

## Epidemiological evidence on potential health properties of flavonoids

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### Propriétés potentielles des flavonoïdes pour la santé: résultats épidémiologiques

#### RÉSUMÉ

Les flavonoïdes sont des polyphénols omniprésents dans les aliments d'origine végétale. L'apport du flavonol quercétine et de quatre autres flavonoïdes voisins varie dans différents pays de 6–64 mg/jour, essentiellement fourni par le thé, le vin rouge, les oignons et les pommes. Certains flavonoïdes tels que les flavonols quercétine et kaempferol inhibent la carcinogenèse induite chimiquement chez les rongeurs.

Les flavonoïdes sont de puissants antioxydants et la quercétine inhibe l'oxydation et réduit la cytotoxicité des LDL *in vitro*. Les LDL oxydées sont athérogènes et il est possible qu'elles soient importantes dans la formation des plaques d'athérosclérose. Certains flavonoïdes tels que la quercétine inhibent également l'aggrégation de plaquettes, amenant à réduire les tendances à la thrombose. Les résultats de la recherche expérimentale suggère donc que l'apport de flavonoïdes de végétaux et de fruits pourrait réduire le risque de cancer et de maladie coronarienne (MC) chez l'homme. Jusqu'à présent, seul un nombre limité de travaux épidémiologiques ont étudié la relation entre l'apport de flavonoïdes, le cancer et la MC. Dans l'étude Zutphen Elderly Study, les informations sur l'alimentation d'environ 800 hommes âgés de 65–84 ans ont été recueillies en 1985, et étudiées en relation avec la survenue de la maladie dans les cinq années qui ont suivi le recueil des données. L'apport de flavonoïdes n'était pas associé avec l'incidence et la mortalité des cancers des voies digestives et respiratoires dans cette étude. Dans l'étude Netherlands Cohort Study, l'apport de flavonoïdes dans les données de base n'était pas, indépendamment d'autres antioxydants, associé avec l'incidence de cancers de l'estomac, des poumons, du côlon et du sein après 4–3 années de suivi chez 120 852 hommes et femmes âgés de 55–69 ans. L'apport moyen de flavonoïdes vers 1960 dans seize cohortes participant à l'étude Seven Countries Study n'expliquait pas non plus les différences dans les taux de mortalité liés aux cancers spécifiques de site entre les cohortes 25 années plus tard. En revanche, l'apport de flavonoïdes était fortement et inversement associé avec la mortalité de la maladie coronarienne dans l'étude Zutphen Elderly Study. Après 5 années de suivi, quarante-trois hommes sur les 800 participant initialement étaient décédés de maladie coronarienne. Les hommes qui avaient un apport de flavonoïdes élevé (>30 mg/jour) avaient un risque moindre de 60% de mourir de MC par rapport aux hommes dont l'apport était inférieur à 20 mg/jour. L'apport de flavonoïdes pourrait aussi expliquer, avec l'apport de graisses saturées et le pourcentage de fumeurs, environ 90% de la variance dans les taux de mortalité 25 ans plus tard dans les seize cohortes participant dans l'étude Seven Countries Study. Le thé et le vin rouge sont d'importantes sources de flavonoïdes dans l'alimentation de l'homme, et tous deux ont été associés avec une réduction du risque de MC. Le vin rouge n'a pas été associé

avec une réduction du risque de cancer, et l'association entre la consommation de thé et le cancer est très variable.

A ce jour, ces conclusions suggèrent que l'apport élevé en flavonoïdes tels que la quercétine pourrait réduire les maladies coronariennes, mais qu'il ne semble pas jouer un rôle important dans la prévention du cancer. Un petit nombre seulement d'études épidémiologiques ont été menées sur les flavonoïdes, et il faudra davantage de résultats de recherche expérimentale et clinique avant de pouvoir tirer des conclusions solides sur les bienfaits des flavonoïdes sur la santé de l'homme.

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Flavonoids are a large group of secondary plant metabolites occurring widely throughout the plant kingdom, including the food plants (Kühnau, 1976). The history of flavonoids has been characterized by controversies regarding their biological effects and significance. In about 1940, flavonoids were thought to have vitamin properties. In the 1970s, it was thought that flavonoids might be mutagens and carcinogens, whereas in the 1980s, much attention was paid to their anti-mutagenic and anti-carcinogenic activities. Finally, in recent years the antioxidant capacities of flavonoids and their potential role in both inhibition of LDL oxidation and platelet aggregation was reported. These findings have resulted in an increased interest in the health aspects of these so-called non-nutritive bioactive compounds. However, until recently experimental findings had not been confirmed in studies involving human subjects. With the determination of the content of five major flavonoids in foods (Hertog *et al.* 1992, 1993c), it became possible to investigate in epidemiological studies the association between intake of these flavonoids, some of their major food sources, such as tea and red wine, and disease occurrence in human subjects. In the present overview, the results of these investigations will be summarized. Particular attention will be paid to flavonols and flavones because of their potentially protective role in carcinogenesis, atherosclerosis and thrombosis.

#### CHEMISTRY OF FLAVONOIDS

The basic structure of flavonoids is the 2-phenyl-benzo- $\gamma$ -pyran or flavane nucleus, represented by the three ring systems (A, B and C) shown in Fig. 1. Flavonoids include flavonols, flavones, flavanones, anthocyanidins, catechins, and biflavans. Over 4000 different types of flavonoids have been described and today this number is still increasing (Markham, 1989). In common with other flavonoids, the most-frequently-found flavonols and flavones are those with B-ring hydroxylation in the 3'- and 4'-positions (Herrmann, 1988). Flavones lack the hydroxyl group at C-3 that characterizes the flavonols. Quercetin and kaempferol are typical flavonols, the corresponding flavones being luteolin and apigenin respectively (Fig. 1). Flavonols and flavones occur in foods usually as *O*-glycosides, with *D*-glucose as the most frequent sugar residue (Herrmann, 1976, 1988). The sugar-free part of the flavonoid molecule is known as the aglycone. Flavonols and flavones occur mainly in the leaves and in the outer parts of the plants, while only trace amounts are found below the soil surface. An exception are onions which contain a large amount of quercetin 4'-*D*-glucosides. In vegetables, quercetin glycosides predominate, but glycosides of kaempferol, luteolin, and apigenin are also present. Fruits almost exclusively contain quercetin glycosides (Herrmann, 1988). Levels of some flavonols in selected foods are shown in Table 1. These data refer to the aglycone

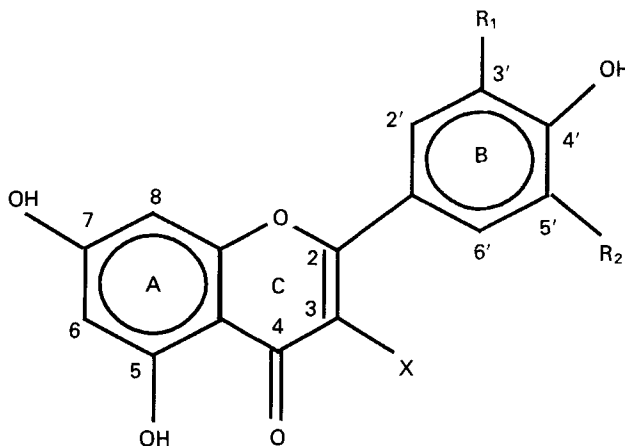


Fig. 1. Structure of flavonoids. Flavonols, where X is OH: quercetin, R<sub>1</sub> is OH, R<sub>2</sub> is H; kaempferol, R<sub>1</sub> is H, R<sub>2</sub> is H; myricetin, R<sub>1</sub> is OH, R<sub>2</sub> is OH. Flavones, where X is H: apigenin, R<sub>1</sub> is H, R<sub>2</sub> is H; luteolin, R<sub>1</sub> is OH, R<sub>2</sub> is H.

level in foods after acid-hydrolysis of the parent glycosides (Hertog *et al.* 1992, 1993c). Total flavonoid intake in the US was estimated to be about 1 g/d, of which about 100 mg (expressed as aglycones) consisted of flavonols and flavones (Kühnau, 1976). However, these estimates were based on food composition data which may have been inaccurate and incomplete. Using our analytical data on the flavonoid content of foods in combination with data on food consumption in the Netherlands provided by the National Food Consumption Survey 1987–8, we calculated the average intake of flavonols and flavones to be approximately 23 mg/d (Hertog *et al.* 1993b). Of these flavonoids, quercetin is the most predominant at 16 mg/d. The main food sources of these flavonoids in the Netherlands were black tea (48% of total intake), onions (29%) and apples (7%).

Table 1. Flavonoid content (mg/kg fresh weight) of selected vegetables, fruits and beverages (From Hertog *et al.* 1992, 1993c)

Food	Quercetin	Kaempferol	Myricetin	Luteolin
Lettuce*	7–30	<2	<1	<1
Onion*	284–486	<2	<1	<1
Endive*	<1	15–95	<1	<1
Red pepper*	<1	<2	<0.5	7–14
Broad beans	20	<2	26	<1
Apples†	21–72	<2	<1	<1
Strawberry*	8–10	120	<1	<1
Black tea (bags)‡§	17–25	13–17	3–5	<1
Red wine‡§	4–16	<1	7–9	<1
Apple juice‡	3	<1	<0.5	<1

\* Range for samples from three seasons.

† Five varieties.

‡ mg/l.

§ Five brands.

|| Below limit of detection.

## FLAVONOIDS AND CARDIOVASCULAR DISEASE

*Experimental studies*

Damage by reactive oxygen species is believed to play an important role in atherogenesis through the generation of oxidized LDL (Steinberg *et al.* 1989). Oxidized LDL can be absorbed easily by macrophages, leading to foam cell formation and ultimately to growth of atherosclerotic plaques (Ross, 1993). Their polyphenolic structure makes flavonoids potent radical scavengers and metal chelators. Their antioxidant activities have been repeatedly investigated and reviewed (Takahama, 1985; Limasset *et al.* 1993). In general, optimum antioxidant activity of flavonoids is associated with multiple phenolic groups (i.e. hydroxyl groups, especially 3' and 4' hydroxyl groups), a carbonyl group at C-4 and free C-3 and C-5 hydroxyl groups (Robak *et al.* 1988). Flavonols, such as quercetin, which combine these features scavenge superoxide anions (Robak & Gryglewski, 1988), hydroxyl radicals (Husain *et al.* 1987), lipid peroxy radicals (Sorata *et al.* 1982), and they form ligands with metal ions (Takahama, 1985). However, quercetin and myricetin also show pro-oxidant actions *in vitro* in the presence of  $\text{Fe}^{3+}$  (Laughton *et al.* 1989). Flavonoids inhibit LDL oxidation by macrophages *in vitro*, probably by protecting  $\alpha$ -tocopherol in LDL from being oxidized by free radicals, by reducing the formation of free radicals in the macrophages or by regenerating oxidized  $\alpha$ -tocopherol (De Whalley *et al.* 1990). Quercetin also reduces the cytotoxicity of oxidized LDL, whereas flavones such as apigenin are completely ineffective (Negre-Salvagyre & Salvagyre, 1992). Quercetin and its rhamnoglucoside, rutin, were modest

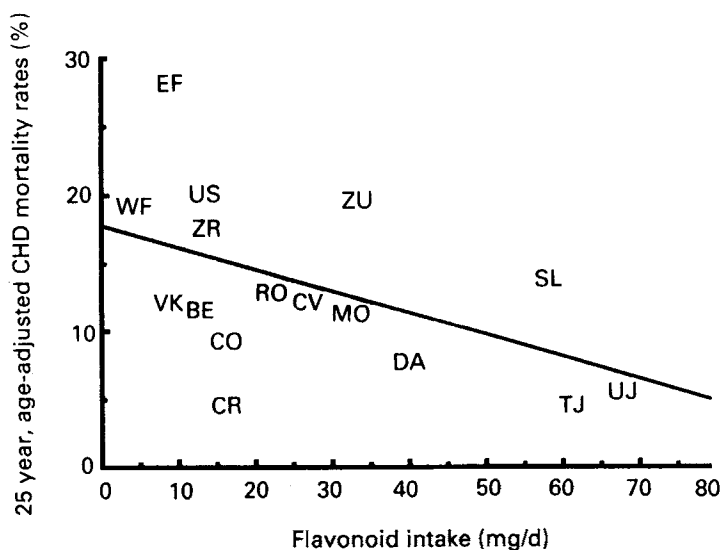


Fig. 2. Average flavonoid intake and age-adjusted mortality from coronary heart disease after 25 years of follow-up (The Seven Countries Study;  $r = -0.50$ ). BE, Belgrade, Serbia; CO, Corfu, Greece; CR, Crete, Greece; CV, Crevalcore, Italy; DA, Dalmatia, Croatia; EF, East Finland; MO, Montegiorgio, Italy; RO, Rome, Italy; SL, Slavonia, Croatia; TJ, Tanushimaru, Japan; US, USA Railroad; UJ, Ushibuka, Japan; VK, Velika-Krsna, Serbia; WF, West Finland; ZR, Zrenjanin, Serbia; ZU, Zutphen, the Netherlands. (From Hertog *et al.* 1995; reprinted with permission from American Medical Association.)

inhibitors of platelet aggregation in platelet-rich plasma *in vitro*, but they are powerful anti-platelet agents *in vivo* (Gryglewski *et al.* 1987).

### *Epidemiological studies*

The results from experimental studies suggest that a diet high in antioxidant flavonoids could reduce cardiovascular disease risk by protecting LDL against oxidation by free radicals and by reducing platelet aggregation and thrombotic tendencies. We investigated whether flavonoids in the diet could contribute to cross-cultural differences in disease mortality using data from the Seven Countries Study, a longitudinal study of risk factors for chronic diseases (Keys *et al.* 1967). Between 1958 and 1964 12 763 men aged 40–59 years from sixteen different cohorts in Japan, Greece, Yugoslavia, Finland, Italy, the Netherlands and the USA were enrolled in the Seven Countries Study and followed for 25 years. The baseline flavonoid intake was determined by chemical analysis of equivalent food composites representing the average diet for 1960 in the cohorts (Hertog *et al.* 1995); flavonoids being defined as the sum of quercetin, kaempferol, myricetin, apigenin, and luteolin. Main sources of flavonoids were tea in Japan and the Netherlands, red wine in Italy, and onions in the USA, Yugoslavia, Greece and Finland. Flavonoid intake was highest in Japan at 64 mg/d, and lowest in Finland at 6 mg/d. After 25 years, only 4.5% of the men in Japan had died from coronary heart disease (CHD), whereas about 28.8% of the men in east Finland had died from CHD. Average flavonoid intake was inversely related to CHD mortality rates across the cohorts after 25 years of follow-up (Fig. 2). About 90% of the total variance in CHD mortality rates across the cohorts could be explained by the combined effects of intake of saturated fat (explaining 73%), percentage of smokers (9%) and flavonoid intake (8%). These results were independent of the intake of alcohol and antioxidant vitamins.

So far, the association between the intake of flavonoids and CHD risk has been investigated in only one prospective cohort study. The Zutphen Elderly Study is a longitudinal investigation of risk factors for chronic diseases and an extension of the former Zutphen Study, the Dutch contribution to the Seven Countries Study. In 1985, 805 men aged 65–84 years living in Zutphen were medically examined and dietary information was collected using the cross-check dietary history method. The food intake data were converted into energy and nutrient values. Mean flavonoid intake was 26 mg/d, and the major flavonoid was quercetin. Men with a high intake of flavonoids generally had a healthier lifestyle, i.e. they smoked less, were more physically active, and had a higher consumption of fruits and vegetables. During 5 years of follow-up thirty-eight men of 693 men initially free of the disease had a first myocardial infarction; forty-three men of the total study population died from CHD. CHD mortality was approximately 65% lower in the highest tertile of flavonoid intake compared with the lowest tertile of flavonoid intake (Fig. 3). The inverse relationship between flavonoid intake and first myocardial infarction was less pronounced. These results were independent of known risk factors for CHD, including antioxidant vitamins (Hertog *et al.* 1993a). Preliminary analysis of the relationship between flavonoid intake and stroke incidence in the Zutphen Study also showed that men with a high intake of flavonoids had a significantly lower risk of stroke (S. Keli, unpublished results). Flavonoids, therefore, seem to lower the risk of events that are particularly associated with thrombotic processes, such as stroke or fatal CHD rather than atherosclerosis alone.

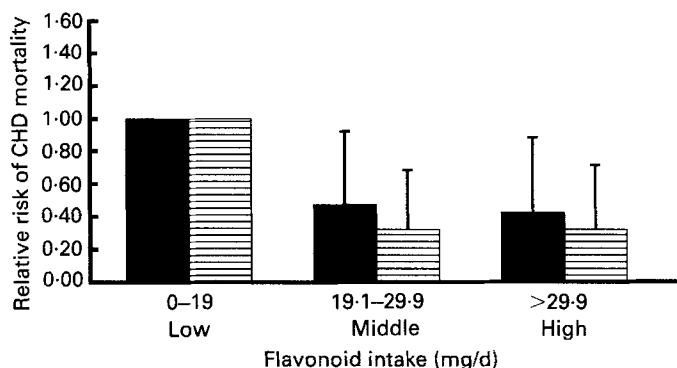


Fig. 3. Flavonoid intake of 805 men aged 65–84 years participating in the Zutphen Elderly Study and mortality from coronary heart disease (CHD; forty-three cases) after 5 years of follow-up. (■), Crude values; (▨), adjusted for age, diet and risk factors. (Based on Hertog *et al.* 1993a.)

Tea was the major source of flavonoids in these studies and in the Zutphen Elderly Study tea consumption was inversely related to both CHD and stroke mortality. Thus, additional indications of potential cardio-protective effects of flavonoids can be derived from epidemiological studies on tea consumption and CHD risk. Only a small number of studies have investigated this issue and most were directed at measuring the possible risk-enhancing effects of caffeine-containing drinks, i.e. coffee and tea and, therefore, did not specifically investigate a protective effect of tea. Brown *et al.* (1993) reported no relationship between tea consumption and the prevalence of CHD in Scottish men and women. In the USA Health Professionals Follow-up Study of 45 589 men there was no relationship between tea consumption and cardiovascular disease risk (Grobbee *et al.* 1990). However, mean tea consumption was very low in this cohort. Stensvold *et al.* (1992) have reported in a Norwegian population study an inverse relationship between tea drinking and serum cholesterol and mortality from CHD, although the latter was not statistically significant. A number of cross-sectional studies, also, have suggested an inverse relationship between black or green tea consumption and serum cholesterol (Green & Harari, 1992; Kono *et al.* 1992; Stensvold *et al.* 1992; Imai & Nakachi, 1995). However, the mechanism by which tea could lower blood lipids is unclear and one cannot exclude the possibility that the reported associations were confounded by other dietary factors that correlate both with tea-drinking habits and with blood lipids, for example dietary saturated fatty acids.

Wine consumption, which makes an important contribution to flavonoid intake, specifically in Mediterranean countries, has been found consistently to be related to a lower risk of CHD. Renaud & de Lorgeril (1992) reported that wine consumption was inversely related to CHD mortality in seventeen selected countries. Similarly, Criqui & Ringel (1994) showed that wine ethanol had the strongest and most consistent negative correlation with CHD mortality in twenty-one developed countries. These findings from cross-cultural comparisons were recently confirmed by a prospective cohort study

conducted in Denmark (Grønbeck *et al.* 1995). Persons who drank three to five glasses of wine per day had, independently from age or education, an approximately 50% lower risk of dying from cardiovascular disease during 12 years of follow-up. Wine drinking was also associated with a lower risk of dying from all causes during the follow-up period. This effect was specific for wine consumption and was not observed for other sources of ethanol. Klurfeld & Kritchevsky (1981) had shown previously that red wine, specifically, reduced coronary atherosclerosis in rabbits. More recently, it was shown in human experimental studies that red wine consumption led to increased antioxidant capacity of serum (Whitehead *et al.* 1995), and reduced oxidizability of LDL (Fuhrman *et al.* 1995). Frankel *et al.* (1993a) showed that phenolic compounds, including quercetin, extracted from red wine inhibited Cu-catalysed oxidation of human LDL *ex vivo* (Frankel *et al.* 1993a), suggesting that flavonoids such as quercetin could partly explain the cardio-protective effect of red wine through a reduction in the level of atherogenic oxidized LDL-cholesterol.

## FLAVONOIDS AND CANCER

### *Experimental studies*

Flavonoids including quercetin inhibited chemically-induced tumours in a number of experimental animal studies. Topical application of quercetin inhibited rat skin tumour promotion induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA; Kato *et al.* 1983; Wei *et al.* 1990), possibly by inhibition of epidermal ornithine decarboxylase (EC 4.1.1.17) activity. Quercetin and other flavonoids also inhibited 7,12-dimethylbenz(a)-anthracene (DMBA)-, benzo(a)pyrene-, 3-methylcholanthrene-, and *N*-methyl-*N*-nitrosourea-induced skin tumourigenesis in mice (Mukhtar *et al.* 1988). Of particular interest are two studies in which the effect of dietary-administered flavonols were investigated. Verma *et al.* (1988) reported that dietary quercetin inhibited mammary tumour initiation by DMBA and tumour promotion by TPA in rats. By using an experimental model of colon cancer, Deschner *et al.* (1991) showed that under low fat intake, dietary quercetin and rutin suppressed hyperproliferation of colonic epithelial cells and ultimately colon tumour incidence. The following mechanisms of action of quercetin and other hydroxylated flavonoids have been suggested by *in vitro* and *in vivo* research; inhibition of the metabolic activation of carcinogens by modulation of the activity of detoxifying enzymes, formation of inactive complexes with ultimate carcinogens, scavenging of reactive oxygen species, and inhibition of arachidonic acid metabolism (Huang & Ferraro, 1992).

### *Epidemiological studies*

Results from experimental research suggest that plant-food-derived flavonoids, specifically the flavonol quercetin, could reduce cancer risk in human subjects. The suggestions are supported by epidemiological studies which consistently show an inverse relationship between the consumption of fruits and vegetables and cancer risk at various sites (Block *et al.* 1992). On average, the consumption of fruits and vegetables was associated with a 50% reduced risk of cancers of the alimentary and respiratory tract. In addition to other anti-carcinogens in those foods, such as vitamin C,  $\beta$ -carotene and dietary fibre, flavonoids such as quercetin could thus contribute to the cancer-inhibiting potential of

fruits and vegetables. In the Seven Countries Study, therefore, we investigated whether flavonoid intake at baseline in sixteen cohorts was associated with cancer mortality rates in these cohorts after 25 years of follow-up. After 25 years, only 8.4% of the men in Belgrade, Serbia and 17.8% of the men in Zutphen, the Netherlands had died from any form of cancer. Average flavonoid intake was not independently related to colo-rectal cancer, lung cancer, and all-cause cancer mortality rates across the cohorts. Flavonoid intake was positively related to mortality from stomach cancer, but this effect was not independent of vitamin C intake and the percentage of smokers in the cohorts. The positive association between flavonoid intake and stomach cancer could have been confounded by infections with *Helicobacter pylori*, as these infections are more common in countries with a high flavonoid intake and less common in countries with a low flavonoid intake.

So far only two prospective epidemiological cohort studies on the cancer protective effects of flavonols and flavones have been conducted. In the Zutphen Elderly Study we investigated whether intake of these flavonoids was related to all-cause cancer mortality and incidence in elderly men. During the 5 years of follow-up, fifty-nine men initially free of the disease developed cancer of the alimentary or respiratory tract, and thirty-four men died from cancer. Flavonoid intake was not related to the incidence of these cancers, nor with cancer mortality. After adjustment for age, diet, and other risk factors (e.g. smoking), the relative risk of the highest tertile of flavonoid intake *v.* the lowest tertile of flavonoid intake was 1.02 (95% CI 0.51–2.04). Similar results were obtained when the relationship between quercetin intake and cancer risk was investigated. The number of cancer cases at specific sites was too small to allow further investigation. The relationship between flavonoid intake, tea consumption and cancer incidence has also been studied in the Netherlands Cohort Study on diet and cancer. This is a study of 120 852 Dutch men and women aged 55–69 years, followed up prospectively since 1986. At baseline, participants completed a mailed food-frequency questionnaire, which was used to calculate individual flavonoid intake. During 4.3 years of follow-up 200 cases of stomach cancer, 650 cases of colon cancer, 764 cases of lung cancer, and 650 cases of breast cancer were registered. Mean intake of flavonoids was 28 mg/d, half of which was provided by tea consumption. Using quintiles of flavonoid intake, relative risks of flavonoid intake and cancer risk at various sites were calculated. An initial inverse relationship between flavonoid intake and lung and stomach cancer risk proved to be confounded by other dietary antioxidants, and disappeared after adjustment for these factors. Flavonoid intake was also not related to breast and colon cancer risk, the relative risks in all categories were close to unity (Goldbohm *et al.* 1995).

Information on cancer protective effects of flavonoids can also be derived from epidemiological studies of tea consumption and cancer. These associations have been investigated in a number of mainly case-control studies. Yang & Wang (1992) in a recent review of epidemiology of tea pointed out that positive and inverse relationships, as well as no relationship, between tea consumption and cancer risk at various sites had been reported. Tea consumption was not associated with cancer risk in the Zutphen Elderly Study (Hertog *et al.* 1994) and preliminary analysis in the Netherlands Cohort Study, also, did not show a relationship between tea consumption and risk of cancer at various sites (S. Goldbohm, unpublished results). A positive relationship between tea consumption and cancer (mainly oesophageal tumours) has been attributed to the drinking temperature of tea, rather than to its chemical constituents (Yang & Wang, 1992). A



small number of studies performed in Asiatic countries have supported a protective effect of green-tea drinking on stomach cancer (Kono *et al.* 1988; Yu *et al.* 1991). However, these findings have not been reproduced consistently (Tajima & Tominaga, 1985). In summary, the results of epidemiological studies reported so far do not show a clear protective effect of tea drinking on cancer risk, but a protective effect of tea consumption on selected cancers in specific populations cannot be ruled out. Alcoholic beverages, including wine, have not consistently been associated with cancer risk, with the exception of a positive relationship between various types of alcoholic beverages and cancers of the upper digestive tract (International Agency for Research on Cancer, 1988). In a meta-analysis involving twenty-seven epidemiological studies, wine consumption was also not associated with risk of colo-rectal cancer (Longnecker *et al.* 1990). It seems, therefore, that wine consumption does not protect against various types of cancers.

Onion consumption is an important source of dietary flavonoids in a number of populations in which tea drinking and wine consumption is low. A large number of case-control studies have quite consistently shown an inverse relationship between consumption of onions and other *Allium* vegetables and cancer risk, particularly cancer of the stomach, colon, and rectum (Dorant, 1994). So far, the Netherlands Cohort Study is the only prospective cohort study in which the relationship between onion consumption and cancer risk at several sites, specifically, was investigated. In most other studies, consumption of onions was categorized in a vegetable group or was not surveyed. In the Netherlands Cohort Study onion consumption was inversely related to stomach cancer risk, but not to lung and colon carcinoma risk (Dorant, 1994). During 3.3 years of follow-up 139 cases of stomach cancer occurred and in comparison with 3123 randomly-chosen healthy cohort members those consuming more than half an onion per d had a 50% reduced risk of stomach cancer. However, it was suggested that this inverse relationship was probably not due to the flavonoid content of onions, because other sources of flavonoids (e.g. tea) were not associated with stomach cancer.

#### DISCUSSION

In the present overview the results of epidemiological studies on flavonols and flavones have been summarized. A limited number of epidemiological studies conducted so far have shown that intake of antioxidant flavonoids (mainly quercetin) could reduce cardiovascular disease risk, but that it does not affect cancer risk. Similarly, important food sources of flavonoids, such as tea and red wine, have been associated, although not consistently, with a lower risk of cardiovascular diseases, whereas these associations are much less apparent for cancer. Thus, the findings from experimental studies are confirmed with respect to the potential cardio-protective effects of these flavonoids, but not with respect to their potential cancer-protective effects.

The discrepancy between experimental cancer research and epidemiology cannot easily be explained. In most animal studies high doses of a carcinogen are used for induction of tumours, and the inhibitory effects of high doses of flavonoids are then investigated. These results, therefore, cannot be extrapolated directly to humans. On the other hand, the follow-up period in the two prospective cohort studies on flavonoids and cancer was only between 4 and 5 years respectively. This period may have been too short to observe an effect of flavonoids on carcinogenesis, which is a process that may take

15–20 years. In addition, fruits and vegetables were thought to be the main sources in the human diet of the flavonoids (mainly quercetin) in the study. Experimental studies on the anti-carcinogenic properties of these flavonoids have also partly been justified by the epidemiological evidence on a protective effect of vegetables and fruits. However, in the Seven Countries Study, tea was the major source of these flavonoids in the Netherlands (about 60%) and in Japan (about 90%), whereas wine was the major single source in Italy (about 40%), and onions and apples in other countries, for example USA and Finland. Thus, the foods often associated with low cancer rates in epidemiological studies, such as green–yellow vegetables and cruciferous vegetables, are not important sources of flavonols and flavones. It seems, therefore, that flavonoids only play a minor role in the explanation of the cancer protective effect of vegetables and fruits. Tea and red wine have not consistently been found to be associated with cancer in epidemiological studies. Although consumption of onions has been found to be inversely related to cancer risk in a number of case–control studies, this effect could also be explained by other potential anti-carcinogens in onions, for example diallylsulphides.

The variance in CHD mortality rates among countries can, in general, partly be explained by well-known risk factors, such as average intake of saturated fatty acids and percentage of smokers; this was shown in the Seven Countries Study. However, some inconsistencies in these relationships have been observed, notably the low CHD mortality among French wine drinkers ('French Paradox'), despite a similar level of saturated fatty acid intake to that in northern European countries. In Japan, CHD mortality rates are very low, possibly due to a low saturated fat intake, but this contrasts with the high percentage of male smokers in Japan. Our studies suggest that the high consumption of tea and red wine, both rich in antioxidant flavonoids partly explains the low CHD rates in Japan and in Mediterranean countries respectively. However, the results of the Seven Countries Study also suggest that intake of these flavonoids has only a moderate impact on cross-cultural CHD mortality rates and that saturated fat intake remains the main determinant. The high intake of saturated fat probably also explains why the UK has one of the highest CHD mortality rates in the world in spite of a very-high average tea consumption. It may also be suggested that due to the custom in the UK of adding milk to tea, binding of tea flavonoids to milk proteins may occur. Possibly flavonoids bound to proteins are less easily absorbed in the gastrointestinal tract, which could in turn result in a low bioavailability. However, there is only limited information on bioavailability of flavonoids and its determinants. A first indication of the absorption of quercetin comes from a pilot study involving nine volunteers which showed that after dietary quercetin supplementation, quercetin levels in plasma rose to approximately 1  $\mu\text{mol/l}$  (P. C. H. Hollman, personal communication). Further studies need to be done on the absorption and metabolism of flavonoids in humans.

The foods that have been associated with a reduced risk of cardiovascular diseases, such as tea and red wine, contain a large variety of compounds that could, in addition to or instead of flavonoids, explain the inverse relationships reported here. Black and green teas contain potent antioxidant catechins such as (-)epicatechin gallate and (-)epigallocatechin gallate. Catechins reduced LDL oxidation *in vitro* (Mangiapane, 1992). Possibly these catechins contribute, alone or together with the flavonols and flavones, to the cardio-protective effects of tea. Similarly, resveratrol is a compound occurring in red and white wine which reduced LDL oxidation and platelet aggregation *in vitro* (Frankel *et al.* 1993b). The importance and role of these compounds in explaining these inverse relationships needs to be studied.

In conclusion, a high intake of flavonoids such as quercetin could reduce the risk of cardiovascular disease, but it does not seem to be an important determinant of cancer risk. Additional experimental, clinical and epidemiological studies are needed to elucidate the mechanisms involved. All prospective cohort studies on the health aspects of flavonoids conducted so far have been conducted in the Netherlands. In order to reduce potential confounding by unknown factors that may be culturally linked to flavonoid intake, and tea and wine consumption, these studies need to be reproduced in populations from other countries and cultures.

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