Annu. Rev. Nutr. 2002. 22:19–34
doi: 10.1146/annurev.nutr.22.111401.144957

DIETARY FLAVONOIDS: Bioavailability, Metabolic Effects, and Safety

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Key Words flavonoids, quercetin, adverse effects, catechins, diet

■ **Abstract** Flavonoids comprise the most common group of plant polyphenols and provide much of the flavor and color to fruits and vegetables. More than 5000 different flavonoids have been described. The six major subclasses of flavonoids include the flavones (e.g., apigenin, luteolin), flavonols (e.g., quercetin, myricetin), flavanones (e.g., naringenin, hesperidin), catechins or flavanols (e.g., epicatechin, gallocatechin), anthocyanidins (e.g., cyanidin, pelargonidin), and isoflavones (e.g., genistein, daidzein). Most of the flavonoids present in plants are attached to sugars (glycosides), although occasionally they are found as aglycones. Interest in the possible health benefits of flavonoids has increased owing to their potent antioxidant and free-radical scavenging activities observed in vitro. There is growing evidence from human feeding studies that the absorption and bioavailability of specific flavonoids is much higher than originally believed. However, epidemiologic studies exploring the role of flavonoids in human health have been inconclusive. Some studies support a protective effect of flavonoid consumption in cardiovascular disease and cancer, other studies demonstrate no effect, and a few studies suggest potential harm. Because there are many biological activities attributed to the flavonoids, some of which could be beneficial or detrimental depending on specific circumstances, further studies in both the laboratory and with populations are warranted.

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INTRODUCTION

Overview

Flavonoids have been known as plant pigments for over a century. The first observation regarding their biological activities was published in 1936 by Rusznyak & Szent-Gyorgyi (59; see also 44). Originally proposed to be required as vitamins, the term "vitamin P" for flavonoids was suggested, although this was later dismissed (44). Flavonoids belong to a vast group of polyphenolic compounds that are widely distributed in all foods of plant origin. Plant polyphenols have been of interest to scientists for decades, originally owing to their importance in plant physiology, specifically for their roles in plant pigmentation and flavor. Polyphenols are involved in plant growth and reproduction, provide resistance to pathogens and predators, and protect crops from disease and preharvest seed germination (5). Recently, interest in the possible health benefits of polyphenols (particularly flavonoids) has increased owing to their antioxidant and free-radical scavenging abilities observed in vitro.

Structure/Subclasses

Polyphenolic compounds refer to one of the most numerous and widely distributed groups of substances in the plant kingdom. They are produced as the result of the secondary metabolism of plants and are frequently found attached to sugars (glycosides), thus tending to be water-soluble. Occasionally, polyphenols also occur in plants as aglycones. Polyphenols arise biogenetically from two main synthetic pathways: the shikimate pathway and the acetate pathway (5). More than 8000 polyphenolic structures are currently known, the common feature of which is an aromatic ring bearing at least one hydroxyl substituent (6). Polyphenols can be divided into at least 10 different classes based upon their chemical structure (5).

Flavonoids are the largest class of polyphenols, with a common structure of diphenylpropanes (C6-C3-C6), consisting of two aromatic rings linked through three carbons (Figure 1). Biogenetically, the A ring usually arises from a molecule of resorcinol or phloroglucinol synthesized from the acetate pathway and has a characteristic hydroxylation pattern at the 5 and 7 position (6). The B ring comes from the shikimate pathway and is usually 4′-, 3′4′-, or 3′4′5′-hydroxylated.

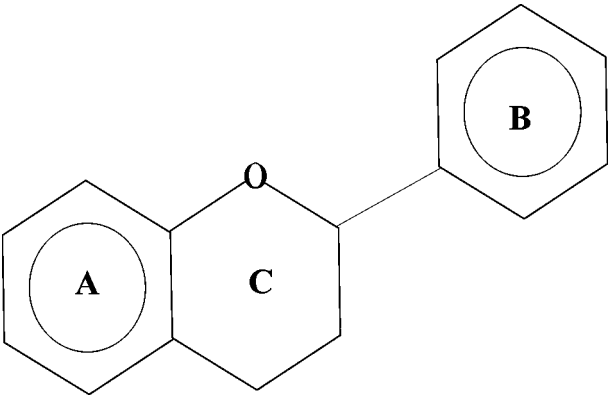


Figure 1 Basic flavonoid structure.

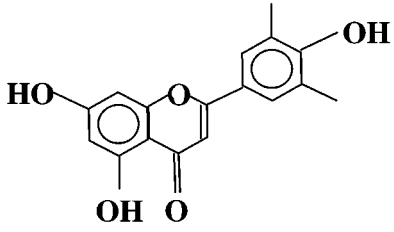
Flavonoids can be further subdivided into six major subclasses, based upon variations in the heterocyclic C-ring including flavones, flavonols, flavanones, catechins, anthocyanidins, and isoflavones (Figure 2). More than 5000 subclasses of flavonoids were identified by 1990 (25).

Food Sources

Flavonoids are widely distributed in foods and beverages of plant origin, such as fruits, vegetables, tea, cocoa, and wine (Figure 2). A large body of literature exists regarding their content in various foods. Within the subgroup of flavonols and flavones, the flavonol quercetin is the most frequently occurring compound in foods. Also common are kaempferol, myricetin, and the flavones apigenin and luteolin. Tea and onions are the main dietary sources of flavonols and flavones. Quercetin is the most abundant flavonol present in onions, whereas tea contains notable amounts of both quercetin and kaempferol.

Intake

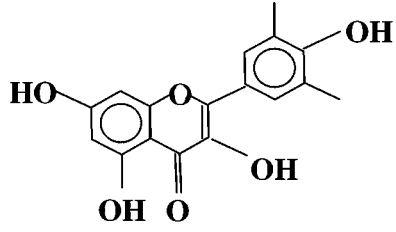
There are several challenges associated with the determination of dietary intake of polyphenols, including flavonoids. First, the formation of flavonoids in plants is influenced by numerous factors including light, plant genetics, environmental conditions, germination, degree of ripeness, and processing and storage, as well as species variety (5). For example, cherry tomatoes contain six times more quercetin per gram fresh weight than do normal size varieties of tomatoes (11). This is likely due to the fact that flavonols are synthesized and stored in the skin of the tomato, and smaller varieties have a higher skin to volume ratio. Hammerstone et al. (24) reported that the procyanidin content of apples varies with the apple variety, with Red Delicious and Granny Smith containing notably higher levels than McIntosh and Golden Delicious. In addition to factors affecting content, there



Flavones

(examples include apigenin, luteolin, diosmetin)

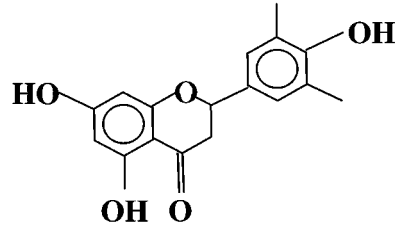
Major Food sources:
parsley, thyme, celery, sweet red pepper



Flavonols

(examples include quercetin, myricetin, kaempferol)

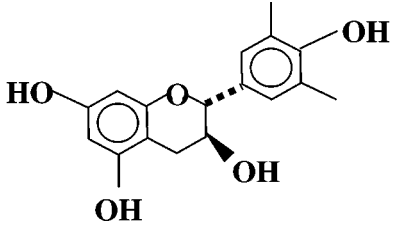
Major Food sources:
onions, kale, broccoli, apples, cherries, fennel, sorrel, berries, tea



Flavanones

(examples include naringenin, hesperedin)

Major Food sources:
citrus foods, prunes

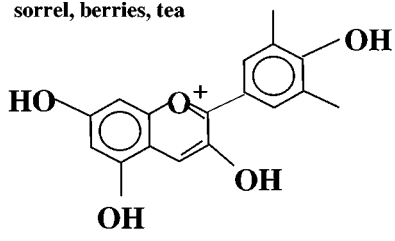


Catechins

(flavanols)

(examples include epicatechin, gallocatechin)

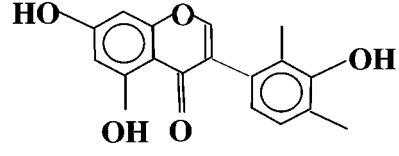
Major Food sources:
tea, apples, cocoa



Anthocyanidins

(examples include pelargonidin, malvidin, cyanidin)

Major Food sources:
cherries, grapes



Isoflavones

(examples include genistein, daidzein)

Major Food sources:
soya beans, legumes

has been a lack of agreement on an appropriate method to analyze the different types of polyphenols. As a consequence, information in the literature on content in plant foods is incomplete and often contradictory. Therefore, estimation of dietary intake of flavonoids is difficult, which can confound the ability to infer epidemiologic relationships regarding health and disease.

Only a few estimations of dietary intake of flavonoids are available. Kuhnau (42) estimated daily intake of flavonoids in the United States to be between 1 and 1.1 g/day, depending on the season. Hertog et al. (29) estimated intake of flavonols and flavones in the Netherlands to be 23 mg/day, and Kuhnau estimated intake of these two subclasses to be 115 mg/day. It has been suggested that Kuhnau's estimate was inflated owing to the unreliability of analytic methods used in the 1970s (33). More recently, Leth & Justesen (45) estimated intake of flavonols, flavones, and flavanones in Denmark to be 28 mg/day, which is similar to that reported by Hertog et al. (29).

ABSORPTION AND BIOAVAILABILITY

There have been several human studies that have investigated the absorption and bioavailability of flavonoids (31). Initially, absorption of flavonoids from the diet was believed negligible, given that the majority of food flavonoids are bound to glycosides. It was expected that the aglycones only could pass freely into the blood stream from the gut wall, because no enzymes are secreted in the gut that could cleave the glycosidic bonds (42). Recent studies, however, have demonstrated that the bioavailability of specific flavonoids is much higher than previously believed.

Hollman et al. (32) conducted a study in nine ileostomy patients to examine the extent of absorption of quercetin. Following a 12-day quercetin-free diet, subjects were randomized to the following supplemented diets over a 12-day period: fried onions (quercetin glucosides), pure quercetin rutinoside (the major quercetin glycoside found in tea), or 100 mg of pure quercetin aglycone. They found that the absorption of orally administered quercetin aglycone was approximately 24%; however, the absorption of quercetin glycosides from onions was 52%, suggesting that the glycoside moiety actually enhanced absorption. Additional studies in healthy individuals confirmed this finding. De Vries et al. (8) conducted a feeding study in which black tea (49 mg quercetin) or fried onions (13 mg of quercetin) were provided to 15 healthy individuals. They reported that quercetin was readily absorbed, but the absorption of quercetin from tea was half that of onions. Similarly, in another study Hollman et al. (33) measured plasma levels of quercetin in nine healthy individuals who were fed a single large dose of onions, apples, or pure quercetin-3-rutinoside. The bioavailability of quercetin from both apples and pure quercetin rutinoside was only 30% compared with onions. Peak quercetin

←

Figure 2 Major subclasses of flavonoids, structure, and food sources (adapted from References 2–4, 12, 18).

plasma levels also varied with a peak of 0.7 hour after ingestion of onions, 2.5 h after apples, and 9 h after rutinoside. Further, the half-lives of elimination were 23 hours for apples and 28 hours for onions, suggesting that repeated consumption of quercetin-containing foods could result in accumulation of quercetin in the blood; others have reported similar results (51). Finally, a recent study suggests that the dietary source of quercetin may affect its bioavailability. De Vries et al. (9) explored the bioavailability of quercetin from red wine, fried onions, and black tea in a randomized cross-over feeding study. Although some of the available flavonols from red wine were absorbed and present in plasma, the levels were lower than those found with onion as the flavonol source. The urinary excretion of quercetin was higher with red wine than with tea. The authors suggested that because one glass of red wine contains fewer flavonols than either a cup of tea or one portion of fried onions, red wine is a poorer source of flavonols.

The bioavailability of other flavonoid compounds has also been explored. Felgines et al. (13), in an animal study, reported that naringenin, the predominant flavonone found in grapefruit, and mainly found in a glycoside form (naringenin-7-rhamnoglucodise and naringenin-7-glucoside), was efficiently absorbed after feeding to rats (although the bioavailability differed with the glycosidic moiety attached). Several investigators have explored the bioavailability of the isoflavones, particularly genistein and daidzein, for which the main dietary source is soya beans (61). Although there is evidence that there is considerable degradation of isoflavones in the gut, measurable plasma concentrations (possibly at bioactive levels) have been reached in human feeding studies (1, 61, 66, 67). Moreover, infants fed soy formulas have plasma isoflavone levels nearly 10-fold higher than concentrations measured in Japanese adults (62). Because isoflavones demonstrate weak estrogenic activity (see below), these levels may be sufficient to exert biological effects.

METABOLIC EFFECTS

Flavonoids have been of interest owing to their observed biological effects in vitro such as free-radical scavenging, modulation of enzymatic activity, and inhibition of cellular proliferation, as well as their potential utility as antibiotic, antiallergic, antidiarrheal, antiulcer, and antiinflammatory agents (5). There is an extensive literature describing each of these biological properties. We have focused on the biological effects of flavonoids, including their antioxidant, antiestrogenic, and antiproliferative activities, which are commonly ascribed to help explain their potential benefit in reducing the occurrence of cardiovascular disease and cancer.

Antioxidant Activity

Diets high in fruits and vegetables are protective against a variety of diseases, particularly cardiovascular disease and some types of cancer (15, 52). Antioxidants and dietary fiber are thought to be the principal nutrients responsible for these protective effects. Reactive oxygen species are formed in vivo during normal aerobic

metabolism and can cause damage to DNA, proteins, and lipids, despite natural antioxidant defense systems. The accumulation of unrepaired damaged products may be critical to the development of cancer, atherosclerosis, diabetes, and chronic inflammation (23). Several in vitro studies have shown that the flavonoids, including flavonols, flavones, isoflavones, flavanols, and anthocyanidins, possess antioxidant activity.

Flavonoids, in conjunction with other antioxidants, including vitamins C and E, are thought to inhibit lipid peroxidation in the phospholipid bilayer caused by reactive oxygen species. In contrast to vitamins C and E, which are concentrated in the aqueous phase and phospholipid bilayer, respectively, flavonoids are likely to be localized between the two phases owing to their hydrophilicity. Flavonoids may trap chain-initiating radicals at the interface of the membranes, thus preventing the progression of the radical chain reaction.

Studies have shown many flavonoids to be effective antioxidants in a wide range of chemical oxidation systems, demonstrated by their ability to scavenge peroxyl radicals, alkyl peroxyl radicals, superoxide hydroxyl radicals, and peroxynite in aqueous and organic environments (11). Recent studies have suggested that dietary flavonoids may protect free-radical–induced damage to DNA by a mechanism other than solely direct free-radical scavenging. Results from pulse radiolysis studies and a plasmid test system have shown that flavonoids can reduce the incidence of single-strand breaks in double-stranded DNA as well as residual base damage through fast chemical repair (2). In addition to free-radical scavenging properties, some flavonoids can chelate those transition metal ions responsible for the generation of reactive oxygen species and therefore inhibit the initiation of the lipoxygenase reaction. Some evidence has suggested that flavonoids also have antioxidant capacity in nontransition metal-dependent oxidation (49).

Flavonoids may also exert antioxidant abilities through protection or enhancement of endogenous antioxidants. Numerous flavonoids have been shown to alleviate oxidative stress by inducing glutathione S-transferase (GST), an enzyme proposed to protect cells against free-radical damage by increasing resistance to oxidative stress caused by hydrogen peroxide (14). Some flavonoids, including quercetin, myricetin, and fisetin, were shown to cause statistically significant increases in GST-specific activity (14). GST is thought to play a protective role against cancer by detoxifying xenobiotics with mutagenic potential (10). Therefore, compounds that upregulate GST may both alleviate oxidative stress and aid in the detoxification of mutagenic xenobiotics.

The antioxidant capacity of phenolic compounds is determined by their structure, in particular the ease with which a hydrogen atom from an aromatic hydroxyl group can be donated to a free radical and the ability of an aromatic compound to support an unpaired electron as the result of delocalization around the M-electron system. Other important structural determinants of the antioxidant capacity of flavonoids appear to be the 4′-OH and 3′-OH groups. The addition of hydroxyl groups to the carbon atoms ortho to the 4-C position appear to further increase antioxidant potential (46). Studies have indicated that the aglycones, including quercetin, luteolin, myricetin, and kaempferol, have greater antioxidant capacity

than do the conjugate flavonoids, such as quercetin-3-glucoside, quercitrin, and rutin (55). Ioku et al. (36) showed that the antioxidant activity of quercetin glycosides is lower than quercetin aglycone in an artificial membrane system, suggesting that glycosidation weakens the antioxidant activity of flavonoids. This decrease may be caused by increased blocking of the phenolic groups responsible for radical scavenging and metal chelation and possibly to a decrease in accessibility of the membranes owing to the large glycoside group. Reaction rate constants in organic media for several flavonoids exceed that of vitamin E. Suggested reasons include that flavonoids have a more extended conjugated system to support an unpaired electron, two or more reactive OH groups, and less stearic hindrance at the site of abstraction. Noroozi et al. (55) demonstrated that, at equimolar concentrations, most flavonoids showed greater antioxidant capacity than did vitamin C. Further, it has been reported that the degree of polymerization of flavonoids may influence antioxidant capabilities, where higher oligomers possess antioxidant capabilities and monomers show little effect (48).

It is important to note that the bioavailability of these compounds determines their activity in vivo. Currently, however, the relevance of in vitro studies to the in vivo situation is unclear. Fremont et al. (17) demonstrated that in rats fed diets high in both monounsaturated and polyunsaturated fatty acids, supplementation with dietary flavonoids significantly reduced the amounts of dienes produced during copper-induced oxidation, indicating increased resistance of very low density lipoproteins and low density lipoproteins to oxidation. Funabiki et al. (18) examined the effects of dietary supplementation of 4-α-glucopyranosylrun (G-rutin), a water-soluble rutin derivative, in rats. Dietary G-rutin significantly inhibited the accumulation of oxidatively damaged DNA and proteins. Terao (64) found that oral administration of (−)-epicatechin and quercetin enhanced the antioxidant capacity of rat plasma, although both flavonoids accumulated mainly as glucuronide and sulfate conjugates in blood plasma. This finding suggests that conjugated metabolites of flavonoids may play a role in the antioxidant defenses of blood plasma.

In humans, Nielsen et al. (54) demonstrated apigenin to be absorbed by subjects fed a diet high in parsley and observed an increase in the concentration of the antioxidant enzymes erythrocyte glutathione reductase and superoxide dismutase. Activities of erythrocyte catalase and glutathione peroxidase, however, were unchanged. In a cross-sectional study in Japan, Arai et al. (3) found total intake of flavonoids among women to be inversely correlated with plasma total cholesterol and low density lipoprotein concentrations, after adjustment for age, body mass index, and total energy intake. Further human studies are needed to explore both the bioavailability of the flavonoids along with biomarkers of antioxidant effects.

Antiestrogenic (and Estrogenic) Properties

Exposure to both endogenous and exogenous estrogens (or hormones) has been identified as a risk factor for some types of cancer. Plant estrogens are quantitatively the most important exogenous estrogens when their hormonal potency is

assessed in vitro (50, 60). Thus far, however, no data exist on the effect of phytoestrogens administered as pure compounds to humans. Indirect evidence based upon phytoestrogen-rich diets has suggested that hormonal effects of these compounds are weak. It is known that average intake of phytoestrogens is higher in countries with lower incidence rates of diseases associated with estrogen exposure, such as breast cancer, hypospadia, and testicular and prostate cancers. However, it is possible that phytoestrogens, including flavonoids, may interact with estrogen receptors and alter concentrations of endogenous sex hormones. Thus, they may alter sex differentiation and increase the risk of reproductive tract tumors or developmental disorders; this may be especially important during fetal development.

Overall, flavonoids are considered to be nonestrogenic or weakly estrogenic. However, some flavones and flavonols (apigenin, kaempferol, and naringenin) act through estrogen-receptor mediated mechanisms and have been shown to have antiestrogenic effects similar to those of the isoflavones in breast cancer cell cultures (50). Apigenin and kaempferol are the most active flavonoids and inhibit estrone reduction at a concentration of 0.12 μmol/L (47). Some flavones have been shown to inhibit the 17β-oxidation of testosterone and estradiol to the less active steroids, androstenedione and estrone. In addition, flavonoids inhibit placental aromatase (35, 38).

Exposure to exogenous estrogens can induce both structural and functional changes in the developing reproductive tract of males. Men exposed to diethylstilbestrol (DES) in utero, for example, experience DES-induced lesions ranging from relatively minor structural alterations to more severe changes such as testicular hypoplasia (21). It is possible that other exogenous estrogens, including flavonoids, may alter reproductive tract development in males.

Newbold et al. (53) examined the carcinogenic potential of genistein in an experimental animal mouse model that experiences a high incidence of uterine adenocarcinomas. They treated pregnant mice with equivalent doses of DES and genistein. At 18 months of age female offspring demonstrated an incidence of 35% uterine adenocarcinoma with genistein and 31% with DES. Given the increasing use and marketing of soy formulas, soy products, and phytoestrogen-containing dietary supplements, further study of the potential detrimental effects is needed.

Antiproliferative Activity

Several in vitro studies have investigated the flavonoids for their inhibition of cellular transformation and proliferation. Franke et al. (16) investigated the effects of dietary flavonoids at inhibiting neoplastic transformation induced by 3-methylcholanthrene in mouse fibroblasts. They examined several flavones, flavonols, flavanones, catechins, and isoflavones and found that the flavonones hesperetin and hesperedin and the catechins exhibited the highest levels of inhibition of transformation. The isoflavones demonstrated the weakest inhibition in this cell culture model. Kuntz et al. (43) screened over 30 flavonoids for their effects

on cell proliferation and potential cytotoxicity in two human colon cancer cell lines. All compounds tested, including specific flavones, flavonols, flavanones, and isoflavonones, demonstrated antiproliferative activity in the absence of cell cytotoxicity. The authors reported no notable structure-activity relationships on the basis of subclass. Wenzel et al. (65) evaluated how the core structure of the flavones, 2-phenyl-4H-1-benzopyran-4-one, affects proliferation, differentiation, and apoptosis in a human colon cancer cell line. In particular, they evaluated the effect of the flavone on the expression of cell-cycle and apoptosis-related genes in the cell line. They reported dramatic changes in mRNA levels of specific genes including cyclo-oxygenase-2, nuclear transcription factor kappaB, and bcl-X. Further, there was high selectively for apoptosis in the transformed cells. The authors concluded that flavones could be a new chemopreventive agent.

In addition to effects on mRNA levels of genes important in cell cycle control and apoptosis, flavonoids have been investigated with respect to their interaction with enzymes associated with DNA topology. DNA topoisomerase II is an enzyme that catalyzes the double-strand breakage and rejoining of DNA; it is pivotal for several cell functions (20). Several flavonoids, including genistein, can inhibit DNA topoisomerase II activity by stabilizing the cleavage complex, thereby facilitating apopotosis (63). Importantly, however, specific flavonoids have been shown to cause single- and double-strand DNA breaks in cultured human cells (12, 63), which suggests that not all cells undergo apoptosis in the presence of specific flavonoids. This observation is extremely important in understanding the potential detrimental effects that may be associated with DNA topoisomerase II inhibition, and further research is underway (58).

OBSERVATIONAL STUDIES/HEALTH EFFECTS

Cardiovascular and Cerebrovascular Disease

Owing to their antioxidant and antithrombotic properties, the intake of flavonoids has been explored in relationship to cardiovascular disease; results of major cohort studies are presented here. Hertog et al. (26) examined data from the Zutphen Elderly Study, which included 805 men aged 65–84 years, who in addition to other data, had their food consumption measured using a diet history method in 1985. The average flavonoid intake was 26 mg/day. After 5 years, their health records were collected and morbidity and mortality data were examined. Hertog et al. (26) reported that high intake of flavonols (particularly quercetin) was associated with a decreased risk of coronary heart disease (CHD) mortality. In a 10-year follow-up of the same group of elderly men, Hertog et al. (28) reported a relative risk (RR) of 0.47 [95% confidence interval (CI) 0.27–0.82] for a coronary event among men in the highest tertile of flavonoid intake compared with men in the lowest tertile. Interestingly, they also reported a decreased risk of all cause mortality with increasing flavonol intake (p for trend $= 0.010$). In a cohort study of 5133 Finnish men and women, Knekt et al. (40) reported similar findings, with a decreased risk

of coronary mortality associated with increasing flavonoid intake. In contrast, a U.S. study of coronary heart disease in male health professionals aged 40–75 found no significant association with flavonoid intake (57). In a study of 1900 Welsh men aged 45–59 years, the authors found a higher risk of dying of ischemic heart disease for men with the highest consumption of tea; men with the highest consumption of tea had an RR of 1.6 (95% CI = 0.0–2.9) of dying of ischemic heart disease compared with men with the lowest consumption (30).

Hertog et al. (27) analyzed data from the Seven Countries Study, which was comprised of 16 cohorts of men (n = 12,763) originally aged 40–59 years, and who were followed for mortality over 25 years. Flavonoid intake was estimated by analysis of equivalent food composites that represented the average diet of the cohorts. Average intake of flavonoids was inversely associated with coronary heart disease. Multivariate analysis revealed that saturated fat intake and percentage of smokers in the cohort explained 73% and 9%, respectively, of the variance in coronary heart disease rates among the 16 cohorts; flavonoid intake accounted for 8% of the variance. However, as Hollman & Katan (34) noted with this study, it is difficult to control for potential underlying confounding factors when making comparisons among widely different populations with respect to diet and lifestyle. Finally, in a 10-year follow-up study of over 34,000 postmenopausal women living in Iowa, total flavonoid intake was associated with a decreased risk of coronary heart disease death after adjusting for age and energy intake [RR = 0.62 (highest to lowest intake); 95% CI = 0.44–0.87] (68).

Fewer of these studies have explored flavonoid intake and risk of stroke. In the Zutphen study, there was an inverse association with increasing dietary flavonoid consumption (mainly quercetin) after adjustment for several confounders, including vitamin intake (37). Tea consumption, which comprised the major source of flavonoid intake, was associated with a decreased risk of stroke; the RR for daily consumption of more than 4.7 cups of tea versus less than 2.6 cups per day was 0.31 (95% CI = 0.12–0.84). Knekt et al. (39) explored quercetin intake and the risk of cerebrovascular disease (CVD) in a cohort study of 9208 Finnish men and women. During a 28-year follow-up period, a total of 824 incident cases of CVD were diagnosed. After adjustment for age, serum cholesterol, body mass index, smoking, hypertension, diabetes, geographical area, occupation, and intake of beta-carotene, vitamin E, vitamin C, fiber, specific fatty acids, and energy, the RR of CVD among the highest quartile of quercetin was 0.99 (95% CI = 0.71–1.38) and 0.95 (95% CI = 0.60–1.21) for men and women, respectively. Thus, the authors concluded that quercetin intake is not associated with CVD. In a cohort study of over 34,000 postmenopausal women, no association was found between flavonoid intake and stroke mortality after 10 years of follow-up (68).

Cancer

There have been a number of studies that suggest that high fruit and vegetable consumption is associated with a decreased risk of human malignancies, including colon, breast, lung, larynx, pancreas, bladder, stomach, esophageal, and oral

cancer (15). Given the antineoplastic effects associated with the flavonoids, several epidemiological studies have examined associations between flavonoid intake (as assessed through a food frequency questionnaire) and the development of malignancy.

Flavonoid intake was evaluated in the Zutphen Elderly Study with respect to cancer development (4, 26). Hertog et al. (29) reported no association between flavonol or flavone intake and total cancer mortality or mortality from specific cancers. In a 10-year follow-up study of the same cohort, Arts et al. (4) evaluated catechin intake and the incidence of epithelial cancer. Although nontea catechins (major source was apples) demonstrated a slight inverse association with lung cancer incidence (RR = 0.66, 95% CI = 0.42–1.05), catechins from tea did not. The authors concluded that it was unlikely that catechins were responsible for this observed inverse trend, although bioavailability among various catechins cannot be ruled out. In contrast, a cohort study from Finland of 9959 men and women reported an inverse association between the intake of flavonoids and the incidence of all cancers combined (41). This association was most apparent for lung cancer (RR = 0.54; 95% CI = 0.34–0.87), even after adjustment for several potential confounding variables. However, in the Netherlands Cohort Study on Diet and Cancer, a large cohort study of 58,279 men and 62,573 women, no associations were reported with flavonol or flavone intake and stomach cancer, colon cancer, or lung cancer after 4 years of follow-up (22). Further, in this same study, black tea consumption was also not associated with the development of colon, lung, or breast cancer. It should be recognized, however, that the follow-up period is extremely short, and as this cohort is followed longer, different patterns may emerge. Finally, Hertog et al. (27) reported that flavonoid intake was not an important determinant of cancer mortality in the Seven Countries Study after 25 years of follow-up.

Case-control studies have also examined these relationships. De Stefani et al. (7), in a study from Uruguay, analyzed dietary data from 541 individuals with lung cancer and 540 hospitalized controls. They reported significant inverse associations with increasing carotenoid, glutathione, flavonoid, and vitamin E intake. Further, total fruit and vegetable consumption was also associated with significant protective effects. In a case-control study from Spain of 354 cases of gastric cancer and 354 matched controls, an inverse association was reported with total flavonoid intake [odds ratio (highest quartile versus lowest quartile) = 0.48; 95% CI = 0.26–0.88] (19).

The epidemiologic data concerning the health benefits of flavonoids in the development of cancer are not convincing. In particular, with the exception of the study from Finland, the cohort studies have been largely negative. Whereas the case-control studies may suggest some positive benefit, the lack of an inverse association observed in the large cohort studies diminishes confidence in interpretation. Given that clear associations have been observed between fruit and vegetable intake and numerous types of cancer, and that fruit and vegetable intake is likely strongly correlated with flavonoid intake, the results of these epidemiologic studies are somewhat surprising. Whereas the lack of an association may be real, other

explanations could include a lack of an adequate measure to assess flavonoid intake (most food frequency questionnaires employed in epidemiologic studies have not been designed to assess phytochemical intake specifically) and the multitude of factors affecting flavonoid content in foods and bioavailability.

SUMMARY

Flavonoids may possess specific properties that could benefit human health. However, as discussed, the experimental and in vitro data have produced conflicting results. The data from epidemiological studies regarding flavonoids in human health are far from convincing. This is especially disconcerting when specific flavonoids such as genistein and quercetin are being marketed as nutritional supplements, in which the concentrations in one dose could far exceed the dose received from a daily vegetarian diet. The results of recent clinical studies using β-carotene supplements (56) should reinforce the need to proceed with caution in using flavonoid supplements. Whereas more studies at all levels are needed, both to characterize the potential health benefits of individual flavonoids and to characterize potential harmful attributes, it is very possible that the sum of the parts (e.g., total fruit and vegetable intake) is more important in providing a health benefit to humans than one plant constituent.

ACKNOWLEDGMENTS

Supported by National Cancer Institute Grant CA-79940 and the Children's Cancer Research Fund. CMK was also supported by National Cancer Institute Training Grant T32-CA09607.

The *Annual Review of Nutrition* is online at http://nutr.annualreviews.org

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