Flavonoids: Diet and Health Relationships

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■ **ABSTRACT**

Flavonoids and/or phenolic acids are the major components responsible for antioxidant capacity in fruits and vegetables. Recent research has shown that flavonoids are absorbed in humans, although the bioavailablity of these dietary components is not completely clear. Some epidemiological evidence demonstrates a reduced risk of cardio- and cerebrovascular diseases in subjects with a high fruit and vegetable (flavonoid) intake. Investigations performed in vitro or in vivo in experimental animals suggest that some of the flavonoids have chemopreventive effects against certain forms of cancers. Flavonoids are potentially beneficial to cardio- and cerebrovascular systems due to their antioxidant activity against low density lipoprotein (LDL) oxidation, inhibition of platelet aggregation, and protection against capillary fragility. Flavonoids showed anti-inflammatory, antiarthritic, and antiallergic activities in vitro, and, thus, may have potential applications in preventing and treating other diseases. *Nutr Clin Care.* 2000;3:279–288 ■

KEY WORDS: ORAC, phenolics, flavonoids, anthocyanins, catechins, proanthocyanidins

Phenolics, the components responsible for antioxidant capacity in fruits and vegetables, constitute one of the most numerous and widely distributed groups of substances in the plant kingdom, with more than 8000 phenolic structures currently known.[1] Natural phenolics can range from simple molecules, such as phenolic acids (Figure 1), to highly polymerized compounds, such as tannins. The term "flavonoid" refers to a specific class of plant phenolics; their basic structure is that of diphenylpropanes (C_6-C_3-C_6) and consists of two aromatic rings linked through three carbons that usually form an oxygenated heterocycle. The common family members of flavonoids include flavones, isoflavones, flavanones, flavanols (Figure 2), catechin, anthocyanins (Figure 3), and proanthocyanidins (procyanidins or condensed tannins). The differences in these classes of flavonoid compounds rests in the presence or absence of a double bond in the C-ring, the orientation of the B-ring (isoflavones), or the lack of an oxygen in position 4 (catechins). (See Figures 2 and 3.) Flavonoids represent the most common and widely distributed group of plant phenolics.

Perhaps the earliest report of a relationship between intake of flavonoids and health was reported by Szent-Gyorgyi in 1938,[2] who considered citrus flavonoids to have vitamin activity, which he named vitamin P. These early reports showed effects of flavonoids on bleeding and capillary fragility. Although these flavonoid components are not currently considered vitamins, there is increasing evidence relating intake of flavonoids to various aspects of health and disease prevention. Some excellent reviews have been published on the impact of flavonoids on mammalian biological systems.[3–7] However, much of the early research reviewed focused on in vitro enzyme and cell systems. This review will highlight some of the areas of research under current investigation, with specific attention to in vivo research.

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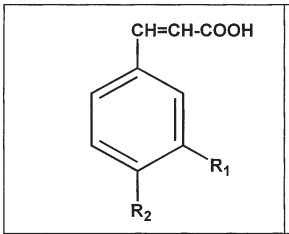
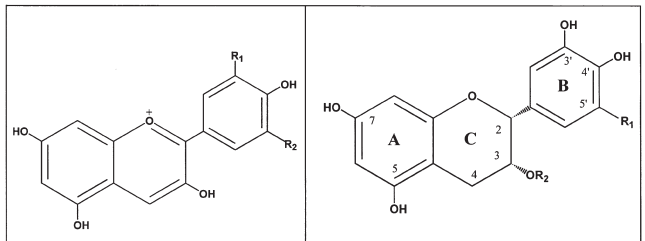


OH-Cinnamic acids	R_1	R_2
Cinnamic acid	H	H
p-Coumaric acid	H	OH
Caffeic acid	OH	OH
Ferulic acid	OCH_3	OH

Phenolic acids	R_1	R_2	R_3	R_4
Gallic acid	H	OH	OH	OH
Protocatechuic acid	H	OH	OH	H

Figure 1. Structures of simple polyphenols.



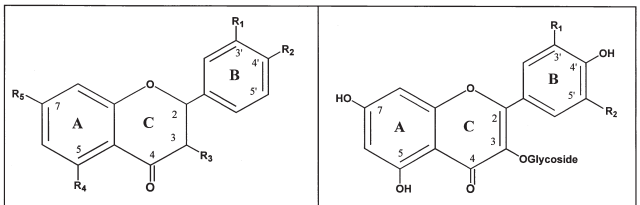
Anthocyanidin	R_1	R_2
Pelargonidin (Pg)	H	H
Cyanidin (Cy)	OH	H
Delphinidin (Dp)	OH	OH
Peonidin (Pn)	O-Me	H
Petunidin (Pt)	O-Me	OH
Malvidin (Mv)	O-Me	O-Me

Catechin		R_1	R_2
Epicatechin	EC	H	H
Epicatechin Gallate	ECG	H	Gallate
Epigallocatechin	EGC	OH	H
Epigallocatechin Gallate	EGCG	OH	Gallate
Catechin	C	H	H
Gallocatechin	GC	OH	H

Figure 3. Structure of anthocyanidins and catechins.

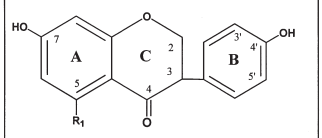
CHEMISTRY, DIETARY SOURCES, AND ABSORPTION OF FLAVONOIDS

Because of the complexity and large numbers of flavonoids in fruits and vegetables and the lack of suitable analytical capabilities, the amount of information available on food content of these substances has been severely lacking until fairly recently. Hertog and coworkers[8] were one of the first groups to publish a fairly comprehensive database on the content of three major flavonols (quercetin, kaempferol, and myricetin) and two major flavones (luteolin and apigenin). Since then, other groups have been actively working on obtaining this data.[9–11] Data on isoflavone content of foods have been published[10] and data are available from Finland[11] on most of the major flavonoids, except anthocyanins. Until such information is available, results from epidemiological studies relating to flavonoid intake and health outcomes will be compromised at best.



Flavanone	R_1	R_2	R_3	R_4	R_5
Hesperetin	OH	OCH_3	H	OH	H
Naringenin	H	OH	H	OH	H
Taxifolin	OH	OH	OH	OH	OH

Flavonol	R_1	R_2
Kaempferol glycoside	H	H
Quercetin glycoside	OH	H
Myricetin glycoside	OH	OH



Isoflavone	R_1
Daidzein	H
Genistein	OH

Figure 2. Structure of flavanones, flavonol glycosides, and isoflavones.

Table 1 highlights some foods that contain significant quantities of key phenolic compounds. However, daily consumption of flavonoids is difficult to determine because of the lack of complete tables on food composition. Until recently, the data most frequently cited were those of Kuhnau,[12] which indicated that total intake was about 1 g/day. More recently, the data from the Netherlands indicated that the daily supply of quercetin, luteolin, kaempferol, apigenin, and myricetin (and their glucoside derivatives) was in the range of 25 mg/day with more than 60% being comprised of quercetin.[13] Recent data from Finland indicated that the average total intake of flavonoids was 55.2 mg/day (excluding any anthocyanins), with fruits contributing 67%, beverages 25%, vegetables 5%, and berries 3% of the total.[11]

Our research has taken a slightly different approach in that we have measured the total antioxidant capacity using the oxygen radical absorbance capacity (ORAC) assay, which includes a measure of the total phenolics in foods that have antioxidant capacity. Antioxidant phenolics are those that have significant hydroxyl groups on the polyphenolic structure. By using a peroxyl radical generator in the ORAC assay, we found that, generally, the more OH substitutions on a flavonoid structure, the higher the ORAC of the compound. Flavones and flavanones that contain multiple OH substitu-

Table 1. Some Dietary Sources of Flavonoids and Phenolic Acids.

Flavonoid Classes and Phenolic Acids	Source
Flavones	
Apigenin, chrysoeriol, diometin, luteolin	Celery, parsley
Flavans	
Catechins, procyanidins, theaflavins, thearubigens	Tea, red wine, chocolate, peanuts, blueberries, cranberries
Flavanones	
Eriodictyol, hesperetin, naringenin	Citrus fruits
Flavonols	
Quercetin, myricetin, kaempferol, isohamnetin	Broccoli, onions, olives, tea, wine, apples, berries
Anthocyanidins	
Cyanidin, delphinidin, malvidin, pelargonidin, peonidin, petunidin	Blueberries, strawberries, cherries, grapes, colored fruits
Isoflavonoids	
Genistein, daidzein, formononetin	Soy, tofu, soymilk
Phenolic Acids	Cereals
Caffeic acid	Grapes, wine, apples, tomatoes, plums, cherries
Chlorogenic acid	Blueberries, plums, prunes, prune juice, coffee

tions have very strong antioxidant activities against peroxyl radicals (Table 2).[14]

Absorption of flavonoids from the diet was long assumed to be negligible as most of the flavonoids, except catechins, are present in plants bound to sugars as glycosides, and these glycosides were considered nonabsorbable. Contrary to the common belief that only flavonoid aglycones can be absorbed, the accumulating evidence indicates that flavonoid glycosides are absorbed in humans and rats without prior hydrolysis by microorganisms. Hollman and coworkers found in ileostomy subjects that the quercetin glycosides from onions were absorbed far better than the pure aglycones.[15] They also found in healthy subjects that the quercetin glycosides from onion were absorbed and were eliminated slowly through the day.[16] Rutin, and other quercetin glycosides, as well as an anthocyanin, were detected simultaneously in plasma from nonsupplemented humans.[17] Similar observations were made in rats on the absorption of flavonoid glycosides.[18] A recent study suggests that quercetin glucosides are capable of interacting with the sodium dependent glucose transport receptors in the mucosal epithelium and may therefore be absorbed by the small intestine in vivo.[19] The absorption of catechin,[20] naringin,[21] hesperidin,[21] genistein,[22] and daidzein[22] have also been reported in human subjects.

Table 2. Oxygen Radical Absorbing Capacity (ORAC) of Selected Flavonoid Compounds[14]

Falvonoid	ORAC*	Hydroxyl Substitution Pattern
Myricetin	4.32	3, 5, 7, 3′, 4′, 5′
Quercetin	3.29	3, 5, 7, 3′, 4′
Luteolin	3.57	5, 7, 3′, 4′
Fustin	3.91	3, 7, 3′, 4′
Eriodictyol	3.41	5, 7, 3′, 4′
Taxifolin	3.59	3, 5, 7, 3′, 4′

*Oxygen radical absorbing capacity expressed as micromoles Trolox equivalents per micromole.

Anthocyanins, one of the subgroups of flavonoids (Figure 3), are the main flavonoids found in red grapes, red wine, berries, and berry-based dietary supplements. Anthocyanins are responsible for the red, violet, and blue colors observed in fruits and berries. As early as 1933, Horwitt observed that the urine of rabbits fed 500 mg of anthocyanin pigment from grapes became highly pigmented; he concluded that small quantities of the grape anthocyanins were absorbed, passed through to the circulation, and appeared to be excreted by the kidney in an unchanged form.[23] This observation has now been confirmed in humans. Paganga and Rice-Evans reported a compound with an absorption spectra similar to anthocyanins in human plasma.[17] This study suggested that flavonoids were absorbed and present in human plasma in the glycosylated form. Rutin and other quercetin glycosides, phlo-

ridzin, as well as an anthocyanin, were detected simultaneously. The polyphenols were detected in plasma from nonsupplemented humans at individual levels in the range of 0.5–1.6 micromol/L. We observed the appearance of the same anthocyanins in plasma as found in an elderberry concentrate (cyanidin-3-glucoside and cyanidin-3,5-diglucoside) at 30 and 60 minutes postconsumption of the elderberry concentrate (1500 mg anthocyanins).[24] We confirmed the identity of the unconjugated forms of these anthocyanins in plasma and urine using HPLC/MS/MS and UV absorption spectra (Prior and Cao, 1999, unpublished). An average of 24 nmol/L of cyanidin-3-glucoside and a trace of cyanidin-3,5-diglucoside were found by Miyazawa and coworkers in human plasma, 30 minutes after the subjects had consumed 2.7 mg of cyanidin-3-glucoside and 0.25 mg of cyanidin-3,5-diglucoside per kg of body weight.[25] Cyanidin is the most common anthocyanidin present in fleshy fruits. Delphinidin is the next most common, followed by peonidin, pelargonidin, petunidin, and malvidin. Lapidot et al[26] observed in human subjects that within 12 hours of drinking 300 mL of red wine, 1% to 5% of the ingested anthocyanins were detected in the subjects' urine.[26]

The absorption of ferulic acid, a phenolic acid and a strong antioxidant found in foods such as apples, blueberries, plums, raspberries, strawberries, and tomatoes, was investigated in humans by Bourne and Rice-Evans. Their results showed that the peak time for maximal urinary excretion of ferulic acid is about 7 hours; the recovery in the urine is 11% to 25% of that ingested.[27]

Thus, there is considerable evidence indicating that phenolics, including flavonoids, are being absorbed, but the absorbed amount is probably a relatively small proportion of the total consumed. However, much has yet to be learned about the metabolism and possible biological functions of these dietary components.

FLAVONOIDS AND IN VIVO ANTIOXIDANT CAPACITY

Phenolic antioxidants function as terminators of free radicals by rapid donation of a hydrogen atom to radicals. They can also act as chelators of transition metals that are involved in free radical production and oxidative reactions.[28,29] We have demonstrated in vitro, along with others, that many flavonoids have antioxidant capacities much stronger than vitamins C and E.[14,30,31] It has been demonstrated that flavonoids can inhibit low-density lipoprotein (LDL) oxidation in vitro induced by free radicals, macrophages, or a transition metal ion, Cu^{2+}.[32–36]

Recently, several dietary phenolic compounds were found to be bound to LDL lipoproteins, adding to the evidence that dietary phenolics are likely to protect LDL lipoproteins from oxidation, thereby delaying the onset or progression of atherosclerotic processes.[37]

Absorbed dietary phenolic antioxidants can improve humans' antioxidant status. We found in 36 healthy nonsmokers that daily intake of the total antioxidants from fruits and vegetables, measured as ORAC, was significantly correlated with fasting plasma antioxidant capacity. Increasing the consumption of fruits and vegetables from the usual 5 servings/day to the experimental 10 servings/day resulted in a significant increase of plasma antioxidant capacity.[38] Increased plasma antioxidant capacity in humans also has been seen after the consumption of strawberries,[39] spinach,[39] grape juice,[40] and red wine.[39,41] A reduced sensitivity to oxidation of plasma and/or LDL was observed by several research groups in human subjects consuming red wine.[42–44]

Reduction of LDL oxidation has been observed after consumption of foods rich in phenolic compounds.[43,45] Morton et al[46] summarized 18 human trials in which some measurement of antioxidant capacity was made following consumption of either wine, fruit juice, or tea phenolics. In 6 of the studies, there was no effect of the treatment compared to control. In most studies, the measure of antioxidant activity was ex vivo LDL oxidation or plasma antioxidant capacity. Although sound rationale was presented for considering polyphenolics as important contributors to dietary antioxidant intake, these authors concluded that additional biomarkers of oxidant damage in vivo are needed before these compounds can be conclusively considered as dietary antioxidants with nutritional benefits.

FLAVONOIDS AND HEALTH OUTCOMES: EPIDEMIOLOGY STUDIES

Epidemiological studies have reported a reduced risk of cardio- and cerebrovascular diseases in sub-

jects with a high flavonoid intake. Tea is a major dietary source for flavonoids in Western populations. Keli and coworkers[47] in an epidemiological study of 552 men studied in the Netherlands concluded that long-term intake of flavonoids and consumption of black tea may protect against stroke. Geleijnse and coworkers[48] studied the association of tea intake with aortic atherosclerosis in a general population in what was known as "the Rotterdam Study." Multivariable analyses showed a significant inverse association of tea intake (more than 500 mL/day) with severe aortic atherosclerosis, but not with mild or moderate atherosclerosis. Flavonoid intake was significantly inversely related to mortality from coronary heart disease and of borderline significance ($P < .08$ for trend) with the incidence of a first fatal or nonfatal myocardial infarction.[8,49] Flavonoid intake has been inversely and significantly associated with death from coronary heart disease and was shown to have an inverse relation with the incidence of myocardial infarction.[13] Knekt et al[50] reported that the incidence of coronary mortality is higher among populations with low dietary intake of flavonoids and that the protective effect of flavonoids was associated with a diet high in apples and onions. However, Hertog et al[51] found that intake of antioxidant flavonols was not inversely associated with ischemic heart disease in the United Kingdom. Rimm and coworkers[52] in the Health Professionals Follow-up Study did not find a strong inverse association between intake of flavonoids and total coronary heart disease, but they did not exclude the possibility that flavonoids have a protective effect in men with established coronary heart disease. However, these early epidemiological studies were based on only 5 flavonoids and, thus, may not give a realistic evaluation of all flavonoids and health outcome. As intriguing as some of these findings are relative to flavonoid action, Muldoon and Kritchesvsky[53] perhaps correctly point out that the evidence of benefit is still fragmentary.

POTENTIAL OF FLAVONOIDS IN DISEASE PREVENTION AND TREATMENT

Flavonoids have been reported to inhibit, and sometimes induce, a large variety of mammalian enzyme systems.[5] Some of these enzymes are involved in important pathways that regulate cell division and proliferation, platelet aggregation, detoxification, and inflammatory and immune response. Thus, it is not surprising that effects of flavonoids have been found on various stages in the cancer process, in atherosclerosis formation, on the immune system, and on hemostasis in cell systems and animals.

Flavonoids and Cancer

Cancer is a major cause of mortality, accounting for more than 7 million deaths per year worldwide.[54] During its early stages, the development of cancer is relatively slow; initial damage of DNA is a prerequisite (initiation step), followed by a prolonged promotion step which completes the various alterations conferring on the cell a capacity to proliferate out of control of normal physiological factors.

Studies performed in vitro, or in vivo in experimental animals, suggest that flavonoids could affect most of the steps involved in carcinogenesis.

During the initiation period, they could prevent genotoxic alterations by various means: (1) inhibition of hepatic microsomal cytochrome P450 enzymes involved in the metabolic activation of procarcinogens, (2) activation of phase II enzymes involved in the detoxification of carcinogens, (3) acceleration of carcinogen movement out of the cells, or (4) direct interaction with the carcinogen itself. It is well known that antioxidant flavonoids can protect against oxidative damage to DNA. For example, flavonoids including quercetin, quercetrin, quercetin-3-glucoside, rutin, luteolin, myricetin, kaempferol, and apeginin were all effective in reducing H_2O_2-induced DNA damage in human lymphocytes.[55] Chlorogenic acid, found in foods like blueberries, plums, prunes, prune juice, and coffee, protected against DNA breakage caused by monochloramine.[56] Microsomal cytochrome P450 enzymes were found to be inhibited by several flavonoids, such as naringenin, quercetin and myricetin.[57,58] The activity of a phase II enzyme, glutathione S-transferase, in the liver, lung, stomach, skin, and small bowel increased significantly in a dose- and time-dependent manner after mice consumed silibinin (a polypenol compound).[59]

Flavonoids also exert growth inhibition in several cell lines, including colorectal carcinoma cells and can induce apoptosis.[60–64] In animals, flavonoids showed a clear chemopreventive effect against var-

ious cancers, such as the 20-methylcholanthrene-induced fibrosarcoma,[65] azoxymethanol-induced colonic neoplasia,[66,67] 4-nitroquinoline 1-oxide-induced tongue carcinogenesis,[68] and 7,12-dimethylbenz [α] anthracene-intiated, and 12-*O*-tetradecanoylphorbol 13-acetate-promoted mouse skin carcinogenesis.[69,70] Ellagic acid, a phenolic acid found in raspberries and strawberries, was also shown to inhibit chemically-induced cancer in the lung, liver, skin, and esophagus of rodents.[71]

Flavonoids and Cardio- and Cerebrovascular Diseases

The antioxidant capacity of flavonoids against LDL oxidation in vitro is well known, as we mentioned above. LDL oxidation has been causally related to atherogenesis.[72,73] Oxidation of LDL appears to occur predominantly in arterial intima in microdomains sequestered from antioxidants of plasma. Recently, antioxidant phenolic compounds (rutin, quercetin-3-glucuronide, plus other unidentified flavonoids) have been detected in human LDL.[37] Phenolics that bind LDL are good candidates for preventing lipid peroxidation and atherosclerotic processes.

Besides their antioxidant capacity, flavonoids have other biological functions which are beneficial to the cardio- and cerebrovascular systems. Proanthocyanidins relaxed the precontracted rabbit aortic rings, possibly through the NO-cGMP pathway.[74] Proanthocyanidins and anthocyanins noncompetitively inhibited the proteolytic enzymes (collagenase and elastase) and glycosidases (β– glucuronidase and hyaluronidase), which are involved in the turnover of the main structural components of the extravascular matrix collagen, elastin, and hyaluronic acid.[75,76] In humans, a commercial crude extract of bilberry (*V. myrtillus*), called *V. myrtillus* anthocyanin (VMA), has been shown to have positive effects in treating various microcirculation diseases resulting from capillary fragility[77,78] and preventing cholesterol-induced atherosclerosis.[79] Effectiveness has been confirmed in trials performed on diabetic and dyslipidaemic patients, on patients with diseases induced by stasis of the lower extremeities such as prevaricose syndrome, essential varices, post–phlebitic syndrome, and with severe arteriosclerotic vascular disease.[76] We found in rats that a blueberry extract rich in anthocyanins was effective in protecting against hyperoxia-induced increase in capillary permeability.[80] Capillary damage has been treated by drugs (mainly diosmin or hesperidin methylchalcone and hydroxyethylrutosides) that are based on flavonoids and derived from hesperidin and rutin. These drugs seem to act primarily on the microvascular endothelium to reduce hyperpermeability and edema.[2]

In the last two decades, it has become apparent that platelets contribute to the development of atherosclerosis and cardio- and cerebrovascular diseases through several mechanisms.[81] Flavonoids, including kaempferol, quercetin, myricetin, fisetin, and morin, markedly inhibited platelet aggregation and ATP release of rabbit platelets induced by arachidonic acid or collagen, and slightly those induced by platelet-activating factor.[82] Thromboxane B_2 formation was also inhibited by flavonoids in platelets challenged with arachidonic acid. In human platelet-rich plasma, quercetin prevented the secondary aggregation and blocked ATP release from platelets induced by epinephrine or ADP.[82] Naringenin,[83,84] crude anthocyanin extracts (reviewed by Morazzoni and Bombardelli[76]), and the sodium salts of ferulic acid[85] also inhibited platelet aggregation. It has been shown that red wine and grape juice, both of which are rich in flavonoids, but not white wine, inhibited platelet aggregation in vivo in a canine model.[86] The anti-antiplatelet effect of flavonoids was thought to be due to both the inhibition of thromboxane formation and thromboxane receptor antagonism.[82]

Flavonoids and Other Diseases

Recently, Bertuglia et al[87] reported that oral administration of delphinidin, one of the aglycones of anthocyanins, at 100 mg/kg body weight in hamsters, was able to prevent microangiopathy in experimental diabetes. The orally administrated delphinidin prevented an increase in microvascular permeability, inhibited adherence of leukocytes, and restored the vessel relaxation response to acetylcholine and sodium nitroprusside in vitro.[87] The supplementation of crude lemon flavonoids, eriocitrin, or hesperidin significantly suppressed lipid peroxidation and DNA damage in diabetic rats.[88]

Flavonoids also possess anti-inflammatory, antiarthritic, and antiallergic activities.[89–92] Quercetin inhibited the expression of inducible intracellular ad-

hesion molecule-1 (ICAM-1) in human endothelial cells in culture through the c-Jun NH_2-terminal kinase (JNK) pathway.[93] ICAM-1 plays a pivotal role in inflammatory responses. Oral administration of a crude anthocyanin extract[76] and grape seed flavonoids[94] also significantly improved visual performance in humans.

RECOMMENDATIONS

The experimental data seem clearly to support the conclusion that increased consumption of fruits and vegetables in the US population will likely lead to improved health outcomes. Consumption of 10 servings per day of fruits and vegetables will provide approximately 3500 micromole Trolox equivalents of antioxidant capacity, or 350 mg of total phenolics, a level that has been shown to increase plasma total antioxidant capacity and that represents a reasonable dietary intake goal. This same level of phenolic intake can be achieved with fewer servings of fruits and vegetables if foods high in antioxidant capacity are selected, such as berries, spinach, broccoli, and/or drinks such as tea or fruit juices. Until we understand more about individual compounds and possible interactions, consumption of a variety of foods is recommended, as the pattern of flavonoids often differs markedly in different foods. Increasing the intake of flavonoids with extracts or other dietary supplements is not recommended at this point in time because of a lack of quality control in many of the commercially available products and incomplete or partial extraction of the flavonoid components such that the extract composition often does not resemble the original food.

SUMMARY

Flavonoids are very common in the plant kingdom and significantly contribute to antioxidant capacity in fruits and vegetables. Recent research has shown that flavonoids are absorbed in humans and that in vivo antioxidant capacity in plasma can be increased in humans with increased dietary consumption of flavonoids and/or fruits and vegetables. Some of the flavonoids have been shown to have chemopreventive effects against various cancers in numerous animal models. Thus, some of the beneficial effects of consumption of fruits and vegetables in reducing cancer risk may be attributed, in part, to the flavonoid content of fruits and vegetables. Although there are epidemiological data indicating an inverse relationship between dietary flavonoid intake and coronary heart disease and stroke, these data are still somewhat fragmentary.

Ronald L Prior, MD and Guohua Cao, MD have indicated no significant relationships with commercial supporters.

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