Dietary Flavonoids and Cancer Risk in the Zutphen Elderly Study

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Abstract

Flavonoids are polyphenolic antioxidants naturally present in vegetable foods. Some flavonoids, such as quercetin, inhibit carcinogenesis in rodents, but their effect in humans is unknown. We measured the flavonoids quercetin, kaempferol, myricetin, apigenin, and luteolin in foods and assessed flavonoid intake in 1985 by dietary history in 738 men aged 65–84 years without a history of cancer, who were then followed for five years. Mean flavonoid intake was 25.9 mg/day. The major sources of flavonoid intake were tea at 61% and vegetables and fruits (mainly onions, kale, endive, and apples) at 38%. Between 1985 and 1990, 75 men developed cancer (all sites) and 34 men died from cancer. Flavonoid intake in 1985 was not associated with incidence of all-cause cancer (p for trend = 0.54) or with mortality from all-cause cancer (p for trend = 0.51). Flavonoid intake was also not associated with risk of cancers of the alimentary and respiratory tract (p for trend = 0.92). Adjustment for age, body mass index, smoking, physical activity, and vitamin C, vitamin E, β-carotene, and dietary fiber intake did not change the relative risks. A high intake of flavonoids from vegetables and fruits only was inversely associated with risk of cancer of the alimentary and respiratory tract (relative risk of highest vs. lowest tertile = 0.51, 95% confidence interval 0.25–1.05); these results suggest the presence of other nonvitamin components with anticarcinogenic potential in these foods.

We conclude that intake of flavonoids, mainly from tea, apples, and onions, does not predict a reduced risk of all-cause cancer or of cancer of the alimentary and respiratory tract in elderly men. The effect of flavonoids on risk of cancer at specific sites needs further investigation in prospective cohort studies.

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Introduction

Flavonoids (Figure 1) are polyphenols ubiquitously present in vegetables, fruits, and beverages of vegetable origin (1). The role of flavonoids in carcinogenesis is controversial. Early studies showed that some major flavonoids (e.g., quercetin, kaempferol, and myricetin) were mutagenic in bacterial test systems (2,3), but quercetin was reported not to be mutagenic
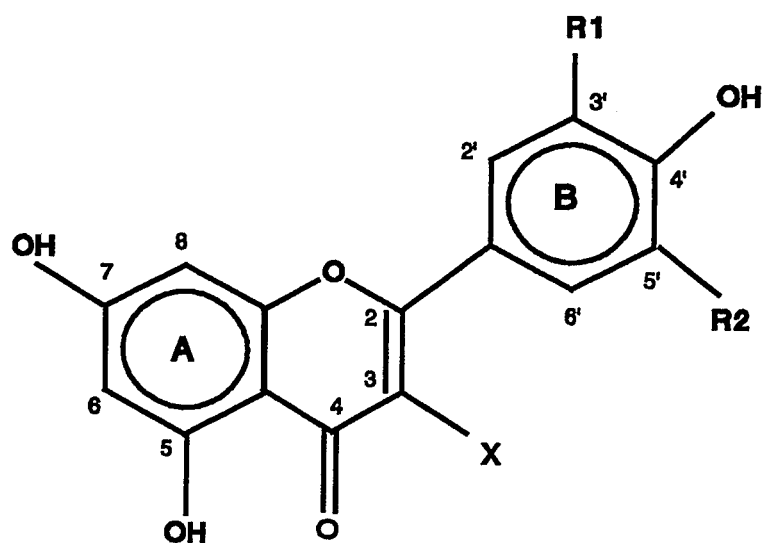
Figure 1. Structure of flavonoids. *Flavonols:* X = OH; quercetin: R_1 = OH, R_2 = H; kaempferol: R_1 = H, R_2 = H; myricetin: R_1 = OH, R_2 = OH. *Flavones:* X = H; apigenin: R_1 = H, R_2 = H; luteolin: R_1 = OH, R_2 = H.

in vivo (4). Quercetin at a level of 2% in the diet was also found to induce bladder tumors in rats (5), but these results were disproved in other studies in which quercetin levels were up to 10% of the diet (6–8). More recent studies, in fact, suggest that flavonoids protect against chemically induced cancer in animals (9–13). Dietary quercetin significantly reduced tumor multiplicity in azoxymethanol-induced colonic neoplasia in mice (9) and in *N*-nitrosomethy-lurea-induced mammary cancer in rats (11). Flavonoids also inhibited skin tumor promotion in mice (10,12). Antioxidant flavonoids such as quercetin also inhibited colonic cell prolifera-tion *in vitro* (14,15). Intake of flavonoids may therefore reduce risk of cancer in humans at several sites. This is supported by epidemiological findings that a high consumption of vegetables and fruits is associated with a reduced risk of cancer of especially the respiratory and alimentary tract (16). Until now, no epidemiological studies on the effects of flavonoids on cancer have been carried out because of a lack of accurate data on flavonoid content of foods.

We selected five major antioxidant food flavonoids (i.e., quercetin, kaempferol, myricetin, apigenin, and luteolin), measured their content in the major types of vegetables, fruits, and beverages consumed in The Netherlands (17–19), and calculated baseline flavonoid intake of the participants of the Zutphen Elderly Study. We reported previously that flavonoid intake predicted a lower rate of coronary heart disease mortality in elderly men (20). Here we report the relation between flavonoid intake and five-year cancer incidence and mortality.

Material and Methods

Flavonoid Analysis

We determined the content of the five major flavonoids, quercetin, kaempferol, myricetin, luteolin, and apigenin, in 28 types of vegetables, 12 types of fruits, and 9 types of beverages (17–19). Together, these foods covered approximately 95% of all vegetable foods commonly consumed in The Netherlands. Each food was purchased in each of the three seasons, spring, summer, and winter, in a supermarket, in a grocery, and in an open-air street market. Separate purchases were combined, and flavonoid content was determined by reverse-phase high-per-formance liquid chromatography with ultraviolet detection. In plants, a large number of flavonoid glycosides originating from the same parent aglycon (sugar-free flavonoid) occur.

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To simplify the analytic procedure and to enhance the limit of detection, glycosides were hydrolyzed, and flavonoid levels are expressed as aglycons. The aglycon is, because of its polyphenolic character, the biologically active part of the flavonoid molecule. Control samples with a known amount of flavonoids were included in every series of analysis. Limit of detection for flavonoids was approximately 1 mg/kg or 0.5 mg/l. Other details have been published elsewhere (17–19).

Population

The Zutphen Elderly Study is a longitudinal investigation of risk factors for chronic diseases (21) and forms the Dutch contribution to the Seven Countries Study (22). It was originally started in 1960 with a cohort of 878 men aged 40–59 years living in the town of Zutphen in The Netherlands. In 1985, 555 men of this cohort were still alive and were invited for new examinations. In addition, a random sample of all other men of the same age group (65–84 yrs) living in Zutphen and not part of the 1960 cohort were approached. This resulted in a target population of 1,266 men. Sixty-two men (4.9%) could not participate because they had moved or could otherwise not be reached, 109 (9.0%) could not be examined because of serious illness, and 156 (12.0%) refused to participate. Hence, in 1985 a total of 939 men (74%) aged 65–84 years entered the study. Complete information on diet and other risk factors was available for 801 participants. Sixty-three participants with a history of cancer (all sites) at baseline were excluded from the analysis, leaving a total of 738 participants.

Examinations

All examinations were carried out between March and June 1985, and a repeat examination was carried out in the same period in 1990. Information on the usual food intake of the participants in the month preceding the interview was collected by experienced dietitians using a cross-check dietary history method adapted to the Dutch situation (23). Each participant, together with the person who prepared the food (usually the wife), was interviewed at home for about one hour about usual food consumption pattern on weekdays and during weekends and about food purchases. Food portions were denoted in household measures. The habitual consumption of foods during a week was determined and verified with the quantities of foods bought per week. This information was combined to calculate the participant's food consumption on a typical weekday. The food intake data were encoded by the dietitians according to The Netherlands Uniform Food Encoding System and converted to energy and nutrient values with use of the 1985 release of The Netherlands nutrient data bank (24) that was updated with 1993 values for β-carotene and vitamin E and with flavonoid data. Flavonoids refer to the sum of quercetin, kaempferol, myricetin, apigenin, and luteolin.

Medical examinations were carried out by trained physicians and included anthropometry and blood measurements. Information on amount and duration of smoking was assessed using a standardized questionnaire and converted to lifetime exposure to cigarettes [(no. of cigarettes/day × 365) × yrs of smoking]. Physical activity was assessed with a questionnaire especially designed for retired men, and minutes of physical activity per week were calculated (25).

Incidence and Mortality Follow-Up

Prevalence of a history of cancer was recorded during the medical examinations in 1985 and 1990 with a standardized questionnaire and verified with written information from the subject's general practitioner, with hospital discharge data, and with information from the cancer registry. For subjects who had died, information on a possible history of cancer was collected from their general practitioners, from hospital discharge data, and from the cancer

registry. All information was uniformly coded by one physician, and the year of first diagnosis of each disease was recorded.

Information on the vital status of all participants up to July 1990 (5-yr follow-up) was obtained from the municipal registries. No one was lost to follow-up. Information on primary cause of death was obtained from the Central Bureau for Statistics, verified by means of hospital discharge, cancer registry data, and information from the general practitioner, and coded (26). All-cause cancer refers to International Classification of Diseases (ICD) 140–208, epithelial cancers of the alimentary and respiratory tract to ICD 140–165 and ICD 188–189, and lung cancer to ICD 162.

Statistical Analysis

Spearman rank correlation coefficients (r_s) were calculated between the intake of flavonoids and baseline variables, and differences in baseline characteristics according to cancer status were evaluated with the Mann-Whitney rank sum test. Relative risks (RR) and their 95% confidence interval (95% CI) of incidence and mortality according to tertiles of flavonoid intake, with the lowest tertile used as reference, were calculated by Cox proportional-hazard (survival) analysis by use of the SAS procedure PHREG (27). Proportional hazard assumptions were checked (28) and found to be valid. P values for trend were calculated from the regression coefficient on a dummy variable for tertiles of flavonoid intake approximating the Mantel-Haenszel chi-square test for trend. Potential confounders were assessed in multivariate analysis. All P values are two-sided, and P values <0.05 were considered statistically significant.

Results

Flavonoid intake of the 738 participants was 25.9 ± 14.6 (SD) mg/day in 1985 and, of the 482 men still alive and participating in 1990, 26.4 ± 13.2 mg/day. The correlation between flavonoid intake in 1985 and 1990 was 0.58 ($p = 0.001$), suggesting that individual intakes were measured with satisfactory precision. The most important flavonoid was quercetin at 16.4 ± 10.2 mg/day (63%), followed by kaempferol at 8.2 ± 5 mg/day (32%). The main source of flavonoids was tea at 61%, whereas vegetables and fruits (mainly onions, endive, kale, and apples) accounted for approximately 38%. Flavonoid intake was significantly (all P values < 0.01) inversely related to lifetime cigarette smoking $(r_s = -0.13)$, positively correlated with the consumption of tea $(r_s = 0.83)$ and, to a lesser extent, with intake of vitamin C $(r_s = 0.28)$, vitamin E $(r_s = 0.16)$, β-carotene $(r_s = 0.26)$, and dietary fiber $(r_s = 0.34)$. Other risk factors were not related to flavonoid intake.

After five years of follow-up (3,266 person-yrs), 75 of the 738 men had developed cancer and 34 men had died from cancer. Cancer cases were older and smoked more than men who did not develop cancer (Table 1). Flavonoid intake was not associated with all-cause cancer incidence (p for trend = 0.54) or with cancer mortality (p for trend = 0.51) (Table 2). Only age and cigarette smoking were significantly associated with cancer incidence. Adjustment for age, diet, and other risk factors did not affect the RR. After exclusion of 15 cancer cases diagnosed within the first two years of follow-up, the adjusted RR of cancer incidence in the highest compared with the lowest tertile of flavonoid intake changed from 1.21 to 1.31 (95% CI 0.65–2.62, P for trend = 0.47).

Fifty-nine of the 75 cancer cases had tumors of the alimentary or respiratory tract and 28 men died from these cancers. Flavonoid intake was not related to incidence (p for trend = 0.91) or with mortality from these cancers (p for trend = 0.95) (Table 3). After adjustment for age, diet, and other risk factors, the RR of incidence of these cancers in the highest tertile vs. the lowest tertile of flavonoid intake was 1.02 (95% CI 0.51–2.04).

Twenty-four men developed lung cancer in this period. No association was observed

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Table 1. Baseline Characteristics of 65- to 84-Year-Old Men According to Cancer Incidence Between 1985 and 1990 (The Zutphen Elderly Study)[a]

| Characteristic | Cancer Incidence | | P Value |
	No (n = 663)	Yes (n = 75)	
Flavonoid intake, mg/day	25.9 ± 14.6	26.2 ± 14.3	0.87
Age, yrs	71.0 ± 5.2	72.5 ± 5.7	0.02
Lifetime cigarette smoking, ×1,000	489 ± 511	604 ± 509	0.03
Body mass index, kg/m^2	25.7 ± 3.1	25.1 ± 3.2	0.10
Physical activity, min/wk	788 ± 725	766 ± 799	0.66
Energy intake, MJ/day	9.5 ± 2.2	9.6 ± 2.0	0.42
Alcohol, g/day	13.1 ± 17.2	14.6 ± 16.4	0.23
Dietary fiber, g/day	25.5 ± 7.7	25.7 ± 7.5	0.72
Vitamin C, mg/day	95.5 ± 45.2	99.4 ± 61.4	0.96
β-Carotene, mg/day	1.37 ± 0.59	1.44 ± 0.82	0.56
Vitamin E, mg/day	8.4 ± 2.6	8.3 ± 2.2	0.82

a: Values are means ± SD of 738 men.

Table 2. Crude and Adjusted RR of Five-Year Incidence and Mortality From Cancer According to Tertiles of Flavonoid Intake in 65- to 84-Year-Old Men (The Zutphen Elderly Study)[a]

| | Flavonoid Intake, mg/day | | | |
	0–19 (Low)	19.1–29.9 (Middle)	>29.9 (High)	P for Trend
No. of men	246	246	246	
All-cause cancer incidence				
No. of cases	21	27	27	
Incidence rate, per 1,000 person-yrs	20.1	24.5	24.2	
RR (95% CI)				
Crude	1.00	1.22 (0.69–2.16)	1.20 (0.68–2.13)	0.54
Adjusted[b]	1.00	1.17 (0.65–2.09)	1.21 (0.66–2.21)	0.54
All-cause cancer mortality				
No. of deaths	9	12	13	
Mortality rate, per 1,000 person-yrs	8.6	10.9	11.6	
RR (95% CI)				
Crude	1.00	1.26 (0.53–3.00)	1.34 (0.57–3.14)	0.51
Adjusted[b]	1.00	1.20 (0.49–2.91)	1.43 (0.58–3.54)	0.44

a: Abbreviations are as follows: RR, relative risk; CI, confidence interval.
b: Age, lifetime cigarette smoking, body mass index, physical activity, and intake of energy, alcohol, vitamin C, β-carotene, vitamin E, and dietary fiber.

between flavonoid intake and lung cancer incidence (*p* for trend = 0.89). The RR of lung cancer in the highest tertile of flavonoid intake vs. the lowest tertile after adjustment for age, diet, and other risk factors was 1.13 (95% CI 0.38–3.40, *p* for trend = 0.40).

In this population, flavonoids were mainly provided by tea, on the one hand, and by vegetables and fruits, on the other hand. We therefore decided to investigate separately the effects of flavonoids derived from tea and flavonoids derived from vegetables and fruits. Flavonoids derived from vegetables and fruits were inversely associated with cancer incidence

Table 3. Crude and Adjusted RR of Five-Year Incidence and Mortality From Tumors of the Respiratory and Alimentary Tract According to Tertiles of Flavonoid Intake in 65- to 84-Year-Old Men (The Zutphen Elderly Study)

	Flavonoid Intake, mg/day			
	0–19 (Low)	19.1–29.9 (Middle)	>29.9 (High)	P for Trend
Incidence of cancer from alimentary and respiratory tract				
No. of men	246	246	246	
No. of cases	17	21	19	
Incidence rate, per 1,000 person-yrs	16.2	19.1	17.0	
RR (95% CI)				
Crude	1.00	1.17	1.04	0.92
		(0.62–2.22)	(0.54–2.00)	
Adjusted[a]	1.00	1.13	1.02	0.96
		(0.59–2.17)	(0.51–2.04)	
Mortality from cancer of alimentary and respiratory tract				
No. of deaths	8	11	9	
Mortality rate, per 1,000 person-yrs	7.8	10.0	8.1	
RR (95% CI)				
Crude	1.00	1.31	1.04	0.94
		(0.53–3.24)	(0.40–2.71)	
Adjusted[a]	1.00	1.26	1.03	0.95
		(0.49–3.20)	(0.37–2.86)	

a: Age, lifetime cigarette smoking, body mass index, physical activity, and intake of energy, alcohol, vitamin C, β-carotene, vitamin E, and dietary fiber.

(RR highest vs. lowest tertile = 0.57, 95% CI 0.31–1.08) (Table 4). A similar inverse association was observed with tumors of the alimentary and respiratory tract (adjusted RR = 0.51, 95% CI 0.25–1.05). Flavonoids derived from tea were not associated with all-cause cancer risk or with risk of cancers of the alimentary and respiratory tract (Table 4).

Discussion

In these elderly men, flavonoid intake is not associated with risk of all-cause cancer or with cancers of the alimentary and respiratory tract. Although the power of our study is too limited to exclude an effect of flavonoids on risk of cancer at specific sites, the absence of any change in cancer mortality and incidence rates across the tertiles of flavonoid intake does not support an important role of flavonoid intake in reducing cancer risk in elderly men. To our knowledge, no previous epidemiological study investigated the relation between flavonoid intake and cancer. Indications for a protective effect of flavonoids on several types of cancer has been provided by experimental studies in which flavonoids inhibited chemically induced tumors in rodents at various sites, including mammary, colon, and skin tumors.

Various explanations for the discrepancies between our findings and the experimental findings can be hypothesized. Tumors at different sites have different etiologies, which may explain why no relation was found between flavonoid intake and mortality of cancers of the alimentary and respiratory tract together. Because of the limited number of cases, we were not able to examine the relation between flavonoid intake and mortality from cancer at specific sites, except lung cancer. In most animal studies, high doses of a carcinogen are used for induction of tumors, and the inhibitory effect of high doses of flavonoids are then investigated. These results can therefore not be extrapolated directly to humans. Clearly, more epidemiological studies on the effect of flavonoids on different cancer sites are needed.

The presence of preclinical diseases in this elderly population may have affected food intake

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Table 4. Crude and Adjusted RR of Five-Year Incidence of Cancer According to Tertiles of Flavonoids From Tea and From Vegetables and Fruit in 65- to 84-Year-Old Men (The Zutphen Elderly Study)

	All Cancer (n = 75)		RR (95% CI)		Cancer of Respiratory and Alimentary Tract (n = 57)		RR (95% CI)	
	No. of men	No. of cases	Crude	Adjusted[a]	No. of men	No. of cases	Crude	Adjusted[a]
Flavonoids from vegetables and fruits								
Low	246	29	1.00	1.00	246	23	1.00	1.00
Middle	246	25	0.81 (0.48–1.39)	0.74 (0.42–1.28)	246	18	0.74 (0.40–1.37)	0.66 (0.35–1.25)
High	246	21	0.65 (0.37–1.13)	0.57 (0.31–1.08)	246	16	0.62 (0.33–1.18)	0.51 (0.25–1.05)
p for trend			0.13	0.08			0.14	0.06
Flavonoids from tea								
Low	246	21	1.00	1.00	246	18	1.00	1.00
Middle	246	27	1.31 (0.74–2.32)	1.27 (0.71–2.26)	246	17	0.95 (0.50–1.84)	0.98 (0.50–1.91)
High	246	27	1.29 (0.73–2.28)	1.31 (0.73–2.35)	246	22	1.22 (0.66–2.28)	1.30 (0.69–2.47)
p for trend			0.41	0.44			0.52	0.41

a: Age, lifetime cigarette smoking, body mass index, physical activity, and intake of energy, alcohol, vitamin C, β-carotene, vitamin E, and dietary fiber.

at the baseline survey. However, exclusion of cancer cases diagnosed in the first two years of follow-up did not result in a significant change of the risk ratios. Selection bias due to selective survival of healthy men up to the age of 65–84 years may have occurred. However, other risk factors such as age and cigarette smoking were still associated with cancer risk. In contrast, intake of other dietary antioxidants with anticarcinogenic potential, such as vitamin C, vitamin E, and β-carotene (16), was also not related to cancer risk in the present study. Possibly the association between dietary antioxidants and the occurrence of cancer, which has been reported for middle-aged persons, is less clear in the elderly. This may explain why we did not find an effect of flavonoids on cancer rates in the present study.

Flavonoids derived from tea and flavonoids from vegetables and fruits had noticeably different effects on cancer rates. Flavonoids from vegetables and fruits were generally associated with a lower risk of cancer, whereas no such effect was observed for flavonoids derived from tea. These results suggest that other components of specific dietary sources of flavonoids, such as onions, endive, kale, and apples, but not tea, may be responsible for the lower cancer incidence rates. Inasmuch as intake of neither vitamin C nor β-carotene was associated with cancer risk, possibly these foods contain other anticarcinogenic compounds such as indoles, which are present in cruciferous vegetables such as kale or organosulfur compounds in onions and garlic. Clearly, prospective studies with detailed information on the consumption of different types of vegetables and fruits are needed to investigate these issues. Epidemiological studies on the effect of tea on cancer risk are scarce, and the results are conflicting (29). Two prospective studies investigated the relation between tea consumption and cancer risk, and both reported a weak positive association between tea consumption and cancer (30,31). In contrast, experimental studies reported inhibitory effects of tea polyphenols, especially those from green tea, on rodent carcinogenesis (32,33). The role of tea in human carcinogenesis therefore deserves more attention.

Mean intake of flavonoids in this population was approximately 26 mg/day, which compares well with the mean intake of 24 mg/day that we found for Dutch men >60 years of age (34). Misclassification of exposure is not likely to have occurred. The cross-check dietary history provides extensive information on all consumed foods, and its validity and reproducibility are well established (23). Also individual flavonoid intake was reproducible over a five-year interval. Moreover our method of flavonoid analysis and our extensive food sampling provided accurate values for the flavonoid content of foods commonly consumed in The Netherlands. Previous estimations reported that the average intake of all flavonoids combined in the United States was approximately 1 g/day, of which about 100 mg consisted of the group of flavonoids investigated in the present study (1). This estimation was based on food analysis techniques now considered inappropriate, which presumably resulted in an overestimation of the flavonoid content of foods. Quercetin was the most important flavonoid, and intake of quercetin was highly correlated with total flavonoid intake ($r_s = 0.98$). Our results can therefore also be interpreted as the relation between quercetin intake and cancer.

We conclude that intake of flavonoids, mainly from tea, onions, and apples, does not predict lower rates of incidence or mortality from all-cause cancer or cancer of the respiratory and alimentary tract in elderly men. The effect of flavonoids on cancer at different sites merits further investigation in other prospective epidemiological studies.

Acknowledgments and Notes

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