Flavonoids: a review of probable mechanisms of action and potential applications[1–3]

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ABSTRACT The aim of this review, a summary of the putative biological actions of flavonoids, was to obtain a further understanding of the reported beneficial health effects of these substances. Flavonoids occur naturally in fruit, vegetables, and beverages such as tea and wine. Research in the field of flavonoids has increased since the discovery of the French paradox, ie, the low cardiovascular mortality rate observed in Mediterranean populations in association with red wine consumption and a high saturated fat intake. Several other potential beneficial properties of flavonoids have since been ascertained. We review the different groups of known flavonoids, the probable mechanisms by which they act, and the potential clinical applications of these fascinating natural substances. *Am J Clin Nutr* 2001;74:418–25.

KEY WORDS Flavonoids, bioflavonoids, antioxidants, French paradox, review, polyphenols

INTRODUCTION

Flavonoids belong to a group of natural substances with variable phenolic structures and are found in fruit, vegetables, grains, bark, roots, stems, flowers, tea, and wine (1). These natural products were known for their beneficial effects on health long before flavonoids were isolated as the effective compounds. More than 4000 varieties of flavonoids have been identified, many of which are responsible for the attractive colors of flowers, fruit, and leaves (2). Research on flavonoids received an added impulse with the discovery of the French paradox, ie, the low cardiovascular mortality rate observed in Mediterranean populations in association with red wine consumption and a high saturated fat intake. The flavonoids in red wine are responsible, at least in part, for this effect (3). Furthermore, epidemiologic studies suggest a protective role of dietary flavonoids against coronary heart disease (2). The association between flavonoid intake and the long-term effects on mortality was studied subsequently (4) and it was suggested that flavonoid intake is inversely correlated with mortality due to coronary heart disease (5).

Until ≈50 y ago, information on the working mechanisms of flavonoids was scarce. However, it has been widely known for centuries that derivatives of plant origin possess a broad spectrum of biological activity (6). In 1930 a new substance was isolated from oranges, which is believed to be a member of a new class of vitamins, and was designated as vitamin P. When it became clear that this substance was a flavonoid (rutin), a flurry of research began in an attempt to isolate the various individual flavonoids and to study the mechanism by which flavonoids act.

Flavonoids can be divided into various classes on the basis of their molecular structure (7). The 4 main groups of flavonoids are listed in **Table 1**, together with the best-known members of each group and the food source in which they are present. The molecular structure of each group of flavonoids is given in **Figure 1**.

The flavones are characterized by a planar structure because of a double bond in the central aromatic ring. One of the best-described flavonoids, quercetin, is a member of this group. Quercetin is found in abundance in onions, apples, broccoli, and berries. The second group is the flavanones, which are mainly found in citrus fruit. An example of a flavonoid of this group is narigin. Flavonoids belonging to the catechins are mainly found in green and black tea and in red wine (2), whereas anthocyanins are found in strawberries and other berries, grapes, wine, and tea.

An important effect of flavonoids is the scavenging of oxygen-derived free radicals. In vitro experimental systems also showed that flavonoids possess antiinflammatory, antiallergic, antiviral, and anticarcinogenic properties (1). The aim of this review was to give an overview of the research in the field of flavonoids. The potential valuable working mechanisms of flavonoids are discussed, followed by present knowledge on the absorption, conjugation, and toxicity of these substances. In the last part of this review, the potential clinical applications of flavonoids are discussed.

WORKING MECHANISMS

Antioxidative effects

The best-described property of almost every group of flavonoids is their capacity to act as antioxidants. The flavones and catechins

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TABLE 1
Main groups of flavonoids, the individual compounds, and food sources

Group	Compound	Food sources
Flavones	Apigenin	Apple skins
	Chrysin	Berries
	Kaempferol	Broccoli
	Luteolin	Celery
	Myricetin	Fruit peels
	Rutin	Cranberries
	Sibelin	Grapes
	Quercetin	Lettuce
		Olives
		Onions
		Parsley
Flavanones	Fisetin	Citrus fruit
	Hesperetin	Citrus peel
	Narigin	
	Naringenin	
	Taxifolin	
Catechins	Catechin	Red wine
	Epicatechin	Tea
	Epigallocatechin gallate	
Anthocyanins	Cyanidin	Berries
	Delphinidin	Cherries
	Malvidin	Grapes
	Pelargonidin	Raspberries
	Peonidin	Red grapes
	Petunidin	Red wine
		Strawberries
		Tea
		Fruit peels with dark pigments

seem to be the most powerful flavonoids for protecting the body against reactive oxygen species. Body cells and tissues are continuously threatened by the damage caused by free radicals and reactive oxygen species, which are produced during normal oxygen metabolism or are induced by exogenous damage (8, 9). The mechanisms and the sequence of events by which free radicals interfere with cellular functions are not fully understood, but one of the most important events seems to be lipid peroxidation, which results in cellular membrane damage. This cellular damage causes a shift in the net charge of the cell, changing the osmotic pressure, leading to swelling and eventually cell death. Free radicals can attract various inflammatory mediators, contributing to a general inflammatory response and tissue damage. To protect themselves from reactive oxygen species, living organisms have developed several effective mechanisms (10). The antioxidant-defense mechanisms of the body include enzymes such as superoxide dismutase, catalase, and glutatione peroxidase, but also nonenzymatic counterparts such as glutathione, ascorbic acid, and α-tocopherol. The increased production of reactive oxygen species during injury results in consumption and depletion of the endogenous scavenging compounds. Flavonoids may have an additive effect to the endogenous scavenging compounds. Flavonoids can interfere with ≥3 different free radical–producing systems, which are described below, but they can also increase the function of the endogenous antioxidants.

Direct radical scavenging

Flavonoids can prevent injury caused by free radicals in various ways. One way is the direct scavenging of free radicals. Flavonoids are oxidized by radicals, resulting in a more stable, less-reactive radical. In other words, flavonoids stabilize the reactive oxygen species by reacting with the reactive compound of the radical. Because of the high reactivity of the hydroxyl group of the flavonoids, radicals are made inactive, according to the following equation (11):

$$\text{Flavonoid(OH)} + \text{R}^{\bullet} > \text{flavonoid(O}^{\bullet}) + \text{RH} \qquad (1)$$

where $\text{R}^{\bullet}$ is a free radical and $\text{O}^{\bullet}$ is an oxygen free radical. Selected flavonoids can directly scavenge superoxides, whereas other flavonoids can scavenge the highly reactive oxygen-derived radical called peroxynitrite. Epicatechin and rutin are also powerful radical scavengers (12). The scavenging ability of rutin may be due to its inhibitory activity on the enzyme xanthine oxidase. By scavenging radicals, flavonoids can inhibit LDL oxidation in vitro (13). This action protects the LDL particles and, theoretically, flavonoids may have preventive action against atherosclerosis.

Nitric oxide

Several flavonoids, including quercetin, result in a reduction in ischemia-reperfusion injury by interfering with inducible nitric-oxide synthase activity (14). Nitric oxide is produced by several different types of cells, including endothelial cells and macrophages. Although the early release of nitric oxide through the activity of constitutive nitric-oxide synthase is important in maintaining the dilation of blood vessels (15), the much higher concentrations of nitric oxide produced by inducible nitric-oxide synthase in macrophages can result in oxidative damage. In these circumstances, activated macrophages greatly increase their simultaneous production of both nitric oxide and superoxide anions. Nitric oxide reacts with free radicals, thereby producing the highly damaging peroxynitrite. Nitric oxide injury takes place for the most part through the peroxynitrite route because peroxynitrite can directly oxidize LDLs, resulting in irreversible damage to the cell membrane. When flavonoids are used as antioxidants, free radicals are scavenged and therefore can no longer react with nitric oxide, resulting in less damage (16). Interestingly, nitric oxide can be viewed as a radical itself, and it is was reported that nitric oxide molecules are directly scavenged by flavonoids (17). Therefore, it has been speculated that nitric oxide scavenging plays a role in the therapeutic effects of flavonoids (17). Silibin is a flavonoid that has been reported to inhibit nitric oxide dose dependently (18).

Xanthine oxidase

The xanthine oxidase pathway has been implicated as an important route in the oxidative injury to tissues, especially after ischemia-reperfusion (19). Both xanthine dehydrogenase and xanthine oxidase are involved in the metabolism of xanthine to uric acid. Xanthine dehydrogenase is the form of the enzyme present under physiologic conditions, but its configuration is changed to xanthine oxidase during ischemic conditions. Xanthine oxidase is a source of oxygen free radicals. In the reperfusion phase (ie, reoxygenation), xanthine oxidase reacts with molecular oxygen, thereby releasing superoxide free radicals. At least 2 flavonoids, quercetin and silibin, inhibit xanthine oxidase activity, thereby resulting in decreased oxidative injury (14, 20, 21). Cos et al (22) carried out a study on structure-function relations in which luteolin (3′,4′5,7- tetrahydroxyflavone) was reported to be the most potent inhibitor of xanthine oxidase.

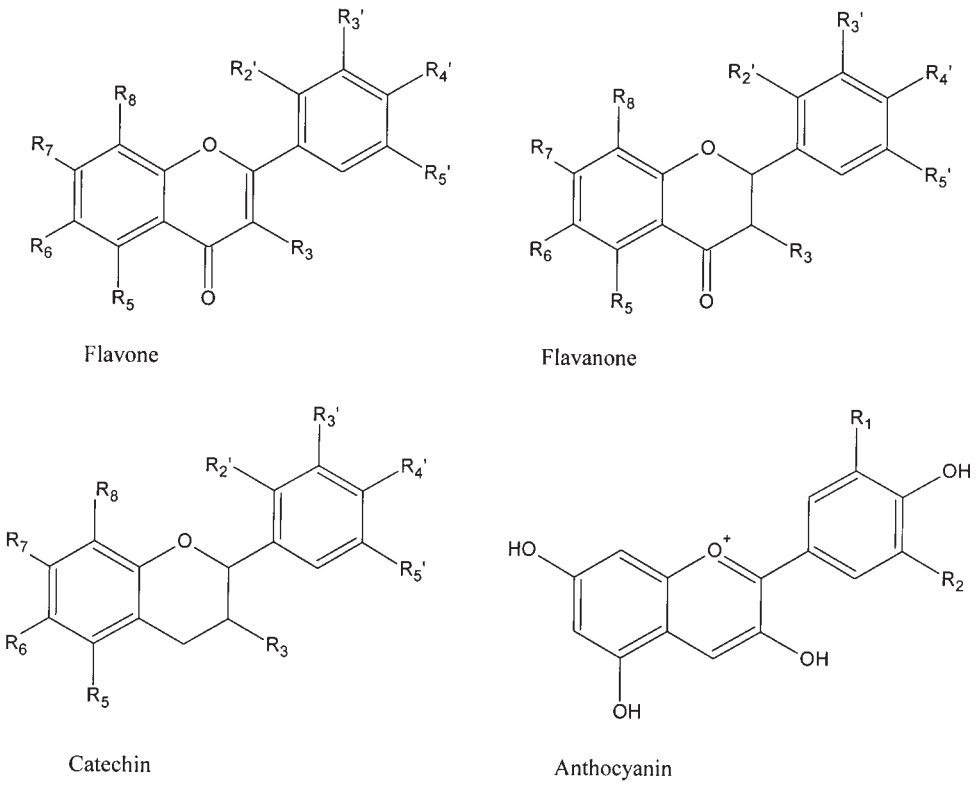

FIGURE 1. The molecular structure of each group of flavonoids.

Leukocyte immobilization

The immobilization and firm adhesion of leukocytes to the endothelial wall is another major mechanism responsible for the formation of oxygen-derived free radicals, but also for the release of cytotoxic oxidants and inflammatory mediators and further activation of the complement system. Under normal conditions, leukocytes move freely along the endothelial wall. However, during ischemia and inflammation, various mainly endothelium-derived mediators and complement factors may cause adhesion of the leukocytes to the endothelial wall, thereby immobilizing them and stimulating degranulation of the neutrophil. As a result, oxidants and inflammatory mediators are released, resulting in injury to tissues. Oral administration of a purified micronized flavonoid fraction was reported to decrease the number of immobilized leukocytes during reperfusion (23). The decrease in the number of immobilized leukocytes by flavonoids may be related to the decrease in total serum complement and is a protective mechanism against inflammation-like conditions associated with, for example, reperfusion injury (23, 24). Some flavonoids can inhibit degranulation of neutrophils without affecting superoxide production (25). The inhibitory effect of some flavonoids on mast cell degranulation was shown to be due to modulation of the receptor-directed Ca^{2+} channels in the plasma membrane (26).

Interaction with other enzyme systems

Compared with research on the antioxidant capacities of flavonoids, there has been relatively little research on other beneficial effects of flavonoids. The major effects of flavonoids (eg, antiallergic effects) may be the result of radical scavenging. Another possible mechanism by which flavonoids act is through interaction with various enzyme systems. Furthermore, some effects may be a result of a combination of radical scavenging and an interaction with enzyme functions.

When reactive oxygen species are in the presence of iron, lipid peroxidation results (27). Specific flavonoids are known to chelate iron (28), thereby removing a causal factor for the development of free radicals. Quercetin in particular is known for its iron-chelating and iron-stabilizing properties. Direct inhibition of lipid peroxidation is another protective measure (29).

Selected flavonoids can reduce complement activation, thereby decreasing the adhesion of inflammatory cells to the endothelium (24) and in general resulting in a diminished inflammatory response. Another feature of flavonoids is a reduction in the release of peroxidase. This reduction inhibits the production of reactive oxygen species by neutrophils by interfering with α_1-antitrypsin activation. A progressive inactivation of proteolytic enzymes was described in neutrophils (30).

Another interesting effect of flavonoids on enzyme systems is the inhibition of the metabolism of arachidonic acid (31). This feature gives flavonoids antiinflammatory and antithrombogenic properties. The release of arachidonic acid is a starting point for a general inflammatory response. Neutrophils containing lipoxygenase create chemotactic compounds from arachidonic acid. They also provoke the release of cytokines.

INTAKE, ABSORPTION, CONJUGATION, AND TOXICITY OF FLAVONOIDS

Intake

The average daily flavonoid intake in the Netherlands is estimated to be 23 mg/d (32). Intakes of flavonoids exceed those of

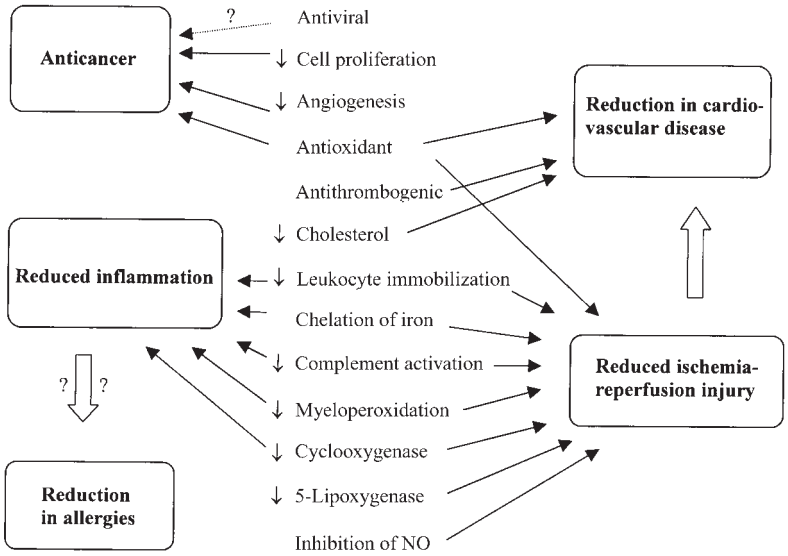

FIGURE 2. Hypothesis of the links between the working mechanisms of flavonoids and their effects on disease. NO, nitrous oxide.

vitamin E and β-carotene, whereas the average intake of vitamin C is 3 times higher than the intake of flavonoids. Flavonoid intakes seem to vary greatly between countries; the lowest intakes (≈2.6 mg/d) are in Finland and the highest intakes (68.2 mg/d) are in Japan (4, 24, 33). Quercetin is the most important contributor to the estimated intake of flavonoids, mainly from the consumption of apples and onions (34). A major problem in cohort studies of flavonoid intakes is that only a limited number of flavonoids can be measured in biological samples, and more importantly, only a relatively small number of fruit and vegetables are used to make an accurate estimation.

Absorption

Data on the absorption, metabolism, and excretion of flavonoids in humans are contradictory and scarce (35–40). Some studies showed that the most intensely studied dietary flavonoid, quercetin, is absorbed in significant amounts (35, 41). Naturally occurring flavones exist predominantly in a glycosylated form rather than in their aglycone form. The form of the flavonoid seems to influence the rate of absorption. Hollman and Katan (39) suggested that the glycosylated forms of quercetin are absorbed more readily than are the aglycone forms; however, this has been questioned by other researchers (40). The role of flavonoid glycosylation in facilitating absorption is questioned by the fact that catechin, which is not glycosylated in nature, is absorbed relatively efficiently (42).

Conjugation

It is generally accepted that the conjugation pathway for flavonoids (catechins) begins with the conjugation of a glucuronide moiety in intestinal cells. The flavonoid is then bound to albumin and transported to the liver (43, 44). The liver can extend the conjugation of the flavonoid by adding a sulfate group, a methyl group, or both. The addition of these groups increases the circulatory elimination time and probably also decreases toxicity.

There are several possible locations for the conjugates on the flavonoid skeleton. The type of conjugate and its location on the flavonoid skeleton probably determine the enzyme-inhibiting capacity, the antioxidant activity, or both of the flavonoid. Recent data suggest that the regular intake of flavonoids results in a more predominant formation of several conjugates, which probably results in greater activity. A detailed example is given in the study by Manach et al (43), in which a high dose of quercetin was administered to a group of rats adjusted to flavonoid intake and to a nonadjusted group. Results of this study indicated that the conjugated compound isorhamnetin was formed in higher quantities in the adjusted group, which is important because it is known to be even more active than is the aglycone form of quercetin on xanthine oxidase inhibition (45).

Concentrations of individual flavonoids and their biologically active conjugates may not be high enough after occasional intake to explain the low mortality rates from cardiovascular disease in Mediterranean countries. However, because the half-lives of conjugated flavonoids are rather long (23–28 h) (41), accumulation may occur with regular intakes, which may in turn result in sufficiently active flavonoid concentrations.

Toxicity

There is much controversy regarding the purported toxic or even mutagenic properties of quercetin. Formica and Regelson (3) gave an interesting overview of the in vitro and vivo studies on quercetin. The early data on toxic side effects are mainly derived from in vitro studies. At a conference of the Federation of American Societies for Experimental Biology in 1984 on mutagenic food flavonoids, carcinogenicity was reported in just 1 of 17 feeding studies conducted in laboratory animals (46, 47). Dunnick and Hailey (48) reported that high doses of quercetin over several years might result in the formation of tumors in mice. However, in other long-term studies, no carcinogenicity was found (49). In contrast with the potential mutagenic effects of flavonoids in earlier studies, several more recent reports indicate that flavonoids, including quercetin, seem to be antimutagenic in vivo (3, 50, 51). A large clinical study by Knekt et al (34), in which 9959 men and women were followed for 24 y, showed an inverse relation between the intake of flavonoids (eg, quercetin) and lung cancer. One possible explanation for these conflicting data is that flavonoids are toxic to cancer cells or to immortalized cells, but are not toxic or are less

toxic to normal cells. If this is true, flavonoids might play a role in the prevention of cancer that is worthy of further investigation.

CLINICAL EFFECTS

An overview of the hypothetical links between the working mechanisms and clinical effects of flavonoids is given in **Figure 2**. The different clinical effects of flavonoids are discussed in greater detail below.

Antiatherosclerotic effects

Because of their antioxidative properties, flavonoids are likely to have a major influence on the vascular system. Oxygen radicals can oxidize LDL, which injures the endothelial wall and thereby promotes atherosclerotic changes. A few clinical studies have pointed out that flavonoid intakes protect against coronary heart disease (4, 52). Hertog et al (4) stated that the flavonoids in regularly consumed foods might reduce the risk of death from coronary heart disease in elderly men. Furthermore, a Japanese study reported an inverse correlation between flavonoid intake and total plasma cholesterol concentrations (53). Oxidative stress and vascular damage are postulated to play a key role in dementia, and the intake of red wine is reported to prevent the development of dementia (54). The intake of flavonoids was reported to be inversely related to the risk of incident dementia (55).

Antiinflammatory effects

Cyclooxygenase and lipoxygenase play an important role as inflammatory mediators. They are involved in the release of arachidonic acid, which is a starting point for a general inflammatory response. Neutrophils containing lipoxygenase create chemotactic compounds from arachidonic acid. They also provoke the release of cytokines. Selected phenolic compounds were shown to inhibit both the cyclooxygenase and 5-lipoxygenase pathways (31, 56, 57). This inhibition reduces the release of arachidonic acid (58). The exact mechanism by which flavonoids inhibit these enzymes is not clear. Quercetin, in particular, inhibits both cyclooxygenase and lipoxygenase activities, thus diminishing the formation of these inflammatory metabolites (6, 59).

Another antiinflammatory feature is the ability of flavonoids to inhibit eicosanoid biosynthesis (3, 60). Eicosanoids, such as prostaglandins, are involved in various immunologic responses (61) and are the end products of the cyclooxygenase and lipoxygenase pathways. Flavonoids also inhibit both cytosolic and membranal tyrosine kinase (3). Integral membrane proteins, such as tyrosine 3-monooxygenase kinase, are involved in a variety of functions, such as enzyme catalysis, transport across membranes, transduction of signals that function as receptors of hormones and growth factors, and energy transfer in ATP synthesis. Inhibition of these proteins results in inhibition of uncontrolled cell growth and proliferation. Tyrosine kinase substrates seem to play key roles in the signal transduction pathway that regulates cell proliferation. Another antiinflammatory property of flavonoids is their suggested ability to inhibit neutrophil degranulation. This is a direct way to diminish the release of arachidonic acid by neutrophils and other immune cells (62, 63).

Antitumor effects

The antitumor activity of flavonoids is still a point of discussion. Antioxidant systems are frequently inadequate, and damage from reactive oxygen species is proposed to be involved in carcinogenesis (64, 65). Reactive oxygen species can damage DNA, and division of cells with unrepaired or misrepaired damage leads to mutations. If these changes appear in critical genes, such as oncogenes or tumor suppressor genes, initiation or progression may result. Reactive oxygen species can interfere directly with cell signaling and growth. The cellular damage caused by reactive oxygen species can induce mitosis, increasing the risk that damaged DNA will lead to mutations, and can increase the exposure of DNA to mutagens.

It has been stated that flavonoids, as antioxidants, can inhibit carcinogenesis (66). Some flavonoids—such as fisetin, apigenin, and luteolin—are stated to be potent inhibitors of cell proliferation (67). A large clinical study suggested the presence of an inverse association between flavonoid intake and the subsequent incidence of lung cancer (34). This effect was mainly ascribed to quercetin, which provided >95% of the total flavonoid intake in that particular study. Quercetin and apigenin inhibited melanoma growth and influenced the invasive and metastatic potential in mice (68). This finding may offer new insights about possible therapies for metastatic disease. Furthermore, it has been speculated that flavonoids can inhibit angiogenesis (67). Angiogenesis is normally a strictly controlled process in the human body. The process of angiogenesis is regulated by a variety of endogenous angiogenic and angiostatic factors. It is switched on, for example, during wound healing. Pathologic, unregulated angiogenesis occurs in cancer (69). Angiogenesis inhibitors can interfere with various steps in angiogenesis, such as the proliferation and migration of endothelial cells and lumen formation. Among the known angiogenesis inhibitors, flavonoids seem to play an important role (67, 70). However, the mechanism behind the antiangiogenetic effect of flavonoids is unclear. A possible mechanism could be inhibition of protein kinases (71). These enzymes are implicated to play an important role in signal transduction and are known for their effects on angiogenesis.

Antithrombogenic effects

Platelet aggregation contributes to both the development of atherosclerosis and acute platelet thrombus formation, followed by embolization of stenosed arteries. Activated platelets adhering to vascular endothelium generate lipid peroxides and oxygen free radicals, which inhibit the endothelial formation of prostacyclin and nitrous oxide. It was shown in the 1960s that tea pigment can reduce blood coagulability, increase fibrinolysis, and prevent platelet adhesion and aggregation (72). Selected flavonoids, such as quercetin, kaempferol, and myricetin were shown to be effective inhibitors of platelet aggregation in dogs and monkeys (73). Flavonols are particularly antithrombotic because they directly scavenge free radicals, thereby maintaining proper concentrations of endothelial prostacyclin and nitric oxide (74). One study showed that flavonoids are powerful antithrombotic agents in vitro and in vivo because of their inhibition of the activity of cyclooxygenase and lipoxygenase pathways (75). It is well known that arachidonic acid, which is released in inflammatory conditions, is metabolized by platelets to form prostaglandin, endoperoxides, and thromboxane A_2, leading to platelet activation and aggregation (76). The main antiaggregatory effect of flavonoids is thought to be by inhibition of thromboxane A_2 formation. Flavonoids affect arachidonic acid metabolism in different ways. Some flavonoids specifically block cyclooxygenase or lipoxygenase, whereas others block both enzymes (77). In

vitro studies showed that flavonoids bind to platelet membranes and may therefore have an accumulative effect over time (78).

Antiosteoporotic effects

In an English study, bone mineral density was compared between older women who consumed tea and those who did not. Women in the study who drank tea had higher bone mineral density measurements than did those who did not drink tea. The flavonoids in tea might be responsible for the prevention of osteoporosis (79).

Antiviral effects

The antiviral activity of flavonoids was shown in a study by Wang et al (80). Some of the viruses reported to be affected by flavonoids are herpes simplex virus, respiratory syncytial virus, parainfluenza virus, and adenovirus. Quercetin was reported to exhibit both antiinfective and antireplicative abilities. The interaction of flavonoids with the different stages in the replication cycle of viruses was previously described (81). For example, some flavonoids work on the intracellular replication of viruses, whereas others inhibit the infectious properties of the viruses. By far, most studies of the effects on viruses were performed in vitro and little is known about the antiviral effect of flavonoids in vivo. There is some evidence that flavonoids in their glycone form seem to be more inhibitory on rotavirus infectivity than are flavonoids in their aglycone form (82).

Because of the worldwide spread of HIV since the 1980s, investigations of the antiviral activity of flavonoids have mainly focused on HIV. Many natural products can inhibit various stages of the replication cycle of the virus. The discovery and development of flavonoids as anti-HIV agents has expanded in the past 2 decades. Most of these studies focused on the inhibitory activity of reverse transcriptase, or RNA-directed DNA polymerase (83), but antiintegrase and antiprotease activities were also described (1). Again, flavonoids have mainly been studied in in vitro experiments; therefore, no clear contribution of flavonoids to the treatment of HIV-infected patients has yet been shown (84).

FUTURE IMPLICATIONS

Some epidemiologic studies suggest a cardioprotective role of flavonoids against coronary heart disease. One large clinical study indicated that flavonoids may reduce mortality from coronary heart disease (52). Various cohort studies indicated an inverse association between flavonoid intakes and coronary heart disease mortality (4, 5, 85). These studies are promising and indicate that flavonoids may be useful food compounds. Flavonoids have received much attention in the literature over the past 10 y and a variety of potential beneficial effects have been elucidated. However, most of the research involved in vitro studies; therefore, it is difficult to draw definite conclusions about the usefulness of flavonoids in the diet.

The study of flavonoids is complex because of the heterogeneity of the different molecular structures and the scarcity of data on bioavailability. Furthermore, insufficient methods are available to measure oxidative damage in vivo and the measurement of objective endpoints remains difficult. There is a need to improve analytic techniques to allow collection of more data on absorption and excretion. Data on the long-term consequences of chronic flavonoid ingestion are especially scarce. In conclusion, the in vivo studies that have been performed do give a hopeful picture for the future. Currently, the intake of fruit, vegetables, and beverages (eg, tea and moderate amounts of red wine) containing flavonoids is recommended, although it is too early to make recommendations on daily flavonoid intakes.

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