

Bioavailability and Health Effects of Dietary Flavonols in Man

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Abstract

Flavonoids are polyphenolic compounds that occur ubiquitously in foods of plant origin. Over 4000 different flavonoids have been described, and they are categorized into flavonols, flavones, catechins, flavanones, anthocyanidins, and isoflavonoids. Flavonoids have a variety of biological effects in numerous mammalian cell systems, as well as *in vivo*. Recently much attention has been paid to their antioxidant properties and to their inhibitory role in various stages of tumour development in animal studies.

Quercetin, the major representative of the flavonol subclass, is a strong antioxidant, and prevents oxidation of low density lipoproteins *in vitro*. Oxidized low density lipoproteins are atherogenic, and are considered to be a crucial intermediate in the formation of atherosclerotic plaques. This agrees with observations in epidemiological studies that the intake of flavonols and flavones was inversely associated with subsequent coronary heart disease. However, no effects of flavonols on cancer were found in these studies.

The extent of absorption of flavonoids is an important unsolved problem in judging their many alleged health effects. Flavonoids present in foods were considered non-absorbable because they are bound to sugars as β -glycosides. Only free flavonoids without a sugar molecule, the so-called aglycones were thought to be able to pass through the gut wall. Hydrolysis only occurs in the colon by microorganisms, which at the same time degrade flavonoids. We performed a study to quantify absorption of various dietary forms of quercetin. To our surprise, the quercetin glycosides from onions were absorbed far better than the pure aglycone. Subsequent pharmacokinetic studies with dietary quercetin glycosides showed marked differences in absorption rate and bioavailability. Absorbed quercetin was eliminated only slowly from the blood.

The metabolism of flavonoids has been studied frequently in various animals, but very few data in humans are available. Two major sites of flavonoid metabolism are the liver and the colonic flora. There is evidence for O-methylation, sulfation and glucuronidation of hydroxyl groups in the liver. Bacterial ring fission of flavonoids occurs in the colon. The subsequent degradation products, phenolic acids, can be absorbed and are found in urine of animals. Quantitative data on metabolism are scarce.

Introduction

A large number of epidemiological studies show a protective effect of vegetables and fruits against cancer (Steinmetz and Potter, 1991; Block et al. 1992). Although not studied as extensively as for cancer, epidemiological studies also suggest a strong protective effect of vegetables and fruits for stroke, and a weaker protective effect for coronary heart disease (Ness and Powles, 1997). Various hypotheses have been suggested to explain these beneficial effects of an increased consumption of vegetables and fruits. An attractive hypothesis is that vegetables and fruits contain compounds that have a protective effect independent of that of known nutrients and micronutrients. This is supported by *in vitro* and *in vivo* studies which show that naturally occurring plant compounds may inhibit various stages in the cancer process (Wattenberg, 1992). In these studies flavonoids have also been studied extensively. Reduced risk of cardiovascular disease is possibly associated with high intakes of dietary antioxidants, of which vitamins have been most frequently studied (Jha et al. 1995).

Flavonoids are polyphenolic compounds that occur ubiquitously in foods of plant origin. Variations in the heterocyclic ring C give rise to flavonols, flavones, catechins, flavanones, anthocyanidins, and isoflavonoids (Fig. 1). In addition, the basic structure of flavonoids allows a multitude of substitution patterns in the benzene rings A and B within each class of flavonoids: phenolic hydroxyls, O-sugars, methoxy groups, sulfates and glucuronides. Over 4000 different naturally occurring flavonoids have been described (Middleton and Kandaswami, 1994). Flavonoids are common substances in the daily diet (Table 1).

Due to their antioxidant properties *in vitro* and to their inhibitory role in various stages of tumour development in animal studies, flavonoids may contribute to the protective effects of vegetables and fruits and dietary antioxidants.

Biological Effects of Flavonoids

A multitude of *in vitro* studies has shown that flavonoids can inhibit, and sometimes induce, a large variety of mammalian enzyme systems. The effects of mainly flavones and flavonols on 24 different enzymes or enzyme systems were described in a review (Middleton and Kandaswami, 1994). 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 in cell systems and animals, on different stages in the cancer process, on the immune system, and on haemostasis (Middleton and Kandaswami, 1994). Worries about the mutagenicity of flavonoids in bacterial systems (Sugimura et al. 1977) triggered much research on the flavonol quercetin. However, mutagenicity of flavonoids *in vivo* in mammals was never found (Aeschbacher et al. 1982; MacGregor et al. 1983). Animal studies of the carcinogenicity of

quercetin were also negative except in one case (Middleton and Kandaswami, 1994). However, the anticarcinogenic and antiproliferative effects of quercetin and other flavonoids are becoming increasingly evident (Huang and Ferraro, 1992).

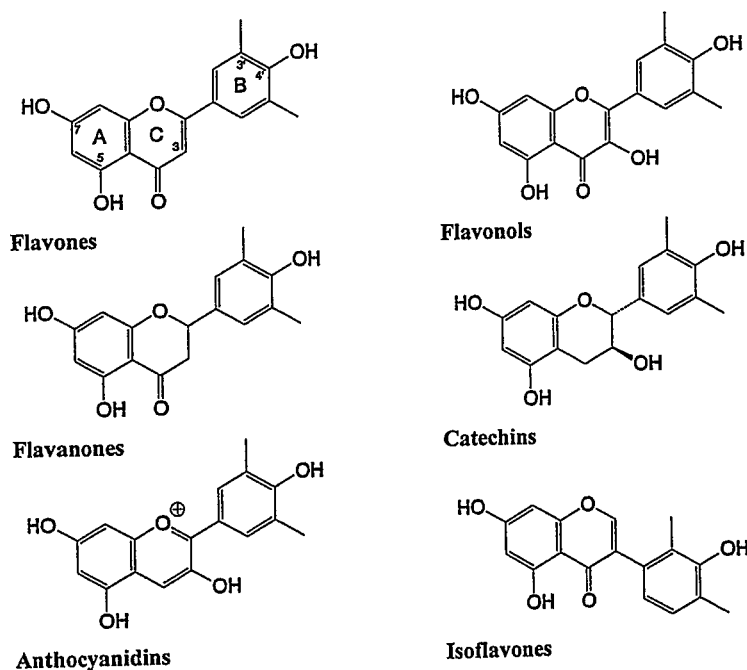


Fig. 1. Subclasses of flavonoids. Classification is based on variations in the heterocyclic ring C.

Recently, it has been hypothesised that their antioxidant properties (Kandaswami and Middleton, 1994) may protect tissues against oxygen free radicals and lipid peroxidation. Oxygen free radicals and lipid peroxidation might be involved in several pathological conditions such as atherosclerosis, cancer, and chronic inflammation (Halliwell, 1994). Quercetin is a powerful antioxidant. The Trolox equivalent antioxidant capacity (TEAC) of quercetin is 4 fold higher than that of the antioxidant (pro)vitamins (Rice-Evans and Miller, 1996). Quercetin prevents oxidation of low density lipoproteins (LDL) *in vitro* (de Whalley et al. 1990). Oxidized LDL has been found in atherosclerotic lesions of humans (Shaikh et al. 1988), and increased plasma concentrations of autoantibodies against oxidized LDL occur in patients with atherosclerosis (Salonen et al. 1992; Bergmark et al. 1995). Quercetin may therefore contribute to the prevention of atherosclerosis (Steinberg et al. 1989), cancer and chronic inflammation (Halliwell, 1994).

Table 1. Occurrence of flavonoids in common foods (Kühnau, 1976; Hertog et al. 1992; Hertog et al. 1993b)

Flavonoid Subclass	Major Food Sources
Flavonols	- onions, kale, broccoli - apples, cherries, berries - tea, red wine
Flavones	- parsley, thyme
Flavanones	- citrus
Catechins	- apples - tea
Anthocyanidins	- cherries, grapes
Isoflavones	- soya beans, legumes

Flavonols in Cancer and Cardiovascular Disease

Reliable data on flavonoid contents of common vegetables and fruits are needed to be able to study the potential role of dietary flavonoids in cancer and coronary heart disease prevention. Such a database did not exist for the Netherlands, and data produced in other countries were fragmentary. In addition, the quality of these data was questionable, because they were obtained with methods now considered obsolete. We undertook to produce a database on flavonoid contents of vegetables and fruits commonly consumed in the Netherlands. We focused on the subgroups of flavonols and flavones, because these flavonoids, including the flavonol quercetin (3,5,7,3',4'-pentahydroxyflavone) occur ubiquitously in plant foods and were the ones most frequently studied in model systems.

We determined the flavonols quercetin, kaempferol, myricetin, and the flavones luteolin and apigenin in 28 vegetables, 9 fruits, and 10 beverages commonly consumed in the Netherlands (Hertog et al. 1992; Hertog et al. 1993b). Quercetin was by far the most important flavonol, followed by kaempferol (3,5,7,4'-tetrahydroxyflavone). Flavones were only found in a few products. These data have been used in a number of prospective cohort studies and in one prospective cross-cultural study on the relation between flavonol and flavone intake and cancer and cardiovascular disease.

Cancer. The intake of flavonols and flavones was calculated in a population of elderly men in the Dutch town of Zutphen, the Zutphen Elderly Study. In 1985 their food consumption was assessed using a dietary history method. A total

number of 805 men aged 65-84 years, entered the study. The intake of flavonols and flavones was on average 26 mg/day. Major sources of flavonols and flavones were tea (61%), onions (13%) and apples (10%). After 5 years, in 1990, their health records were collected, and morbidity and mortality data were studied. Differences in baseline characteristics of these men between tertiles of flavonol and flavone intake were evaluated, and relative risks were calculated. No associations were found between flavonol and flavone intake and total cancer mortality. Also specific forms of cancer, such as lung cancer were not associated with flavonols and flavones (Hertog et al. 1994).

In a large cohort study, The Netherlands Cohort study, consisting of 120 850 men and women aged 55-69 years, also no association was found with flavonol and flavone intake and stomach cancer, colon cancer and lung cancer during 4.3 years of follow-up (Goldbohm et al. 1995).

The Zutphen Study cohort is one of the cohorts of the Seven Countries Study, a cross-cultural study of diet, lifestyle and disease. In 1987 the foods that represented the baseline diet as per 1960 of each cohort were bought locally. The foods were combined into food composites that represented the average daily food intake of each cohort. In these food composites flavonols and flavones were determined. The intake of flavonols and flavones ranged from 3 mg/day in a Finnish cohort to 70 mg/day in a Japanese cohort. The major dietary sources of flavonols and flavones varied substantially between cohorts. In the Japanese and Dutch cohorts the major source was tea, while red wine was the major source in Italy. Onions and apples were the predominant sources in the United States, Finland, Greece and former Yugoslavia. Again, no association with cancer mortality was found (Hertog et al. 1995). Thus, no association with cancer mortality was found in these three studies (Table 2).

Cardiovascular Disease. As for cancer, the only studies relating the intake of dietary flavonoids to risk of cardiovascular disease have been observational in nature. We determined the average dietary flavonol and flavone intake as it was around 1960 in 16 cohorts participating in the Seven Countries Study. The average flavonol and flavone intake was inversely correlated to mortality rates of coronary heart disease after 25 years of follow-up (Hertog et al. 1995). The intake of flavonols and flavones, together with smoking and the intake of saturated fat, explained about 90% of the variance in coronary heart disease mortality rates across the 16 cohorts.

Five prospective within-population cohort studies have been carried out. Coronary heart disease mortality was strongly inversely associated with flavonol and flavone intake in the Zutphen Elderly Study (Hertog et al. 1993a) with a reduction in mortality risk of more than 50% being recorded in the highest tertile of flavonol intake. Average flavonol intake in the highest tertile was 42 mg/day, and in the lowest 12 mg/day. Recently, the ten year follow-up of the Zutphen Elderly Study was completed with results strengthening the findings of the five year follow-up (Hertog et al. 1997a). Unlike the findings of the five year follow-up, a clear dose-response relationship between flavonol intake and coronary heart disease mortality was now recorded.

Table 2. Summary of epidemiological prospective studies on flavonol and flavone intake and cancer risk

Population	Age (y)	Follow-up (y)	Relative Risk ^a (95% Confidence Interval)
<i>Cohort studies</i>			
805 men; Zutphen (The Netherlands) (Hertog et al. 1994)	65-84	5	1.2 (0.7 - 2.2)
120 852 men + women; Netherlands Cohort Study (Goldbohm et al. 1995)	55-69	4	1
<i>Cross-cultural study</i>			
12 763 men; Seven Countries Study (Hertog et al. 1995)	40-59	25	r = 0.39 (P = 0.14)

^a Relative risk of highest versus lowest flavonol intake group, adjusted for age, diet and other risk factors for cancer.

The association between flavonol and flavone intake and risk of stroke was studied in a cohort of 550 middle-aged men (Keli et al. 1996). These men were followed for 15 years, and the men in the highest quartile of flavonol and flavone intake (>30 mg/day) showed a considerably reduced risk of the disease of about 60%.

Mortality from coronary heart disease was weakly inversely associated with flavonol and flavone intake in a cohort of 5130 Finnish men and women aged 30 - 69 years followed over a 20 years period (Knekt et al. 1996). The relative risks of mortality from coronary heart disease between the highest (>5 mg/day) and lowest quartiles (<2.5 mg/day) of flavonol and flavone intake were 0.73 for women and 0.67 for men.

Recently, in male US health professionals a modest, but non-significant, inverse association between flavonol and flavone intake and coronary mortality was found only in men with previous history of coronary heart disease (Rimm et al. 1996). Median flavonol intake in the highest quintile was 40 mg/day and 7 mg/day in the lowest.

In contrast to the above studies, increased mortality of ischaemic heart disease was found in Welsh men (Hertog et al. 1997b) in all quartiles of high flavonol intake compared to the lowest quartile. Mean flavonol intake in the highest quartile was 43 mg/day, and 14 mg/day in the lowest quartile.

To summarize (Table 3), a protective role for flavonols in cardiovascular disease was found in 3 out of 5 prospective cohort studies, in addition to one cross-cultural study. One prospective cohort study showed no association, and one a weakly positive association between flavonol intake and coronary heart disease. So far, the epidemiological evidence points to a protective effect of antioxidant flavonols in cardiovascular disease but it is not conclusive.

Table 3. Summary of epidemiological prospective studies on flavonol and flavone intake and coronary heart disease (CHD) and stroke risk

Population	Age (y)	Follow-up (y)	Relative Risk ^a (95% Confidence Interval)
<i>Cohort studies</i>			
CHD, 805 men; Zutphen (The Netherlands) (Hertog et al. 1993a)	65 - 84	5	0.32 (0.15 - 0.71)
CHD, 5133 men + women; Finland (Knekt et al. 1996) :	30 - 69	20	0.73 (0.41 - 1.32) 0.67 (0.44 - 1.00)
CHD, 34 789 men Health Professionals (U.S.A.) (Rimm et al. 1996)	40 - 75	6	1.08 (0.81 - 1.43)
CHD, 1900 men Caerphilly (U.K.) (Hertog et al. 1997b)	49 - 59	14	1.6 (0.9 - 2.9)
Stroke, 552 men; Zutphen (The Netherlands) (Keli et al. 1996)	50 - 69	15	0.27 (0.11 - 0.70)
<i>Cross-cultural study</i>			
CHD, 12 763 men Seven Countries Study (Hertog et al. 1995)	40 - 59	25	r = -0.50 (P = 0.01)

^a Relative risk of highest versus lowest flavonol intake group, adjusted for age, diet and other risk factors for coronary heart disease

Absorption and Metabolism of Flavonoids

These epidemiological data support a role of flavonols as antioxidants in coronary heart disease prevention. However, absorption from the diet is a prerequisite for a causal relation between flavonols and coronary heart disease. In addition, metabolism of flavonols after absorption should not substantially inhibit their antioxidant capacity. The absorption and subsequent distribution, metabolism and excretion of flavonoids in humans is little studied. Absorption of flavonoids from the diet was long considered to be negligible, as they are present in foods bound to sugars as β -glycosides (with the exception of catechins). Only free flavonoids without a sugar molecule, the so-called aglycones, were considered to be able to pass the gut wall, and no enzymes that can split these predominantly β -glycosidic bonds are secreted into the gut or present in the intestinal wall (Kühnau, 1976; Griffiths, 1982). Hydrolysis only occurs in the colon by microorganisms, which at the same time degrade dietary flavonoids extensively (Kühnau, 1976). Thus, only a marginal absorption of dietary flavonoids is to be expected. However, research on the mechanisms for aglycone transfer across the gut wall is lacking. Although only flavonoid aglycones were considered to be able to pass the gut wall, the orally administered aglycone of quercetin was poorly absorbed (< 1%) in a human trial (Gugler et al. 1975). In contrast, absorption of about 20% was demonstrated in rats after oral administration of quercetin aglycone (Ueno et al. 1983). We recently confirmed these results in a human study with ileostomy subjects: absorption of orally administered quercetin aglycone was 24% (Hollman et al. 1995). The absorption of quercetin glycosides from onions in this study was 52%, and 17% for pure quercetin rutinoside, a common glycoside in foods (Hollman et al. 1995). These data show that humans absorb appreciable amounts of quercetin and that absorption of glycosides in the small intestine is possible.

After absorption of flavonoids, the subsequent metabolism of flavonoids is rather well known from animal studies (Griffiths, 1982; Hackett, 1986), but practically no data in humans are available. Hydroxyl groups are conjugated with glucuronic acid or sulfate in the liver. In addition O-methylation may occur. Excretion in bile of glucuronides and sulfates seems to be important. Bacteria in the colon hydrolyse conjugates which is supposed to enable absorption of the liberated aglycones. However, these microorganisms also substantially degrade the flavonoid moiety by cleavage of the heterocyclic ring. Three main types of ring scission for catechins, flavonols, and flavones and flavanones, each leading to various phenolic acids or their lactones, have been postulated. These primarily produced phenolic acids are prone to secondary reactions such as β -oxidation, reduction, demethylation, dehydroxylation, and decarboxylation. The phenolic acids are absorbed and excreted in the urine. The significance of these results for humans is not clear.

Bioavailability of Quercetin Glycosides from Foods

We studied the time course of the plasma quercetin concentration in healthy subjects after supplementation of major food sources of quercetin: onions, apples and pure quercetin rutinoid. Quercetin rutinoid is a major glycoside of tea. The subjects ingested a single dose of about 4 times the average intake of flavonols and flavones in the Netherlands (Hollman, 1997). Peak plasma levels of 225 ng/ml (= 0.8 μ M) were reached after 0.7 h for the onions supplement, 90 ng/ml after 2.5 h for the apples, and 90 ng/ml after 9 h for the rutinoid. Disposition of quercetin in plasma was biphasic for onions and apples, with an elimination half-life of about 25 h. This implies that repeated dietary intake of quercetin throughout the day would lead to a build-up of the concentration in plasma. The bioavailability of quercetin from apples and the rutinoid was about one third of that from onions. Thus, dietary quercetin glycosides are absorbed in man. Absorption kinetics and bioavailability is probably governed by the type of glycoside. The dietary antioxidant quercetin could significantly increase the antioxidant capacity of blood plasma.

Conclusions

Flavonoids are bioactive polyphenols that occur ubiquitously in plant foods. Animal studies and *in vitro* studies suggest that dietary flavonols could inhibit cancer in humans. However, so far no association with cancer mortality was found in three epidemiological studies. In contrast, intake of flavonols and flavones was inversely associated with cardiovascular disease in three prospective cohort studies and in a prospective cross-cultural study. However, in one large prospective cohort study no association with coronary heart disease was found. Antioxidant effects of flavonoids possibly could explain these results. Dietary quercetin, the major flavonol in foods, is absorbed in humans and is only slowly eliminated throughout the day. Quercetin could thus contribute significantly to the antioxidant defences present in blood plasma. The metabolism of flavonoids in humans is little studied and pharmacokinetic data are scarce, probably because selective and sensitive analytical methods for these compounds in body fluids are lacking.

The role of dietary flavonols and flavones in cardiovascular disease prevention is promising. Epidemiological research in other countries and cultures, studies on biological mechanisms and bioavailability, and intervention studies are needed to fully evaluate their role in human health.

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