standard chemicals. They reported results for the most prominent aglycones, glycosylated flavonoids, and phenolic acids for 63 vegetables, fruits, and teas. Robust methods exist for procyanidins and the flavonols, epicatechin and catechin (15,16), and thus these compounds can now be measured in different foods (11).

Bioavailability and bioefficacy. There are several definitions of bioavailability. One defines bioavailability as "the rate and extent to which an ingredient is absorbed and becomes available to the site of action"; this definition includes an element of bioefficacy. The alternative definition, which is often used in nutrition, is "the fraction of the consumed ingredient that appears in the blood circulation." Regardless of which definition is used, the bioavailability of the polyphenols and flavonoids varies among the subclasses.

The process of bioavailability for flavonoids is the liberation and digestion in the stomach and the gastrointestinal tract, transport across the intestinal membrane into the blood, tissue distribution, metabolism and efficacy (biological effects), and last, elimination. During elimination, most polyphenols are absorbed and excreted completely. Current evidence suggests that there are no long-term stores of polyphenols in the body. However, flavonoids do concentrate in tissues at measurable concentrations (17,18), and these concentrations have biological effects.

Several methods have been used to determine the bioavailability of nutrients in humans. These include the postprandial test, the oral-intravenous balance, the oral-fecal balance, and observation of the effects of chronic consumption. The postprandial test is the most frequently used method; it determines the area under the curve (AUC) in the blood after a single dose. The other methods are not useful or suitable for polyphenols.

Digestion, metabolism, absorption, and excretion. Digestion and absorption of polyphenols begin in the stomach. Absorption can depend on dietary fat intake (19), the form ingested (e.g., different extracts containing EGCG), dose, gut transit time, and fecal degradation rate. The aglycones of many polyphenols may be absorbed in the stomach. One exception is anthocyanins (the glycosylated form of anthocyanidins), which are absorbed intact in the stomach (20,21).

Most flavonoid glycosides reach the small intestine intact. The intestinal epithelium, rich in drug-metabolizing enzymes, is important in the metabolism of the different flavonoids. Flavonoid aglycones and flavonoid glucosides (bound to glucose, but the attached glucose is removed during absorption, except for anthocyanins) are absorbed in the small intestine, where they are rapidly taken up and metabolized to form methylated, glucuronidated, or sulfated metabolites (5,22–26). Bacteria that normally colonize the colon also play an important role in flavonoid metabolism and absorption. Flavonoids or flavonoid metabolites that reach the colon may be further metabolized by bacterial enzymes and absorbed. Colonic degradation by the microflora is extensive for procyanidins, the flavonol quercetin, and flavan-3-ols.

Polyphenols known to be particularly well absorbed in humans are isoflavones, followed by quercetin glucosides (flavonol). Proanthocyanidins and the flavan-3-ol EGCG appear to be among the least well-absorbed polyphenols, but this may be a result of instability once absorbed and not of poor absorption (27).

The flavonoids that are absorbed sufficiently to exert a possible effect on cardiovascular parameters in vivo include isoflavones, flavonols, flavanones, and the flavan-3-ols. For these flavonoids, a dose of 50 mg would give rise to a maximum plasma concentration (C_{max}) between 0.4 μM ((−)epicatechin) to 2.5 μM (genistein) (27). Four classes of flavonoids (flavonols, flavanones, isoflavones, and flavan-3-ols) can be predicted to be absorbed in sufficient amounts to exert biological effects even as conjugated forms (28). Some flavanol conjugates, such as quercetin (29) and flavan-3-ols (30,31), substantially inhibit LDL oxidation in vitro (29). The concentrations measured in vivo of intact anthocyanidins and proanthocyanidins are typically thought to be insufficient to exert a predicted biological effect. There are some demonstrated effects in vivo, but only at higher concentrations, and these may be caused by secondary metabolites (22,32).

Metabolites of polyphenols are "lost" from the body via urinary and biliary excretion. Urinary excretion is an important pathway for flavanones, isoflavones, and flavan-3-ols (10% or more of the dose is excreted via urine). Biliary excretion is important for all polyphenols. Flavan-3-ols demonstrate the most variability because EGC has an unusually high C_{max} compared with its AUC value, and EGCG has a very low urinary excretion. However, this may be partially a result of the conversion of EGCG into EGC.

Dietary intake of flavonoids

Consumption. The structural diversity of plant flavonoids, their wide distribution in foods, and variations in their content in a given food are some of the factors that contribute to the difficulty in estimating dietary consumption (33). There are 2 ways to measure flavonoid intake: absolute and relative intakes. Absolute intake is obtained by gathering data on the flavonoid content of foods and calculating the amount that has been ingested by humans. Accurate and comprehensive food composition tables are needed to calculate absolute intakes. Relative intakes are inferred using biomarkers of flavonoid intakes in a biological framework. For example, if blood genistein were a good biomarker of isoflavone intake, then an individual with genistein blood levels twice as high as another individual could be assumed to have higher relative isoflavone intakes, all other things being equal.

In 1976, Kuhnau estimated an average dietary intake of polyphenols in the United States of 1 g/d, which exceeds the intake of other common antioxidants such as vitamin C (90 mg/d), carotenoids (5 mg/d), and vitamin E (12 mg/d). The main sources of polyphenols were fruits and beverages such as tea, coffee, and wine (34). Although these data are from 3 decades ago, they continue to serve as a reference for daily polyphenol intake, even though they are now known to be incomplete, imprecise, and inaccurate. More recent studies of food composition values and dietary records have been used to calculate the dietary intake of individual classes of polyphenols (see **Table 2**).

Although progress has been made in the development of polyphenol profiles of certain foods, the accurate determination of polyphenol intake is hindered by the lack of comprehensive food composition databases. These data are essential to determine the contribution of the different food sources to the total polyphenol consumption and health outcomes. They are also needed to develop epidemiological studies on polyphenols and to compare polyphenol intake to that of other antioxidants or bioactive food components with related biological properties.

Similarly, determination of dietary flavonoid intakes is limited by the accuracy of both the food composition data and the dietary intake data. The latter often include neither the spectrum of individual food items of relevance nor the amounts consumed. However, to the best of current knowledge based largely on Western European data, the primary dietary sources of flavonoids include tea, red wine, fruits, cocoa, chocolate, vegetables, and legumes. Total flavonoid intake varies considerably. In Western populations, crude estimates of average intake appear to be ~65–250 mg/d (5).

Isoflavone intake in Western countries does not usually exceed 1–3 mg/d, but it is somewhat higher in vegetarians consuming soy milk or yogurt and in women treated with isoflavone supplements. The mean daily isoflavone intake among Japanese adults ranges from 25 to 50 mg (expressed as aglycone equivalents). Intake in Hong Kong and Singapore is lower than in Japan, and significant regional intake differences exist for China, although intake in Shanghai appears to be similar to that in Japan (35).

Table 2 presents estimates of daily dietary intake of individual classes of flavonoids. Flavonol intake varies from 13 to 64 mg/d. Flavone intake is low (1–2 mg/d). An intake of 21 to 29 mg/d of flavanones was calculated from 2 German cohorts. Large variations are reported for intakes of flavan-3-ols (catechin/epicatechin), ranging from 4 to 120 mg/d. A consumption of 58 mg proanthocyanidin per day was determined for an average American consumer. Very few data for anthocyanins and proanthocyanidins have been published to date.

TABLE 2 Estimates of polyphenol consumption by adults in different countries

Polyphenols	Country[1]	Intake, *mg/d*	Reference
Flavonols	Netherlands	21–29	Hertog et al. (99); Hertog et al. (267); Geleijnse et al. (108)
	Germany	13.1	Linseisen et al. (268)
	Germany	22.6	Radtke et al. (42)
	Japan	15.3	Kimira et al. (269)
	Japan	15.6	Arai et al. (270)
	Japan	64	Hertog et al. (271)
	Korea	24.6	Park et al. (272)
	UK	26	Hertog et al. (104)
	U.S.	9.4	Chun et al. (266)
Flavones	Netherlands	1.6	Hertog et al. (267)
	Japan	1	Kimira et al. (269)
	Japan	1.1	Arai et al. (270)
	Korea	2.1	Park et al. (272)
	U.S.	1.3	Chun et al. (266)
Flavanones	Germany	21	Linseisen et al. (268)
	Germany	29.4	Radtke et al. (42)
	Finland	20	Knekt et al. (148)
	Greece	31–78	Lagiou et al. (156,273,274)
	Italy	31	Bosetti et al. (275)
	Australia	23	Lyons-Wall et al. (276)
	U.S.	2.7	Chun et al. (266)
Isoflavones	Netherlands	0.9	Boker et al. (277)
	Ireland, Italy, Netherlands, UK	<1	van Erp-Baart et al. (278)
	UK	<1	Jones et al. (279)
	UK	3	Clarke et al. (280)
	Finland	0.8	Valsta et al. (281)
	U.S.	0.5	Chun et al. (266)
	U.S.	0.8	de Kleijn et al. (282)
	U.S.	2.9	Horn-Ross et al. (283)
	U.S.	11	Kirk et al. (284)
	U.S.	14–25	Rimm et al. (103); Yochum et al. (105); Sesso et al. (110)
	Japan	31.7	Wakai et al. (285)
	Japan	39.4	Kimira et al. (269)
	Japan	46.5	Arai et al. (270)
	Japan	63.9	Arai et al. (286)
	Korea	24.6–38	Park et al. (272)
	Finland	4–6	Hertog et al. (271); Knekt et al. (148)
Flavan-3-ols (monomeric)	Germany	11	Linseisen et al. (268)
	Netherlands	50	Arts et al. (151)
	U.S.	4–25	Gu et al. (11); Arts et al. (287)
	U.S.	121	Chun et al. (266)
Anthocyanidins	Germany	6.5	Linseisen et al. (268)
	U.S.	1.3	Chun et al. (266)
Proanthocyanidins	U.S.	58	Gu et al. (11)

[1] UK, United Kingdom; U.S., United States.

Another source of polyphenols is natural or synthetic phenolic food additives, which are added to food for color (e.g., anthocyanin extracts prepared from elderberry and grape skin extracts), flavor (e.g., vanillin), or preservation (e.g., rosemary). The added value differs widely according to the compounds

(e.g., 10 to 20 mg/kg of food for vanillin but up to 3 g/kg of food for anthocyanins).

Food composition tables. Accurate and comprehensive databases are essential for assessing the health impact of flavonoids (2,36). Databases for phytochemicals are particularly critical because the paradigms for characterizing the health effects for the "classical nutrients" and for phytochemicals are somewhat different. In the past, food composition tables were developed to allow assessment of the adequacy of the diet and to support nutritional planning. Each of the nutrients analyzed was included because it was known to be essential for health. The thrust was to prevent deficiencies in individuals and groups as the result of inadequate intakes of the essential nutrients. Today, as we study the relation between food composition and health, databases for estimating nonnutrient compounds such as phytochemicals remain a critical step in determining which food components may promote health and confer disease prevention.

There are 5 major challenges to developing a food composition table for polyphenols. These include structural diversity of the compounds, the large number of dietary sources, the large variability in polyphenol content for a given source, the diversity of analytical methods, and, in some cases, the lack of suitable analytical methods. A sixth and even more serious challenge is the lack of dedicated resources in government, academia, and industry to develop methods and data.

The USDA assembles and maintains the national databases for food composition. Currently, there are 3 special-interest databases for the polyphenols: flavonoids, proanthocyanidins, and isoflavones (37–39). The flavonoid database contains 26 of the most abundant compounds within 5 predominant subclasses of flavonoids (i.e., flavonols, flavones, flavanones, flavan-3-ols, and anthocyanidins) and presents analyses on ~200 foods and teas in various preparation forms.

The Institut National de la Recherche Agronomique in France is currently developing a database on polyphenol content in food that will include content values for over 400 phenolic compounds. This database includes information on flavonoids, phenolic acids, lignans, stilbenes in the form of aglycones, glycosides, and esters (40).

Biomarkers of flavonoid intake. Urine or plasma biomarkers of intake are commonly used in epidemiological studies to compare individual exposures to various nutrients. Often, these biomarkers do not provide absolute values of intake but only relative values and may be influenced by factors affecting bioavailability and interindividual genetic and physiological differences in absorption. However, they do sometimes allow more precise comparisons of relative intakes between and among individuals because they are not dependent on subjectively collected diet records.

The reliability of plasma biomarkers of intake for the polyphenols is often insufficient because of their rapid absorption and elimination following ingestion. This has been clearly shown for isoflavones (41). Modest correlations have been observed between dietary intake from 7-d food records and flavonol and flavanone concentrations in fasting plasma concentrations (42). Microbially produced metabolites of polyphenols formed in the colon (e.g., enterolactone) may be more relevant as plasma biomarkers because their intestinal absorption is spread over a longer period of time (41,43).

Urinary biomarkers may be more useful for comparing individual intakes or exposures to flavonoids. Positive correlations between urinary excretion of specific polyphenols and intake of polyphenol-rich foods have been observed. These include isoflavones with soy foods (44,45), hesperetin with fruit juice (46), a sum of various flavonoids (quercetin, kaempferol, isorhamnetin, tamarixetin, naringenin, hesperetin, and phloretin) with fruits, berries, and vegetables (46,47), and quercetin with vegetables (46).

A rapid liquid chromatography tandem mass spectrometry (LC-MS-MS) method was developed to simultaneously analyze 15 polyphenols including flavonols, flavanones, phloretin, phenolic acids, and mammalian lignans in urine (48) and to identify correlations between urinary excretion (24-h urine or overnight urine) of these polyphenols and the consumption of several foods in 53 free-living adults. Positive correlations were observed between kaempferol and phloretin and apple consumption; herperetin and naringenin and citrus fruit and juice consumption; chlorogenic acid and coffee consumption; and gallic acid and tea and wine consumption (49).

Markers have not been agreed on for anthocyanins or proanthocyanidins. However, a known microbial metabolite of proanthocyanidins, 3-hydroxyphenylpropanoic acid, may be a useful biomarker of proanthocyanidin intake. Its urinary excretion was significantly increased after consumption of 2 proanthocyanidin sources, chocolate and a grape seed extract (50,51). These results suggest that it is possible to use urinary biomarkers to compare individual intake or exposure to flavonols, flavanones, isoflavones, and flavan-3-ols. It is envisioned that urinary biomarkers can complement food composition tables to compare polyphenol intakes in populations.

Risk assessment and safety evaluation of flavonoid intake

Hazard (the potential for causing adverse effects), risk (the probability that adverse effects will occur at a specified dose/level), and safety (the practical certainty that no adverse effects will be observed) must be considered in evaluating the health effects of specific foods. Tools to assess hazard, risk, and safety are available.

Flavonoids can affect a wide range of functions at the tissue, cellular, and molecular levels. However, the hazards and risks of consuming high intakes of these compounds are not well understood because of 1) the large number of compounds that exist in the flavonoid family, 2) the lack of accurate information on dietary flavonoid intake, and 3) the lack of studies aimed at assessing hazard, risk, and safety (52). Although the risk of consuming high doses of polyphenols from naturally polyphenol-rich foods may be low, the negative effects of other ingredients in these foods, such as the alcohol in wine, must be considered. Foods can be fortified with polyphenols; however, they should be consumed by the target populations for which they are designed and not by populations that are potentially at risk, such as children and pregnant women. Dietary supplements that contain high (i.e., pharmacologic) doses of polyphenols can be developed. The intake of polyphenols may then easily reach very high levels; in such cases, toxicologic testing may be required to ensure safe levels of intake (52).

Observational epidemiological studies in appropriate populations with good exposure measurements can assess risks associated with intakes within the "usual" or observed ranges. Epidemiological research offers the strongest evidence currently available on the safety of existing levels of consumption for health outcomes that have been studied, but adverse events are often not tracked in such studies. Because many foods are naturally rich in many of the flavonoids, they are generally recognized as safe (GRAS). If amounts of flavonoid consumption

are maintained at low levels, it is unlikely that detrimental effects will be present.

Hazard identification. Adverse effects of different flavonoids have been observed. These hazards include antinutritional effects, thyroid toxicity, drug interactions, genotoxicity/carcinogenicity, and developmental effects.

Antinutritional effects. Very high concentrations of flavonoids in the diet (e.g., myricetin, quercetin, and EGCG) have been postulated to have antinutritional effects, including inhibition of proteolysis within the gut, reduced glucose uptake, impaired food utilization, and impaired mineral absorption. In one study, 1 mM ECG inhibited d-glucose uptake by 75% in everted rabbit jejunal sacs (53). These effects are most apparent in experimental animals and isolated systems. High flavonoid intakes may also negatively influence lipid and carbohydrate metabolism (53,54). Flavonols inhibited the activity of catechol-O-methyltransferase, resulting in increased norepinephrine concentrations and activation of fat oxidation (55,56). Polyphenols from green tea (e.g., EGCG, ECG) inhibited glucose uptake in rat intestinal everted sacs by competing for the sodium glucose cotransporter 1 (53). Similarly, Song et al. (57) found a flavonoid-mediated inhibition of the facilitated diffusion glucose transporter 2. Daily subcutaneous injections of EGCG for 7 consecutive days significantly reduced blood levels of leptin, insulin, insulin-like growth factor-1 (IGF-1), glucose, and triglycerides in male Sprague-Dawley and Zucker rats. These endocrine and metabolic changes were thought to be secondary to an anorectic effect of EGCG (58). However, the metabolic changes observed following the injection of flavonoids may not reflect those that occur following the ingestion of flavonoids by oral routes. For example, high dietary flavonoid intake is typically associated with increases in leptin production (59) rather than decreases as observed by Kao et al. (58). Although some researchers have contended that the effects of certain flavonoids on glucose uptake may represent a problem, an alternative interpretation is that high amounts of flavonoids in the diet may be beneficial and can result in a slower absorption of glucose from a meal. In theory, this could provide protection against diabetes mellitus and the metabolic syndrome (60).

Although binding and sequestering of metals by flavonoids are often associated with positive health effects, specific flavonoids may also affect metal homeostasis in a negative manner (61). Unlike quercetin and catechin, the isoflavone genistein was not found to bind metal ions in Caco-2 cells but was shown to induce the expression of metallothionein, a metal-binding protein that influences the metabolism of several metals (62). Hurrell et al. (63) have shown that the acute consumption of flavonol-rich beverages such as cocoa, coffee, herb tea, black tea, and red wine significantly impairs dietary nonheme iron absorption in humans after a test meal. They found that consumption of beverages containing 20 to 50 mg total polyphenols per serving reduced iron absorption by 50–70%, whereas those containing 100 to 400 mg total polyphenols per serving reduced iron absorption by 60–90%. It has been suggested that a high consumption of flavonoids may increase the risk of iron depletion in populations characterized by marginal iron status (64). Dietary ascorbate partially prevents the effect of dietary flavonoids on iron uptake. In Western populations consuming heme iron, there is no evidence that dietary flavonoid intake has any effect on iron status or is a risk for anemia (65). In theory, the consumption of large amounts of some flavonoids may also

have a negative effect on the absorption of certain other essential metals, including zinc, copper, and manganese.

Thyroid toxicity. Similar to synthetic antioxidants, several flavonoids have been shown to inhibit thyroid peroxidase and interfere with the biosynthesis of thyroid hormone via free radical iodination (66–68). It has been suggested on the basis of in vitro and animal data that a high intake of flavonoids, particularly isoflavones, increases the risk of thyroid cancer during critical stages of life (69) and can precipitate the development of goiter (69,70). The antithyroid effects of genistein observed in animals are more pronounced when iodine status is inadequate. However, there is no evidence that in healthy adults either soy foods or isoflavones affect thyroid function (71). Although a few cases of goiter resulted from the consumption of soy flour–based infant formula, no cases of goiter have been attributed to soy formula among the ~20 million infants who have used this formula since it was fortified with iodine and the protein source switched from soy flour to isolated soy protein. Furthermore, although Kimura et al. (72) found that the addition of soybean to iodine-deficient diets increased malignant goiter in Wistar rats, Son et al. (73,74) found no effect of isoflavones on thyroid carcinogenesis in male and female rats. Also, in a U.S. case-control study conducted by Horn-Ross et al. (75), isoflavone intake was inversely related to risk of thyroid cancer. Although soy consumption as estimated by isoflavone intake was quite low in this population study, the notion that soy intake could increase risk of thyroid cancer has little foundation.

Drug interactions. Flavonoids may affect drug bioavailability and pharmacokinetics. The most notable of these is naringenin (present in grapefruit juice), which can inhibit cytochrome P450 3A4 (CYP3A4) activity (76–78). Although this alteration does not produce a toxic effect per se, it underscores the need to consider potential drug-flavonoid interactions, which can potentially enhance or reduce drug potencies.

Genotoxicity/carcinogenicity. Some polyphenols may have carcinogenic and genotoxic effects at high doses and concentrations (52). In experimental animals, caffeic acid (79), quercetin (80,81), and tannins (82) are capable of inducing both effects. The genotoxic effects of quercetin observed in vitro may be attributed in part to the fact that at very high concentrations polyphenols can act as strong prooxidants (52). The prooxidant effect of flavonoids in vitro is catalyzed by free copper, ascorbate, and peroxidase activity. In vivo copper is not generally in the free form, and peroxidase is compartmentalized; therefore, the in vitro effects are not likely to occur in vivo. Moreover, it is important to note that the concentrations used in most in vitro studies significantly exceed those that can occur in vivo. Dose-response studies are needed to determine the toxic levels of various polyphenols in animals and cautious studies required in humans.

Developmental effects. The extent to which the inclusion of flavonoids in the maternal diet poses a nutritional problem to the reproductive and developmental processes is poorly understood. Early reports of infertility in sheep and cattle grazing in pastures rich in isoflavones and coumestrol (83) have not been confirmed in nonhuman primates fed soy isolates for 6 mo (84). Also, a recent epidemiological study evaluating the reproductive capacity in adults who were fed soy formula in infancy showed no effects on fertility, miscarriage, abortion, or ectopic pregnancy rates (83). Furthermore, there is much species variation in the

metabolism of, and biological response to, isoflavones, and it is likely that this variation accounts for the infertility problems observed in certain species (85,86). It is well established, for example, that the rodent chow diets used by animal breeders lead to normal reproduction and yet contain large amounts of isoflavone-rich soy meal and produce serum isoflavone levels as high as those found in people who eat soyfoods (87). Also, it is important to recognize in regard to in utero exposure that, in contrast to the rodent, the human fetus is exposed to very high estrogen levels (88). Therefore, it is unlikely that maternal consumption of weakly estrogenic dietary compounds will affect the human fetus, although it may affect the rodent fetus.

Combined results from 3 epidemiological studies showed a positive association between a high consumption of certain foods that contain high levels of potential topoisomerase II inhibitors (e.g., fresh vegetables, herbal tea, cocoa) in mothers and the incidence of acute childhood leukemia in the United States and Canada (89). However, it is important to note that in these studies the consumption of other foods also classified as rich in topoisomerase inhibitors (e.g., black tea, soy products) was not associated with an increased risk.

In the most recent epidemiological study on this issue, flavonoid intakes were associated with an increased risk of infant acute myeloid leukemia. However, the overall risk for all leukemias was reduced (90). Based on the early studies, some researchers have suggested that flavonoid supplements should be restricted during pregnancy and that women should consider restricting their intake of fruits and vegetables during pregnancy (91–93). More than 80% of all infant acute lymphoid and myeloid leukemias are characterized by a rearrangement of the myeloid-lymphoid-leukemia (MLL) gene at chromosome 11, including chromosomes 4 and 9 (94–97). The MLL gene rearrangements seem to result from an inhibition of DNA topoisomerase II activity (91). In a study in which a variety of flavonoids were tested at supraphysiological concentrations ($>25\ \mu M$) in primary hematopoietic cell lines as well as in hematopoietic progenitor cell lines, the inhibition of the activity of DNA topoisomerase II and the induction of MLL gene rearrangements were observed (98). These effects were observed with flavonoids sharing a common conformational structure (i.e., flavones, flavonols, and isoflavones). Preliminary work suggests that epicatechin and catechin do not influence DNA topoisomerase at physiologically relevant levels. Because only select flavonoids have been reported to potentially affect the MLL gene in vitro, more research is needed on the effects of consumption of flavonoid-rich diets during pregnancy, which could promote aberrant chromosomal translocation by inhibiting DNA topoisomerase II activity in the embryo. This research should be limited to foods containing high levels of the specific flavonoids shown to have topoisomerase II inhibitory activity.

Flavonoids and cardiovascular disease

Epidemiological data. Epidemiological studies have examined both the relation between foods rich in flavonoids (such as tea, berries, cocoa, chocolate, and wine) and cardiovascular disease (CVD) and the relation between total flavonoid intake and the risk for CVD (99–114). Epidemiological and experimental evidence is suggestive of a protective relation between consumption of foods rich in flavonoids and risk of CVD. The study of individual flavonoids in an epidemiological context is hindered by the limited amount of food composition data, variability in flavonoid content within a given food, and the intercorrelations among various flavonoids from common food sources. Thus, intake of flavonoid-rich foods is addressed here.

Subsequent sections discuss the relation between individual flavonoids and CVD.

Tea, particularly black tea, consumption is related to coronary heart disease (CHD) and myocardial infarction (MI) in epidemiological studies (107). Heterogeneity of effects across countries is evident with a reduction of CHD risk most evident in continental Europe, perhaps because of differences in the tea used or dose from country to country. Tea consumption across the United States and continental Europe has been estimated to reduce CVD risk by an average of 11% per increase of 3 cups per day (107). However, in the United Kingdom, tea was positively correlated with CVD occurrence as well as with total mortality and cancer deaths (107,115). This is probably a consequence of confounding of results by socioeconomic class because the amount of tea consumed by the upper classes in the United Kingdom is lower than amounts consumed by those in lower socioeconomic classes (104). Fewer studies have examined tea consumption and ischemic stroke or intercranial hemorrhage, but those that have done so suggest protective effects at adequate doses (4 or more cups of tea per day) (102,111,116–119).

Epidemiological studies of wine consumption suggest a consistent dose-response cardiovascular preventive effect (120). This may be stronger and more consistent than that seen with tea in part because of more accurate intake measurement of wine consumption. Most individuals, because of the relatively high cost of wine and a standard bottle size, can estimate their intakes reasonably accurately, resulting in little response bias except at very high intake. Variation then results largely from the differences in flavonoid content between grapes and wine-making methods. With tea, the desired entity is the amount of dry tea matter used to make the tea, which is only crudely expressed when cups of consumption are reported because of differences in cup size, tea type and amount, and dilution factors including the ice in iced tea. The impact of this potential error in epidemiological studies would lead to a corresponding dilution of effect estimates because of the greater measurement error.

Pathogenesis of cardiovascular disease events. Atherosclerosis is a chronic inflammatory disease (121). Atherosclerotic lesions may be present and clinically silent for decades before becoming active and producing clinical events, such as acute MI, unstable angina, or cerebrovascular accident. These events are caused by plaque rupture or erosion, which lead to acute formation of platelet-rich thrombi, which occlude or partially occlude the arterial lumen and go on to produce infarction or ischemia. The underlying causes of plaque vulnerability and rupture remain incompletely understood. However, pathological studies suggest that local inflammation within the plaque, thinning of the fibrous cap, and accumulation of plaque lipid are important factors (122). The extent of thrombosis formation and acute changes in vascular tone may determine the level of ischemia/function.

Many studies have shown that flavonoids demonstrate protective effects against the initiation and progression of atherosclerosis. Studies of grape flavonoids began much earlier than those of tea, chocolate, or pomegranate because of the "French paradox," which was originally described in 1979 (123). The "paradox" is that the French appeared to have a much lower rate of heart attacks despite consumption of diets high in saturated fat. It was postulated that the daily consumption of red wine with meals provided some unexpected cardiovascular protection. Many studies commenced around 1990 to investigate potential explanations for the protective effects of red wine. It is now thought that the flavonoids from red wine, largely flavan-3-ols,

anthocyanins, flavonols, and proanthocyanidins, and not solely the alcohol are the primary protective components. It was demonstrated in vivo that red wine, but not equal amounts of pure alcohol, and alcohol-free purple grape juice inhibited in vivo platelet-mediated experimental coronary thrombosis (124). Grape flavonoids also inhibited ex vivo platelet aggregation in humans (125). Similar to red wine and purple grape juice, the consumption of flavonoid-rich cocoa and chocolate has been associated with a reduction in platelet activity (126).

Endothelial function. Impaired endothelial function may contribute to the development of atherosclerosis and to the conversion from quiescent to active disease (127). The endothelium regulates vascular homeostasis by producing factors that act locally in the vessel wall and lumen, including nitric oxide (NO), a potent vasodilator. NO also prevents adherence of leukocytes to the endothelial surface and prevents platelet adhesion and aggregation (128). In atherosclerosis, effective release of NO is reduced. This change in endothelial function is accompanied by other changes in endothelial phenotype that promote atherosclerosis.

Cardiovascular disease risk factors also have adverse effects on the endothelium. Dyslipidemia, hypertension, diabetes mellitus, smoking, aging, physical inactivity, systemic inflammation, infectious processes, hyperhomocysteinemia, and the postmenopausal state are all associated with endothelial dysfunction (129). Genetic and environmental factors such as diet may also influence the effects of CVD risk factors on endothelial function. Therefore, flavonoid intake may be one of many factors that are important in determining the risk for CVD events.

Prospective studies have shown that endothelial dysfunction is associated with an increased risk of CVD events (130–138). Interventions shown to reduce CVD risk have the ability to reverse endothelial dysfunction, including lipid-lowering therapy (e.g., statins), angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, aldosterone antagonists, weight loss, insulin-sensitizing agents, antioxidants, estrogen, smoking cessation, and exercise (127). These findings suggest that endothelial function may serve as a surrogate marker for CVD risk that is useful for evaluating potential interventions for the prevention or treatment of CVD (139).

Endothelial dysfunction predicts accelerated atherosclerosis and future cardiac events and can be proatherogenic. Improvements in endothelial function have been observed in patients with known coronary disease following daily consumption of flavonoids from purple grape juice (which includes flavan-3-ols, flavonols, proanthocyanidin, and anthocyanins) (140) and from black tea (129,141,142). Similarly, in healthy young adults, endothelial function has been reported to improve following the acute consumption of a flavonol-rich cocoa (143). In vitro studies have shown that the flavonoids in red wine (e.g., flavan-3-ols, flavonols, proanthocyanidin, and anthocyanins) improve endothelial function by up-regulating endothelial nitric oxide synthase (eNOS) expression and increasing production of endothelial cell NO (144).

Postprandial increases in oxidative stress and inflammation. In the postprandial period, lipemia and hypertriglyceridemia increase the production of free radicals. This causes the activation of proinflammatory cells by inducing the translocation of nuclear factor-κB (NF-κB) to the nucleus of circulating mononuclear cells. Consumption of the flavonoids in red wine but not vodka (which has alcohol but lacks any flavonoids) with the meal prevents the activation of NF-κB in circulating

monocytes (145). This result shows that the alcohol has no beneficial effect during the postprandial period but that flavonoids do.

During the postprandial period in people with diabetes, there are even larger increases in free radical production, which depletes serum antioxidant defenses. The grape flavonoids in red wine, when taken with a meal, significantly preserved plasma antioxidant defenses and reduced LDL oxidation (146).

The abnormal proliferation and migration of vascular smooth muscle cells are also a part of atheroma development and intimal thickening. Grape flavonoids retard cell cycle progression of the S phase of mitosis, in part by decreasing expression of the Cyclin A gene. They also inhibit platelet-derived growth factor and the subsequent vascular smooth muscle cell migration by inhibiting the activation of the phosphatidylinositide 3-kinase (PI3K) and p38 MAPK pathways (147).

Science of the flavonoid classes and coronary heart disease protection
Flavonols, flavones, flavanones, and flavan-3-ols.

Observational studies. Epidemiological studies, when well designed with high-quality exposure measurement and appropriately analyzed, can confirm or negate theories about the importance of flavonoids in the diets of free-living individuals. Thus, they are useful to evaluate the human health effects of long-term exposure to physiological concentrations of flavonoids without proving causality. Reliable data on flavonoid contents of foods are needed for epidemiological studies on the potential role of dietary flavonoids in CHD prevention. Comprehensive exposure data are available only for the flavonoid subclasses of flavonols (quercetin, kaempferol, and myricetin), flavones (luteolin and apigenin), and flavan-3-ols [(+)-catechin, (+)-gallocatechin, (−)-epicatechin, (−)-epigallocatechin, (−)-epicatechin gallate, and (−)-epigallocatechin gallate]. The flavonol, flavone, and flavan-3-ol data used in most epidemiological studies were based on analyses conducted in the Netherlands (3,4,8,108) but have been supplemented in some studies with data for additional food items. Recently, data became available for flavanones (hesperetin, naringenin, and eriodictyol) in Finnish foods (148). Except for one study from the United Kingdom (149), all prospective cohort studies of flavonols, flavones, flavan-3-ols, and flavanones are from the Netherlands, Finland, or the United States.

To date, a number of cohort studies on flavonol, flavone, and flavan-3-ol intake and the risk of CHD have been published. Eight of these studies found protective effects of flavonols, flavones, or flavan-3-ols with respect to fatal or nonfatal CHD; risk of mortality was reduced by as much as 65%. These studies were as follows: the Zutphen Elderly Study (flavonols and flavones), with a small cohort of 805 men in the Netherlands (99,149) and a subgroup of 470 men (150); the Dutch Elderly Study (flavan-3-ols) (151); the Iowa Women's Health Study (flavonols and flavones), a cohort study of 34,500 women in the United States (105); the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (flavonols and flavones) among 25,000 male smokers (152); the Rotterdam Study (flavonols and flavones) in the Netherlands, a cohort study of 4800 men and women (108). In a large cohort study of 35,000 male health professionals in the United States, reduced coronary mortality rates were associated with high flavonol intake (flavonols and flavones) only among men with a previous history of CHD (relative risk: 0.63; 95% CI 0.33, 1.20) (103). In the Finnish Mobile Clinic Health Examination Survey (flavonols and flavones), a significant protective effect was found only among men (101). Moreover, Ding et al.

(153) showed in a meta-analysis that flavonoids from chocolate and cocoa may lower CHD mortality.

In contrast, a trend for increased CHD mortality rates (P for trend = 0.12) was found in the Caerphilly Study of Hertog et al. (flavonols and flavones), a cohort study of 1900 Welsh men (104). It was suggested that the English habit of adding milk to tea (the major source of flavonols for this cohort) might inhibit the absorption of flavonols, explaining the lack of protection of tea flavonols against CHD. Proteins bind phenols efficiently and, therefore, might inhibit absorption from the gastrointestinal tract when they are consumed together with flavonoids. However, other studies have suggested that the absorption of flavonols is not impaired with the addition of milk (154,155). Residual confounding by socioeconomic status and lifestyle factors might have affected evaluation of the results of this study among Welsh men.

The only published prospective study on flavanones is The Finnish Mobile Clinic Health Examination Survey (148), which showed no effects of hesperetin or naringenin on CHD risk. In addition, a case-control study in Greece did not show any effects for flavanones on CHD risk (156).

Intervention studies. Eight human intervention studies examined the effects of flavonols on markers for CHD. Studies used pure quercetin aglycone (157,158), pure quercetin glycosides (159,160), onions (161–163), or a variety of flavonol-rich foods (164). Duration of the interventions varied from 1 to 42 d, and the number of subjects from 6 to 19. The daily dose of quercetin equivalents varied from 20 mg, slightly above an average dietary intake, to 250 mg. Favorable effects on a variety of antioxidant biomarkers, such as antioxidant enzymes, plasma antioxidant capacity, resistance to LDL oxidation, reduced DNA damage, and reduced 8-OH-2′-deoxyguanisin were found in several studies (158,159,162,164). However, 1 study showed that other antioxidant biomarkers such as ferric-reducing antioxidant potential (FRAP), malondialdehyde, and isoprostanes as well as resistance to LDL oxidation were not affected (159). It is difficult to explain these discrepancies because of the many differences in study designs, sources of flavonoids, and biomarkers. Plasma lipid profiles, LDL, HDL, and triglycerides, were not affected by the supplements (157,158). Quercetin was shown to inhibit platelet aggregation (160). Possibly the high plasma quercetin concentration of 4.7 μM was necessary to invoke the effect because no effect on platelet aggregation was found in another study where a plasma concentration of only 1.5 μM was reached (163). In a study with parsley, a rich source of apigenin, no effect on platelet aggregation was found (163). However, in another parsley intervention study, some antioxidant biomarkers (glutathione reductase and superoxide dismutase) were favorably affected, whereas others (catalase, glutathion peroxidase, and a plasma protein oxidation marker) did not change (165).

Orange juice and grapefruit juice did not have an effect on platelet aggregation (166). A high intake (750 mL) of orange juice improved plasma lipid profile by significantly increasing HDL without changing LDL in subjects with moderate hypercholesterolemia (167). Citrus peels decreased serum levels of apoprotein B (the structural protein of LDL cholesterol) and triglycerides in animals (6).

The epidemiological data suffer from measurement error, which tends to underestimate effects. Existing studies, particularly those based on individual foods, which are likely to have less measurement error, suggest protective effects of high intakes of flavonols, flavones, and flavan-3-ols on CHD. Flavonols and flavones have been studied most frequently. The only 2 studies on flavanones showed no effects.

In summary, the epidemiological studies that suggest that higher intakes of flavan-3-ols, flavonols, and flavones are associated with a lower risk of CVD should be considered with caution. The studies are food-based analyses of population data, and there is limited ability to do the accurate component-based analysis required to conclude that the observed dietary benefits are truly associated with the flavonoids found in the diet. This is impossible when correlations between flavonoids and other components are high (114).

The human clinical studies aimed at demonstrating the role of dietary flavonoids in cardiovascular health have been aimed at identifying potential mechanistic functions for a role of flavonoids in promoting heart health. These studies show with some consistency that flavonoids may have a role in promoting healthy blood vessel function and in helping to reduce platelet activity. Although these studies are encouraging, no endpoint studies have been conducted, and, in that respect, the clinical evidence is not sufficient to support a role for dietary flavonoids in reducing risk of CVD.

Isoflavones. Although isoflavones are referred to as phytoestrogens, in vivo they often fail to exert estrogen-like effects (168). At the molecular level, isoflavones affect many genes differently—and many different genes than estrogen—and it is well established that estrogen receptor (ER)-binding ligands often have very different, and sometimes opposite, biological effects (169).

Arguably, isoflavones are more accurately classified as selective ER modulators (SERMs) than as phytoestrogens (170,171). The selectivity of isoflavones may stem in part from their preferential binding to and activation of ER-β in comparison to ER-α (172–174). Furthermore, isoflavones, especially genistein, have a variety of nonhormonal properties that affect cell function in ways that are potentially relevant to protection against CHD (175–178). Consequently, even classifying isoflavones as phyto-SERMs is an incomplete characterization.

Initial speculation about the possible coronary benefits of isoflavones can be attributed to research that suggests that isoflavones lower cholesterol (179,180) and to the reported protective effects of estrogen against CHD (181). Although there is conflicting evidence regarding the impact of isoflavones on the serum cholesterol-lowering effects of soy protein (182–184), there is little support for independent hypocholesterolemic effects of isoflavones (185,186). Furthermore, recent clinical trials have failed to find that hormone therapy [estrogen alone (187) or estrogen plus progestin therapy (188)] reduces CHD risk. Nevertheless, animal and epidemiological data suggest that isoflavones may exert coronary benefits. For example, soyfood intake has been found to be inversely related to a coronary event, nonfatal MI in 2 cases (189,190), and fatal CHD in 1 case (191). In 1 prospective study of 65,000 women from Shanghai, the risk of nonfatal MI was reduced by 86% (190). Clearly, the modest cholesterol-lowering actions of soy protein alone could not be responsible for this effect; thus, these findings suggest involvement of isoflavones. Although soy protein may have coronary benefits independent of cholesterol reduction, such as by decreasing blood pressure (192), animal data show that isoflavones directly reduce atherosclerosis (193–195).

Impact of isoflavones on specific CHD risk factors. There are several possible mechanisms by which isoflavones may favorably impact CHD risk. For example, some research suggests isoflavones directly improve the health of the coronary vessels. Walker et al. found that in both men and pre- and

postmenopausal women, genistein, but not daidzein, infused into the brachial artery produced a dose-dependent increase in forearm blood flow (196). Furthermore, equimolar concentrations of 17β-estradiol caused similar vasodilation to genistein, and the responses to both agents were inhibited by a NO synthase inhibitor. Similarly, Italian researchers found that both at 6 mo and after 1 y, oral genistein significantly enhanced endothelium-dependent vasodilation of the brachial artery in comparison to the placebo group and that plasma genistein levels were directly correlated with plasma levels of NO end products (197,198). In agreement with these findings, Cuevas et al. (199) found that vessel dilation increased 9.4% after the consumption of 40 g soy protein containing 80 mg isoflavones in 18 postmenopausal women with hypercholesterolemia, compared with the prestudy value of 5.3% ($P < 0.05$), whereas after consuming 40 g casein there was no change in vascular reactivity. The design of the above study, however, does not permit ascribing the beneficial effects to isoflavones. Furthermore, in contrast to the results from the 3 previously discussed studies, other trials have failed to find that isoflavone-rich soy protein or isolated isoflavones affect endothelial function (200–202). Similarly, although 3 studies found that isoflavones (40 to 80 mg/d) enhanced systematic arterial compliance in postmenopausal women to a similar extent as estrogen (200,203,204), such findings have not been confirmed in other trials (186).

Isoflavones and their metabolites exhibit antioxidant effects in vitro (205), and several studies (206,207) but not all (208–210) suggest that isoflavone-rich soy protein inhibits LDL-cholesterol (LDL-C) oxidation. LDL-C oxidation has been viewed as a factor in CHD etiology, although recent evidence has called this hypothesis into question (211). Interestingly, although Wiseman et al. (207) found that isoflavone-rich soy protein inhibited LDL-C oxidation in comparison to soy protein nearly devoid of isoflavones, isoflavone supplements have not been shown to reduce LDL-C oxidation (212,213), even though they have exhibited antioxidant activity in vivo in human subjects (214). Finally, there are speculative in vitro (215) and animal data suggesting that isoflavones inhibit platelet aggregation (216,217), but thus far this work has not been confirmed in humans (218,219).

Studies have produced inconsistent results regarding the coronary benefits of isoflavones. Thus, no firm conclusions can be made at this time, and further research is clearly warranted. Some inconsistency is not unexpected considering the small sample size of most trials and the marked differences in isoflavone metabolism among subjects (220). Furthermore, many different and possibly unequal isoflavone-related products have been used. Perhaps more consistent results will be obtained if greater emphasis is placed on examining the effects of isoflavones in subjects at high risk of CHD in whom physiological processes related to the etiology of CHD are perturbed.

Anthocyanins. In recent years, numerous studies have shown that anthocyanins display a wide range of biological activities (221,222), including antioxidant (22,223–225), antiinflammatory (226,227), antimicrobial (228), and anticarcinogenic (229–231) activities; improvement of vision (232,233); induction of apoptosis (230); and neuroprotective effects (234,235). In addition, anthocyanins display a variety of other effects on blood vessels (236,237) and platelets (124,238) that may reduce the risk of CHD (123).

Anthocyanidins are potent antioxidants, stronger than classical antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene, and α-tocopherol (223,225). Glycosylation of

anthocyanidin decreases radical scavenger activity compared with the aglycone because it reduces the ability of the anthocyanidin radical to delocalize electrons. In accordance with this, Fukumoto and Mazza (225) reported increased antioxidant activity with an increase in the hydroxyl groups and decreased antioxidant activity with glycosylation of anthocyanidins.

The antioxidant activity (scavenging free radicals, metal chelation, protein binding) of anthocyanins, including the protection of LDL against oxidation, has been demonstrated in a number of different in vitro systems (239–241). Recently, pelargonidin, cyanidin, delphinidin, peonidin, malvidin, malvidin 3-glucoside, and malvidin 3,5-diglucosides were found to have strong inhibitory effects on NO production in LPS/IFN-γ– activated RAW 264.7 macrophage. At the range of 16 to 500 μM, these compounds inhibited NO production by >50% without showing any cytotoxicity (227). Their inhibitory effects were comparable to that of quercetin, which has been extensively studied and shown to exert antiinflammatory and antioxidant effects. Anthocyanin-rich berry extracts also showed considerable inhibitory effects on NO production, and their inhibitory effects were significantly correlated with the content of total anthocyanins. Additionally, Cao et al. (242) reported increased serum antioxidant capacity measured as oxygen radical absorbency capacity, trolox equivalent antioxidant capacity, and total reactive antioxidant potentials after the consumption of strawberries or red wine; and Mazza et al. (22) found that the concentration of anthocyanins in the serum of male subjects who had consumed 1.2 g anthocyanins from freeze-dried blueberries was positively correlated with serum antioxidant capacity.

The association between grape phenolics and CHD has been ascribed in part to the presence of anthocyanins in red wine (243,244). In addition, several epidemiological studies have shown that CHD mortality can be decreased by moderate consumption of red wine (245–247). The primary mechanisms believed to be responsible for this reduction in risk include decreased platelet coagulation (248,249) and elevated circulating high-density lipoprotein cholesterol concentration (246,247). Other mechanisms such as inhibition of lipoprotein oxidation, free-radical scavenging, and modulation of eicosanoid metabolism (250–253) are also thought to play a role in the reduction of atherosclerosis.

A study of the relations among vasodilation capacity, antioxidant activity, and phenolic content of 16 red wines reported that total phenol content correlated well with vasodilation capacity and antioxidant activity of the wines, but only anthocyanins were correlated with vasodilation capacity (254). Other recent studies support the hypothesis that vasodilation activity is connected to skin-derived compounds in the grapes. Andriambeloson et al. (236) found that only the anthocyanin and oligomeric condensed tannin-containing fractions of red wine showed vasorelaxant activity comparable to the original polyphenol fraction of the red wine. The phenolic acid derivatives, hydroxycinnamic acids, and flavonol classes that were tested failed to induce this type of response.

It is apparent that anthocyanins have diverse effects in vitro, which suggest potential health benefits in general, particularly a reduction of CHD. However, until the absorption and metabolic fate of anthocyanins in vivo are unraveled, it would be unwise to conclude that a high consumption of anthocyanins will reduce the risk of chronic disease, including heart disease.

Definite proof can be obtained only by large long-term properly designed intervention trials. In the meantime, the evidence for the benefits connected with consumption of

anthocyanin-rich products should include a more complete knowledge of the identity of anthocyanin metabolites and their tissue distribution using molecular, cell biology, animal, and epidemiological studies. Future in vitro investigations to identify the physiological effects of anthocyanins/flavonoids should be conducted with chemical structures that exist in the circulation (i.e., parent compound and metabolites) and at similar concentrations.

Proanthocyanidins and flavan-3-ols. Proanthocyanidins and flavan-3-ols found in foods such as grapes increase the release of NO and decrease the production of superoxide in aggregating platelets, which limits the size of a developing platelet aggregate (255–257). They also reduce the release of a proinflammatory mediator, CD40-L, from activated platelets (258). This in turn inhibits the proinflammatory responses in the arterial wall (259). Moreover, these flavonoids inhibit the oxidation of both LDL and HDL in vitro (260) and the oxidation of LDL in vivo (259). Proanthocyanidins and flavan-3-ols together may protect LDL from oxidative modification by acting as free radical scavengers or by chelation of transition metals, which generate free radicals (261,262). They also protect and increase serum HDL-C paroxonase by reducing macrophage oxidative stress through inhibition of cellular oxygenases such as nicotinamide adenine dinucleotide phosphate, reduced-form NADPH oxidase, or myeloperoxidase (263).

Activation of matrix metalloproteinases inhibits enzymes involved in plaque weakening leading to plaque rupture and the expression of intercellular adhesion molecule 1 and vascular cell adhesion molecule 1 by endothelial cells, reducing monocyte and granulocyte adhesion (264).

Many studies have been conducted on the combination of proanthocyanidins and flavan-3-ols, but some have failed to show any potential antiatherogenic benefit. This may have resulted from an insufficient amount of total polyphenolic content in these flavonoid classes, the low dose administered, population differences, and/or different risk factors measured.

There is a considerable range of polyphenolic substances in various food sources such as red wine, grape juice, grape extracts, and cocoa. In addition, red wine contains alcohol, which is proinflammatory and a prooxidant. Therefore, it may be that unless the wine has a high content of antioxidant polyphenolics to counteract the negative alcohol effect, it may not be effective. In 1 study, a red wine was given to a group of patients with coronary artery disease and who were known to have endothelial dysfunction. Endothelial function, as measured by flow-mediated dilatation of the brachial artery, became significantly worse in the 2 h following red wine consumption. When the same 15 subjects were given the same wine a week later, but with the alcohol removed, they showed significant improvement in endothelial function (265).

This workshop examined the state-of-the-science on flavonoids and their relation to heart health, and it also identified gaps in the science to provide guidance in the direction of future research. **Table 3** summarizes the current state of knowledge and gaps in common subclasses of flavonoids discussed in this article.

Dietary flavonoids are a subclass of food-based polyphenolic compounds with a wide diversity. They are found in many common foods and herbs. The main dietary sources of flavonoids include cocoa and chocolate, tea (black and green), purple grapes and grape juice, red wine, soy, and citrus fruits. The composition of flavonoids in foods and beverages and the daily dietary intake of individual classes of flavonoids have been the subject of much recent work; however, research gaps remain. The most recent analyses based on NHANES II suggests that flavonoid intake in the American diet is about 120 mg/d, with about 90% from flavan-3-ols (266).

Flavonoids, particularly the flavan-3-ols, flavonols, anthocyanins, and isoflavones, are bioavailable to various degrees. Blood levels are relatively low, reaching only 1–5 μM concentrations. Although these compounds have a relatively short half-life, measurable tissue levels in humans appear to be sufficient for biological activity, and there do not appear to be any safety risks from typical dietary intakes of flavonoid-rich foods. Safety

TABLE 3 State of knowledge and gaps in common subclasses of flavonoids

Flavonoid subclass	Prominent food flavonoids	Common food sources	Database available (yes/no)	Sources of dietary intake data[1]	Biomarkers of intake available (yes/no)
Flavonols	Isorhamnetin, kaempferol, myricetin, quercetin	Fruits, vegetables, tea	Yes	Japan, U.S., UK, Germany, Netherlands, Finland	Yes
Flavones	Apigenin, luteolin	Green leafy spices (e.g., parsley; celery)	Yes	Japan, Korea, Netherlands	Yes
Flavanones	Eriodictyol, hesperetin, naringenin	Citrus fruits	Yes	Germany, U.S., Italy, Finland, Greece, Australia	Yes
Isoflavones	Daidzein, genistein, glycitein, total isoflavones	Soybeans, soy foods, legumes	Yes	UK, U.S., Finland, Japan, Italy, Korea, Netherlands, Ireland	Yes
Flavan-3-ols	(+)-Catechin, (+)-gallocatechin, (−)-epicatechin, (−)-epigallocatechin, (−)-epicatechin 3-gallate, (−)-epigallocatechin 3-gallate	Tea, red grapes, apples, red wine, cocoa, grape juice, chocolate	Yes	Germany, Netherlands, U.S.	Yes
Anthocyanidins	Cyanidin, delphinidin, malvidin, pelargonidin, peonidin, petunidin	Red, purple, and blue berries, grape wine and juice	Yes	Germany	Yes
Proanthocyanidins	Procyanidins	Fruits, vegetables, nuts, cereals, chocolate, grape juice	Yes	U.S.	No

[1] UK, United Kingdom; U.S., United States.

may be an issue for purified flavonoids; however, this would be addressed when GRAS status for food use of these materials is sought.

Epidemiological analyses have found that increasing intake of foods containing dietary flavonoids is associated with a reduced risk of CVD and stroke. However, the epidemiological studies are only suggestive and are not sufficient to conclude that dietary flavonoids are the active agent in these foods responsible for the decreased risk. With improved food composition and food intake data, detailed populated-based studies can be conducted.

Clinical studies on flavonoid-rich foods have not focused on disease endpoints, and most studies have used foods rather than purified flavonoids. These studies have shown that foods rich in dietary flavonoids such as cocoa, tea, purple grape juice, and soy have biological effects within the cardiovascular system. Moreover, most studies have focused on foods rich in flavan-3-ols and flavonols and suggest that these flavonoids are associated with improved endothelial function via improving the NO signal pathway and decreasing platelet function (both in vivo and in vitro) and may have a role in blood pressure control—all toward promoting cardiovascular health. However, these findings are not sufficient to conclude that consumption of foods rich in dietary flavonoids will help reduce CVD risk.

Although an extensive amount of information has been presented on flavonoids, the need for further research in this area is clearly evident. With the expected growth in interest in flavonoids, a better understanding and clearer recommendations concerning the role of flavonoids in heart health endpoints are necessary. Thus, the recommendations for future research are as follows:

Flavonoid basics.

- Develop reference standards and reference libraries for commonly consumed flavonoids.
- Develop sufficient data to set a DRI or to allow for public health recommendations for 1 or more of the subgroups of flavonoids.
- Determine whether a nutrient model, such as the DRI, is appropriate for bioactive components such as flavonoids. This includes, but is not limited to, determining the risk of deficiency and/or excess in certain instances (e.g., specific tissues or special populations).
- Increase data on the pure compounds/metabolites, the flavonoid constituents in foods, and the foods and food patterns. Foods are complex mixtures. Therefore, there is the need to know whether *1*) purified constituents have different effects; *2*) processing increases or decreases the effects; and *3*) doses of compounds have a physiological effect at a certain level.
- Investigate the potentially selective breeding of plants and formulation of foods to increase intakes of various bioactive constituents such as flavonoids.
- Identify the target populations [e.g., the general population at low risk (using a food model) or individuals at high risk (using a drug model)].
- Determine the role the liver plays in the enterohepatic circulation of flavonoids.
- Identify methods to determine the availability of compounds to target tissues.
- Identify whether the flavonoid compounds themselves, their conjugates, or their low-molecular-weight phenolic metabolites cause specific biological effects.

Dietary intake of flavonoids.

- Continue expanding the food composition databases to include new flavonoids, new food sources, cooking effects, fortification, and to show the variability in flavonoid content within food (cultivating, geography, processing).
- Obtain knowledge of the quantitative intake of classes of polyphenols, particularly anthocyanins and proanthocyanidins.
- Evaluate the effects of complete mixtures as compared with those of individual compounds, including bioavailability and the role of the food matrix in bioavailability.
- Identify and validate intake biomarkers for classes of flavonoids that lack them and determine whether these intake biomarkers are sensitive enough to estimate the absolute amounts of flavonoids present in various tissues and fluids.
- Determine if the intake biomarkers are influenced by other modifiers of uptake or metabolism such as smoking, alcohol consumption, gut microflora, or genetic polymorphisms.
- Compare flavonoid intake in different populations following varied diets to identify the main dietary sources in the flavonoid classes.

Risk assessment and safety evaluation of flavonoid intake.

- Determine potential chronic toxicity, carcinogenicity, immunotoxicity, reproductive toxicity, genotoxicity, and intergenerational effects of common flavonoids.
- Identify the metabolic and safety consequences of altering intakes of various flavonoids.

Flavonoids and CVD.

- All flavonoids are not the same regarding health effects. A more precise indication of which flavonoids are expected to affect CVD endpoints needs to be developed.
- Refine theories about the mechanism of action of flavonoids, which specific flavonoid compounds are involved, and develop experimental designs to test for CHD, CVD, and stroke outcomes.
- Determine which flavonoids play a role (and where) in the primary prevention of plaque buildup and in the secondary prevention of later events in CVD development.
- Identify the possible mechanisms for modes of action of flavonoids (e.g., oxidation/antioxidant, platelet aggregation, inflammation, endothelial dysfunction, blood flow).
- Identify valid biomarkers of intake and surrogate markers of health outcomes.

Science of the flavonoid classes.

- Develop a matrix that assesses the evidence and tests all major compounds in the flavonoid classes and their effects on various biomarkers of health outcomes.

Flavonols, flavones, and flavanones.

- Conduct in vitro studies to determine physiological doses of both foods and compounds present in plasma.
- Develop more studies on flavanones and health outcomes.

Isoflavones.

- Develop an efficacy needs assessment for isoflavones.

Anthocyanins.

- Obtain intake data on anthocyanins.

- Develop or update a biomarker for anthocyanin intake.
- Determine the active metabolite(s) of anthocyanin.

Proanthocyanidins and flavan-3-ols.

- Utilize the model system with other flavonoids.
- Develop clinical studies on flavan-3-ols and polymers to determine health outcomes.
- Study the unique effects in the postprandial period.
- Obtain intake data on proanthocyanidins.
- Develop or update a biomarker for proanthocyanidin intake.

Author Disclosures

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