Tea Flavonols in Cardiovascular Disease and Cancer Epidemiology (44365)

PETER C. H. HOLLMAN,*[,1] EDITH J. M. FESKENS,† AND MARTIJN B. KATAN‡

State Institute for Quality Control of Agricultural Products (RIKILT-DLO), Bornsesteeg 45, The Netherlands; National Institute for Public Health and the Environment,†3720 BA Bilthoven, The Netherlands; Wageningen Center for Food Sciences (WCFS) and Wageningen Agricultural University, Department of Human Nutrition and Epidemiology,‡ NL-6703 HD Wageningen, The Netherlands*

Abstract. **Tea is an important dietary source of flavonols in countries such as the Netherlands, the United Kingdom and Japan. Flavonols may have beneficial health effects because of their antioxidant properties and their inhibitory role in various stages of tumor development in animal studies. The association between flavonol intake and cancer risk was investigated in three prospective studies (Zutphen Elderly Study in the Netherlands, a Finnish cohort, and the Netherlands Cohort Study). Only one study (Finnish cohort) showed an inverse association with cancer mortality. The intake of flavonols with subsequent cardiovascular disease was studied in six prospective epidemiological studies. In some populations (Seven Countries Study, Zutphen Elderly Study, a Finnish cohort) a clear protective effect was observed. In a large US cohort, a protective effect was only found in a subgroup with previous history of coronary heart disease, whereas in Welsh men, flavonol intake, mainly from tea, was associated with an increased risk of coronary heart disease. These conflicting results may be due to confounding by coronary risk factors associated with tea consumption. The question of whether flavonols protect against cardiovascular disease remains still open; a protective effect of flavonols against cancer is less likely.**

[P.S.E.B.M. 1999, Vol 220]

Flavonols belong to the large group of flavonoids that are polyphenolic compounds ubiquitously present in foods of plant origin. Over 4000 different naturally occurring flavonoids have been described (1). Flavonoids are classified into flavonols, flavones, catechins, flavanones, anthocyanidins, and isoflavonoids, based on variations in the heterocyclic ring C (Fig. 1). Flavonoids are common substances in the daily diet (Table I).

A multitude of *in vitro* studies has shown that flavonoids can inhibit, and sometimes induce, a large variety of mammalian enzyme systems (1). 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 (2), on the immune system, and on hemostasis in cell systems and animals systems (1).

It has been hypothesized that the antioxidant properties of flavonoids (3) 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 (4). Thus, flavonoids, which by their chemical nature are antioxidants, might contribute to the prevention of atherosclerosis, cancer, and chronic inflammation. Oxidation of low-density lipoproteins (LDL) is thought to play an important role in atherosclerosis. Oxidized LDL has been found in atherosclerotic lesions of humans (5), and increased plasma concentrations of autoantibodies against oxidized LDL occur in patients with atherosclerosis (6, 7). A whole range of antioxidants has been shown to prevent LDL-oxidation when added to isolated LDL. Not surprisingly, flavonoids (8–12) also show this protective action towards *in vitro* LDL-oxidation; however, the clinical relevance of these laboratory findings is still obscure.

This work was supported by grants from the Foundation for Nutrition and Health Research and the Netherlands Heart Foundation, (94.128).

[1] To whom requests for reprints should be addressed at State Institute for Quality Control of Agricultural Products (RIKILT-DLO), Bornsesteeg 45, NL-6708 PD Wageningen, The Netherlands. E-mail: p.c.h.hollman@rikilt.dlo.nl

0037-9727/99/2204-0198$10.50/0

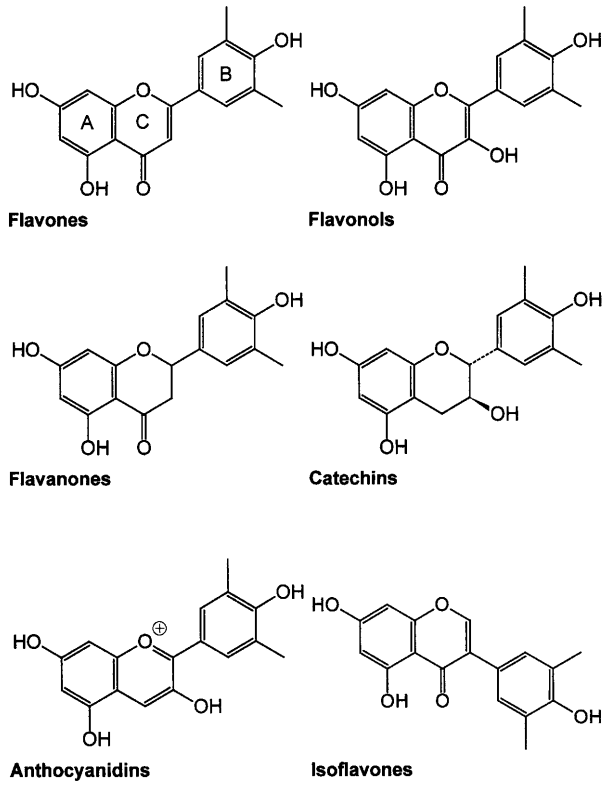

Figure 1. Subclasses of flavonoids. Classification is based on variations in the heterocyclic C-ring.

Here we summarize prospective epidemiological studies on the relation between the intake of flavonols and subsequent cardiovascular disease and cancer risk.

Epidemiology of Flavonols

Dietary Intake of Flavonoids. Kühnau (13) estimated that the total intake of flavonoids in the United States was 1 g/day (expressed as glycosides) or 650 mg/day (expressed as aglycones). Flavonols would contribute 115 mg/day. However, this estimate is not very accurate because only limited data on flavonoid contents of foods were available. We determined flavonols and flavones of vegetables, fruits, and beverages commonly consumed in the Netherlands (Table II) (14, 15); With these data, we were able to calculate the intake of flavonols and flavones in a representative sample of 6000 individuals in the Netherlands (16). Tea turned out to be the major source in this population (48% of total intake) followed by onions (29%) and apples (7%). The average intake of flavonols and flavones was 23 mg/day, of which the flavonols quercetin, myricetin and kaempferol contributed 21 mg/day. These data on flavonol contents have been used in a number of prospective studies. Average daily intake of flavonols ranged from 4–68 mg/day in these studies (Table III). Tea was the major source of flavonols in the Netherlands, the UK and Japan.

Thus, the estimated intake of flavonols of 115 mg/day in the United States (13) is most certainly too high. We estimate the daily intake of all flavonoids to be a few hundreds of milligrams per day expressed as the aglycones (17). Flavonols comprise only a small fraction. However, reliable quantitave data on the intake of other flavonoids such as catechins are not yet available.

Table I. Occurrence of Flavonoids in Common Foods

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	—Soy beans and soy products

Table II. Flavonol[a] Contents of Common Vegetables, Fruits, and Beverages

Flavonol contents	Foods
Low (<10 mg/kg or <10 mg/l)	—Cabbage, spinach, carrots, peas, mushrooms
	—Peaches, strawberries
	—Orange juice, white wine, brewed coffee
Medium (<50 mg/kg or <50 mg/l)	—Lettuce, broad beans, red pepper, tomato
	—Apples, grapes, cherries
	—Tomato juice, red wine, tea beverages
High (>50 mg/kg or 50 mg/l)	—Broccoli, endive, kale, French beans, celery, onions
	—Cranberries

[a] Sum of quercetin, kaempferol, and myricetin

Table III. Average Intake and Dietary Sources of Flavonols in Various Populations

Population	Average intake (mg/day)	Dietary sources
Finland (20)	4	Apples + onions (64%)
		Fruits, juices, berries (36%)
Health Professionals (USA) (26)	20	Black tea (25%)
		Onions (25%)
		Apples (10%)
		Broccoli (7%)
Zutphen (The Netherlands) (22)	26	Black tea (61%)
		Onions (13%)
		Apples (10%)
Caerphilly (UK) (27)	26	Black tea (82%)
Croatia (21)	58	Mainly onions and apples
Japan (21)	68	Green tea (>80%)

Flavonols in 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. 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. Specific forms of cancer, such as lung cancer were also not associated with flavonols and flavones intake (18).

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 between flavonol and flavone intake and stomach cancer, colon cancer, or lung cancer during 4.3 years of follow-up (19).

A prospective study involving about 10,000 men and women aged 15–99 years was carried out in Finland (20). After 24 years of follow-up, a reduction in risk of lung cancer of about 50% was found in the highest quartile of flavonol intake. No reduction in risk of cancer at other sites was found.

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 68 mg/day in a Japanese cohort. The major dietary sources of flavonols and flavones varied substantially between cohorts. No association with total or site-specific cancer mortality was found (21).

Thus, no association with cancer mortality was found in two cohort studies, whereas in only one cohort study a reduction of lung cancer risk was apparent (Table IV).

Flavonols in Cardiovascular Disease. In the Seven Countries Study the average flavonol and flavone intake was inversely correlated with mortality rates of coronary heart disease after 25 years of follow-up (21). 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 (22) 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 10-year follow-up of the Zutphen Elderly Study was completed with results strengthening the findings of the 5-year follow-up (23). Unlike the findings of the 5-year follow-up, a clear dose-response relationship between flavonol intake and coronary heart disease mortality was now recorded.

The association between flavonol and flavone intake and risk of stroke was studied in a cohort of 550 middle-aged men (24). 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 70%.

Mortality from coronary heart disease was weakly inversely associated with flavonol and flavone intake in a cohort of 5,130 Finnish men and women aged 30–69 years followed over a 20-year period (25). 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.

In male US health professionals, a modest, but statistically not significant, inverse association between flavonol and flavone intake and coronary mortality was found only in men with previous history of coronary heart disease (26). 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 (27) 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. The authors argued that milk proteins could have inhibited absorption of tea flavonoids, because these Welsh men used milk in their

Table IV. Summary of Epidemiological Prospective Studies on Flavonol and Flavone Intake and Cancer Mortality[b] or Cancer Incidence[c] Risk

Population	Age (y)	Follow-up (y)	Relative risk[a] (95% Confidence interval)
Cohort studies			
805 men; Zutphen (The Netherlands) (18)	65–84	5	1.2[b] (0.7–2.2)
120,852 men + women; Netherlands Cohort Study (19)	55–69	4.3	1[c]
9959 men + women; Finland (20)	15–99	24	0.5[c] (0.3–1.0)

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

Table V. Summary of Epidemiological Prospective Studies on Flavonol and Flavone Intake and Coronary Heart Disease (CHD) and Stroke Incidence Risk

Population	Age (y)	Follow-up (y)	Relative risk[a] (95% Confidence interval)
Cohort studies			
CHD, 805 men; Zutphen (The Netherlands) (22)	65–84	5	0.32 (0.15–0.71)
CHD, 5133 men + women; Finland (25)	30–69	20	♀: 0.73 (0.41–1.32) ♂: 0.67 (0.44–1.00)
CHD, 34,789 men; Health Professionals (USA) (26)	40–75	6	1.08[b] (0.81–1.43)
CHD, 1900 men; Caerphilly (UK) (27)	49–59	14	1.6 (0.9–2.9)
Stroke, 552 men; Zutphen (The Netherlands) (24)	50–69	15	0.27 (0.11–0.70)
Cross-cultural study			
CHD, 12,763 men Seven Countries Study (21)	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.
[b] Fatal and nonfatal CHD.

tea. However, plasma concentrations of catechins (28) and flavonols (Hollman, unpublished data) in volunteers given tea with milk did not differ from those when given tea without milk. A second explanation offered by the authors is confounding. In contrast to the other studies, tea consumption was associated with a less healthy lifestyle (smoking and high-fat intake) and with lower social class. Although Hertog *et al.* adjusted for these confounders, they probably could not be completely eliminated by multivariate analysis (29).

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

Conclusions

Tea is an important dietary source of flavonols in countries such as the Netherlands, the United Kingdom, and Japan. Animal studies and *in vitro* studies suggest that dietary flavonols could inhibit cancer in humans. However, so far in only one epidemiological study, an inverse association with cancer was found. Therefore, flavonols probably do not play an important role in cancer protection.

Flavonoids showed a protective action toward oxidation of LDL *in vitro*. The intake of flavonols 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 apparent, whereas a weakly positive association was found in one cohort study. These studies are not conclusive for a protective effect.

The role of dietary flavonols and flavones in cardiovascular disease prevention is in the process of being defined. Epidemiological research in other countries and cultures, studies on biological mechanisms and metabolism, and the development of biomarkers for *in vivo* oxidation are needed to fully evaluate the role of flavonoids in human health. Only clinical trials with validated intermediate disease end points or real end points (cases) will give a definitive answer.

We thank Prof. D. Kromhout, who took the initiative for the epidemiological studies. We are grateful to Dr. M. G. L. Hertog, Prof. J. G. A. J. Hautvast, and J. H. M. de Vries for valuable discussions, and to John M. P. van Trijp, M. N. C. P. Buysman, D. P. Venema, and B. v. d. Putte for technical assistance.

1. Middleton E, Kandaswami C. The impact of plant flavonoids on mammalian biology: Implications for immunity, inflammation, and cancer. In: Harborne JB, Ed. The Flavonoids, Advances in Research Since 1986. London: Chapman & Hall, pp619–652, 1994.
2. Wattenberg LW. Inhibition of carcinogenesis by minor dietary constituents. Cancer Res **52 (Suppl):**2085S–2091S, 1992.
3. Kandaswami C, Middleton E Jr. Free radical scavenging and antioxidant activity of plant flavonoids. Adv Exp Med Biol **366:**351–376, 1994.
4. Halliwell B. Free radicals, antioxidants, and human disease: Curiosity, cause, or consequence? Lancet **344:**721–724, 1994.
5. Shaikh M, Martini S, Quiney JR, Baskerville P, La Ville AE, Browse NL, Duffield R, Turner PR, Lewis B. Modified plasma-derived lipoproteins in human atherosclerotic plaques. Atherosclerosis **69:**165–172, 1988.
6. Bergmark C, Wu R, de Faire U, Lefvert AK, Swedenborg J. Patients with early-onset peripheral vascular disease have increased levels of

autoantibodies against oxidized LDL. Arterioscler Thromb Vasc Biol **15:**441–445, 1995.

7. Salonen JT, Ylä-Herttuala S, Yamamoto R, Butler S, Korpela H, Salonen R, Nyyssönen K, Palinski W, Witztum JL. Autoantibody against oxidised LDL and progression of carotid atherosclerosis. Lancet **339:**883–887, 1992.
8. Mangiapane H. The inhibition of the oxidation of low-density lipoprotein by (+)-catechin, a naturally occurring flavonoid. Biochem Pharmacol **43:**445–450, 1992.
9. Nègre-Salvayre A, Salvayre R. Quercetin prevents the cytotoxicity of oxidized LDL on lymphoid cell lines. Free Radic Biol Med **12:**101–106, 1992.
10. de Whalley C, Rankin SM, Hoult JRS, Jessup W, Leake DS. Flavonoids inhibit the oxidative modification of low-density lipoproteins by macrophages. Biochem Pharmacol **39:**1743–1750, 1990.
11. Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med **20:**933–956, 1996.
12. Wu T-W, Fung K-P, Wu J, Yang C-C, Lo J, Weisel RD. Morin hydrate inhibits azo-initiator-induced oxidation of human low-density lipoprotein. Life Sci **58:**17–22, 1996.
13. Kühnau J. The flavonoids. A class of semi-essential food components: Their role in human nutrition. World Rev Nutr Diet **24:**117–191, 1976.
14. Hertog MGL, Hollman PCH, Katan MB. Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. J Agric Food Chem **40:**2379–2383, 1992.
15. Hertog MGL, Hollman PCH, van de Putte B. Content of potentially anticarcinogenic flavonoids of tea infusions wines and fruit juices. J Agric Food Chem **41:**1242–1246, 1993.
16. Hertog MGL, Hollman PCH, Katan MB, Kromhout D. Intake of potentially anticarcinogenic flavonoids and their determinants in adults in the Netherlands. Nutr Cancer **20:**21–29, 1993.
17. Hollman PCH, Katan MB. Absorption, metabolism, and bioavailability of flavonoids. In: Packer L, Rice-Evans C, Eds. Flavonoids in Health and Disease. New York: Marcel Dekker Inc., pp483–522.
18. Hertog MGL, Feskens EJM, Hollman PCH, Katan MB, Kromhout D. Dietary flavonoids and cancer risk in the Zutphen Elderly Study. Nutr Cancer **22:**175–184, 1994.
19. Goldbohm RA, van den Brandt PA, Hertog MGL, Brants HAM, van Poppel G. Flavonoid intake and risk of cancer: A prospective cohort study. Am J Epidemiol **141 (Suppl):**s61, 1995.
20. Knekt P, Järvinen R, Seppänen R, Heliövaara M, Teppo L, Pukkala E, Aromaa A. Dietary flavonoids and the risk of lung cancer and other malignant neoplasms. Am J Epidemiol **146:**223–230, 1997.
21. Hertog MGL, Kromhout D, Aravanis C, Blackburn H, Buzina R, Fidanza F, Giampaoli S, Jansen A, Menotti A, Nedeljkovic S, Pekkarinen M, Simic BS, Toshima H, Feskens EJM, Hollman PCH, Katan MB. Flavonoid intake and long-term risk of coronary heart disease and cancer in the Seven Countries Study. Arch Intern Med **155:**381–386, 1995.
22. Hertog MGL, Feskens EJM, Hollman PCH, Katan MB, Kromhout D. Dietary antioxidant flavonoids and risk of coronary heart disease: The Zutphen Elderly Study. Lancet **342:**1007–1011, 1993.
23. Hertog MGL, Feskens EJM, Kromhout D. Antioxidant flavonols and coronary heart disease risk. Lancet **349:**699, 1997.
24. Keli SO, Hertog MGL, Feskens EJM, Kromhout D. Flavonoids, antioxidant vitamins and risk of stroke: The Zutphen study. Arch Intern Med **156:**637–642, 1996.
25. Knekt P, Jèrvinen R, Reunanen A, Maatela J. Flavonoid intake and coronary mortality in Finland: A cohort study. Br Med J **312:**478–481, 1996.
26. Rimm EB, Katan MB, Ascherio A, Stampfer MJ, Willett WC. Relation between intake of flavonoids and risk for coronary heart disease in male health professionals. Ann Intern Med **125:**384–389, 1996.
27. Hertog MGL, Sweetnam PM, Fehily AM, Elwood PC, Kromhout D. Antioxidant flavonols and ischemic heart disease in a Welsh population of men: The Caerphilly Study. Am J Clin Nutr **65:**1489–1494, 1997.
28. van het Hof KH, Kivits GAA, Weststrate JA, Tijburg LBM. Bioavailability of catechins from tea: The effect of milk. Eur J Clin Nutr **52:**356–359, 1998.
29. Katan MB. Flavonoids and heart disease. Am J Clin Nutr **65:**1542–1543, 1997.