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Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study

Michaël G L Hertog, Edith J M Feskens, Peter C H Hollman, Martijn B Katan, Daan Kromhout

Summary

Flavonoids are polyphenolic antioxidants naturally present in vegetables, fruits, and beverages such as tea and wine. In vitro, flavonoids inhibit oxidation of low-density lipoprotein and reduce thrombotic tendency, but their effects on atherosclerotic complications in human beings are unknown.

We measured the content in various foods of the flavonoids quercetin, kaempferol, myricetin, apigenin, and luteolin. We then assessed the flavonoid intake of 805 men aged 65–84 years in 1985 by a cross-check dietary history; the men were then followed up for 5 years. Mean baseline flavonoid intake was 25 9 mg daily. The major sources of intake were tea (61%), onions (13%), and apples (10%). Between 1985 and 1990, 43 men died of coronary heart disease. Fatal or non-fatal myocardial infarction occurred in 38 of 693 men with no history of myocardial infarction at baseline. Flavonoid intake (analysed in tertiles) was significantly inversely associated with mortality from coronary heart disease (p for trend = 0·015) and showed an inverse relation with incidence of myocardial infarction, which was of borderline significance (p for trend = 0 08). The relative risk of coronary heart disease mortality in the highest versus the lowest tertile of flavonoid intake was 0·42 (95% CI 0·20–0·88). After adjustment for age, body-mass index, smoking, serum total and high-density-lipoprotein cholesterol, blood pressure, physical activity, coffee consumption, and intake of energy, vitamin C, vitamin E, beta-carotene, and dietary fibre, the risk was still significant (0·32 [0·15–0·71]). Intakes of tea, onions, and apples were also inversely related to coronary heart disease mortality, but these associations were weaker.

Flavonoids in regularly consumed foods may reduce the risk of death from coronary heart disease in elderly men.

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Introduction

Flavonoids are a large group of polyphenolic antioxidants that occur naturally in vegetables and fruits and in beverages such as tea and wine.[1,2] The most important groups of flavonoids are anthocyanins, flavonols, flavones, catechins, and flavanones. Flavonols are scavengers of superoxide anions,[3] singlet oxygen,[4] and lipid peroxy radicals,[5] and they can sequester metal ions through liganding.[6] Quercetin, a major flavonol, inhibits oxidation and cytotoxicity of low-density lipoproteins (LDL) in vitro.[7,8] Oxidised LDL are atherogenic, and are thought to be important in the formation of atherosclerotic plaques.[9] Flavonols and flavones also inhibit cyclo-oxygenase, leading to lower platelet aggregation and reduced thrombotic tendencies.[10] Flavonoids have been studied in relation to their improvement of vascular fragility, increasing cellular permeability, and vitamin-C-sparing activities.[1] However, no clear evidence for physiological effects of flavonoids in human beings has been reported, and their in-vivo effects on atherosclerotic complications are unknown.

We selected five major antioxidant food flavonoids—the flavonols quercetin, kaempferol, and myricetin and the flavones apigenin and luteolin. We measured the content of these flavonoids in the vegetables, fruits, and beverages most commonly consumed in the Netherlands.[11,12] We report the relation between baseline intake of flavonoids and subsequent coronary heart disease mortality and the incidence of myocardial infarction in the Zutphen Elderly Study.

Subjects and methods

The Zutphen Elderly Study is a longitudinal investigation of risk factors for chronic diseases in elderly men.[13] It is an extension of the Dutch contribution to the Seven Countries Study.[14] The study started in 1960 with a cohort of 878 men then aged 40–59 years, who had lived for at least 5 years in Zutphen, in the eastern Netherlands. In 1985, 555 of the original cohort were alive and were invited for new examinations. In addition, we approached a random sample of all other men of the same age (65–84 years) living in Zutphen. The target population was 1266 men. 62 (4·9%) men had moved or could not be reached, 109 (9·0%) could not be examined because of serious illness, and 156 (12·0%) refused to take part. Thus in 1985, 939 (74%) men aged 65–84 years entered the study; complete information on diet and risk factors was available for 805.

We measured the flavonoid content of 28 vegetables, 12 fruits, and 9 beverages commonly consumed in the Netherlands.[11,12] Each food was purchased in each of three seasons in a supermarket, in a grocery, and in an open-air street market. Separate purchases from each site were combined, and flavonoid content was measured by reverse-phase high-performance liquid chromatography with ultraviolet detection.[15] Identity and purity of the flavonoids were confirmed by diode-array detection on-line. In plants many flavonoid glycosides originate from the same parent aglycon

	Flavonoid intake (mg daily)			p (ANOVA)
	0–19 0	19 1–29 9	>29 9	
Number of men	268	268	269	
Men with history of myocardial infarction	36	40	36	0·84
Mean (SD)				
Flavonoid intake (mg daily)	12 0 (4 8)	23 9 (3 2)	41 6 (12 4)	.
Age in 1985 (yr)	71 0 (5·2)	71 8 (5 4)	71 0 (5 1)	0 14
Lifetime cigarette smoking (× 1000)	216 (191)	167 (164)	174 (195)	0 007
Body-mass index (kg/m²)	25 4 (3 5)	25 3 (2 9)	25·8 (3 0)	0 13
Systolic blood pressure (mm Hg)	149 (20)	152 (21)	151 (22)	0 32
Serum total cholesterol (mmol/L)	6·16 (1 16)	6 09 (1 13)	6 11 (1 03)	0 72
Serum HDL cholesterol (mmol/L)	1 15 (0 35)	1 10 (0 26)	1 11 (0 27)	0 14
Physical activity (min per week)	720 (693)	816 (785)	805 (713)	0 26
Energy intake (mJ daily)	9 1 (2 2)	9 4 (2 1)	9 9 (2 2)	0 0005
Saturated fatty acids (g daily)	43 4 (15 0)	43 3 (13 5)	44 5 (15 1)	0 54
Dietary cholesterol (mg daily)	334 (111)	336 (119)	347 (130)	0 40
Vitamin C (mg daily)	83 1 (40 0)	92 9 (42 7)	112 2 (51·3)	0 0001
Vitamin E (mg daily)	8 1 (2 6)	8 2 (2 4)	8 9 (2 6)	0 0002
Beta-carotene (mg daily)	1 2 (0 5)	1 4 (0 6)	1 6 (0 7)	0 0001
Coffee consumption (mL daily)	508 (300)	407 (220)	392 (286)	0 0001

Table 1: **Baseline characteristics of participants by tertile of flavonoid intake**

(sugar-free flavonoid). To simplify the analytical procedure and to enhance the limit of detection, glycosides were hydrolysed, and flavonoid concentrations 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 each series. The limit of detection was about 1 mg flavonoids per kg or 0·5 mg/L.

Dietary and medical examinations were carried out between March and June, 1985, and again in the same months in 1990. Usual food intake of the participants in the month before the interview was recorded by trained dietitians who used a cross-check dietary history method adapted to the Dutch setting.[16] Each participant was interviewed at home for about 1 h together with the person who prepared the food (in most cases the wife) about his usual food consumption pattern on weekdays and at weekends, and about food purchases. The habitual consumption of foods during a week was assessed 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. Quantities of foods were estimated by the dietitians, with a portable scale if necessary. The food intake data were encoded by the dietitians according to the Netherlands Uniform Food Encoding System, and converted into energy and nutrient values by the 1985 release of the Netherlands nutrient data bank,[17] updated with 1993 data for beta-carotene and vitamin E, and with flavonoid data. The flavonoid content was defined as the sum of quercetin, kaempferol, myricetin, apigenin, and luteolin. Seasonal variability was low,[11] and the average flavonoid content measured in three seasons was used in the analyses. Flavonoid content was known for about 95% of all foods of vegetable origin that are commonly consumed in the Netherlands.[2]

Venous blood samples were taken during the medical examinations, and physicians measured height and weight with the participants in underclothes only. Blood pressure was measured in duplicate at the end of the medical examination. Total cholesterol and high-density-lipoprotein (HDL) cholesterol were measured enzymically.[18,19] Information on amount and duration of smoking was collected on a standard questionnaire, and lifetime exposure to cigarettes was calculated as 365 multiplied by the number of cigarettes daily and the years of smoking. Minutes of physical activity (mainly walking, cycling, gardening, sports, hobbies, and work) per week were calculated by a questionnaire specially designed for retired men.[20]

Information on the occurrence of cerebrovascular accident, diabetes mellitus, chronic non-specific lung disease, and cancer was obtained by a standard medical questionnaire during the medical examinations in 1985 and 1990. Information on history of myocardial infarction, angina pectoris, and intermittent claudication was obtained from a separate questionnaire developed at the London School of Hygiene and Tropical Medicine.[21] Diagnosis of each disease was verified with hospital discharge data and with written information from general practitioners. All information was uniformly coded by one physician, and the year of first diagnosis of each disease was recorded. Myocardial infarction was assumed to be present when two of these three criteria were satisfied: a specific medical history (eg, severe chest pain lasting for more than 20 min and not disappearing at rest); characteristic electrocardiographic changes (eg, major Q wave and major T wave findings), and higher than normal values for serum cardiac enzymes.

Information on the vital status of all participants up to July, 1990, was obtained from municipality registries. No man was lost to follow-up. Information on the primary cause of death was obtained from the Central Bureau for Statistics, and verified by means of hospital discharge data and information from the general practitioner. The primary cause of death was coded according to the International Classification of Diseases, 9th Revision; Coronary heart disease was taken as codes 410–414.

Differences in baseline characteristics of the participants between tertiles of flavonoid intake were assessed by one-way analysis of variance (ANOVA: SAS, release 6·03; SAS Insitute, North Carolina, USA). Spearman rank correlation coefficients (r_s) were calculated for the intake of flavonoids and other dietary variables. Crude and adjusted relative risks of mortality and incidence according to tertiles of flavonoid intake were calculated by Cox proportional hazard (survival) analysis by the SAS procedure PHREG. Proportional hazard assumptions[22] were satisfied. Probability values for trend were calculated with Mantel-Haenszel chi-square statistics. We checked whether the association between flavonoid intake and coronary heart disease mortality was modified by a history of myocardial infarction by including an interaction term in the regression models. No such interaction was found ($p=0·58$). We therefore included men with previous myocardial infarction at baseline in the mortality analyses, and incorporated the prevalence of previous myocardial infarction at baseline (yes or no) as a covariate in the regression models. All p values are two-sided.

Results

Mean flavonoid intake in the 805 participants in 1985 was 25·9 (SD 14·5) mg daily and in the 509 men alive and participating in 1990, 26·6 (13·2) mg daily. The correlation between flavonoid intake in 1985 and 1990 was 0·57 ($p=0·001$), which suggests that individual intakes were measured with satisfactory precision. Quercetin made up most of the flavonoid intake (16·3 [10·1] mg daily = 63% of flavonoid intake) followed by kaempferol (8·2 [5·0] mg daily = 32%). The main sources of flavonoids in this population were black tea (61% of flavonoid intake), onions

	Flavonoid intake (mg daily)			p for trend
	0–19 0	19 1–29 9	>29 9	
Mortality from coronary heart disease (n = 805)				
Number of men	268	268	269	
Deaths	22	11	10	
Mortality rate (per 1000 person-years)	18 5	8 7	7 8	
Crude RR (95% CI)	1 00	0 47 (0 23–0 97)	0 42 (0 20–0 88)	0·015
RR adjusted for age and diet*†	1 00	0 34 (0 16–0 73)	0 34 (0 15–0·79)	0 008
RR adjusted for age, diet, and risk factors†‡	1 00	0 32 (0 15–0 68)	0 32 (0 15–0 71)	0 003
Incidence of fatal and non-fatal first myocardial infarction (n = 693)§				
Number of men	231	231	231	
Cases	16	14	8	
Mortality rate (per 1000 person-years)	16 2	13 8	7 6	
Crude RR (95% CI)	1 00	0 85 (0·42–1 75)	0·47 (0 20–1 09)	0 08
RR adjusted for age and diet*	1 00	0 87 (0·41–1 84)	0 49 (0 19–1 25)	0·15
RR adjusted for age, diet, and risk factors‡	1 00	0 89 (0 43–1 87)	0 52 (0 22–1 23)	0 15

RR = relative risk.
*Intake of total energy, saturated fatty acids, cholesterol, alcohol, coffee, vitamin C, vitamin E, beta-carotene, and dietary fibre.
†History of myocardial infarction in 1985 included as additional covariate.
‡Intake of total energy, saturated fatty acids, physical activity, body-mass index, smoking, serum total and HDL cholesterol, and systolic blood pressure.
§Only men with no history of myocardial infarction at baseline.

Table 2: **Relative risk of mortality from coronary heart disease and incidence of myocardial infarction by tertile of flavonoid intake**

(13%), and apples (10%). Mean daily consumption of tea was 427 (319) mL or 3·4 (2·5) cups; mean daily consumption of onions was 9·4 (20·9) g and of apples 68·8 (70·3) g. Flavonoid intake was correlated highly with consumption of tea ($r_s = 0·83$, $p = 0·001$), but less strongly with consumption of onions ($r_s = 0·32$, $p = 0·001$) and apples ($r_s = 0·27$, $p = 0·001$). Similar associations were observed with the total vegetable consumption ($r_s = 0·31$, $p = 0·001$) and total fruit consumption ($r_s = 0·23$, $p = 0·001$).

Lifetime cigarette smoking and coffee consumption were highest among men in the lowest tertile of flavonoid intake (table 1). Intake of energy, vitamin C, vitamin E, and beta-carotene increased significantly from the lowest to the highest tertile of flavonoid intake. Flavonoid intake was inversely associated with fat intake ($r_s = -0·11$, $p = 0·009$) and positively related to intake of carbohydrates ($r_s = 0·16$, $p = 0·001$) and dietary fibre ($r_s = 0·34$, $p = 0·001$).

After 5 years of follow-up (3727 person-years) 185 men had died, 43 from coronary heart disease. 20 of these men had no history of myocardial infarction at baseline. Of the 693 men with no history of myocardial infarction at baseline, 38 had myocardial infarctions during the study, which were fatal in 13. Flavonoid intake showed a significant inverse relation with mortality from coronary heart disease (p for trend = 0·015) and an inverse association with incidence of a first fatal or non-fatal myocardial infarction, which was of borderline significance (p for trend = 0·08) (table 2). Relative risks of mortality from coronary heart disease and incidence of a first myocardial infarction were about 50% lower in the highest tertile of flavonoid intake than in the lowest tertile. In the initial multivariate model, estimated relative risks were adjusted for age and dietary variables. Only age, history of myocardial infarction, flavonoid intake, and intake of saturated fatty acids were significantly related to incidence of myocardial infarction and death from coronary heart disease. The effect of flavonoid intake on mortality from coronary heart disease became more pronounced after such adjustment (p for trend = 0·008). After further adjustment in the final model for non-dietary risk factors, the relation between flavonoid intake and coronary mortality remained unchanged (p for trend = 0·003). The effect of flavonoids on relative risks of incidence of myocardial infarction did not change with these adjustments (table 2). Adjustment for total consumption of fruits (200 [141] g daily) and vegetables (177 [73] g daily) did not affect the strength of the relation of flavonoid intake with myocardial infarction incidence or coronary heart disease mortality.

We also calculated relative risks of mortality from coronary heart disease in the 693 men with no history of

	Tertile of intake*			p for trend
	Low	Middle	High	
Number of men	268	268	269	
Tea consumption				
Deaths	21	10	12	
Mortality rate (per 1000 person-years)	17 1	8 1	9 5	
Crude RR (95% CI)	1 00	0 48 (0 22–1 01)	0 55 (0 27–1 13)	0 081
RR adjusted for age and diet†	1 00	0 39 (0 18–0 85)	0 44 (0 20–0 96)	0 033
RR adjusted for age, diet, and risk factors‡	1 00	0 38 (0 18–0 82)	0·45 (0 22–0 93)	0 024
Apple consumption				
Deaths	17	16	10	
Mortality rate (per 1000 person-years)	13 7	13 2	7 9	
Crude RR (95% CI)	1 00	0 97 (0 49–1 92)	0 58 (0 26–1 26)	0 18
RR adjusted for age and diet†	1 00	0 86 (0 43–1 75)	0 50 (0 21–1 19)	0 13
RR adjusted for age, diet, and risk factors‡	1 00	0 90 (0 45–1 82)	0 51 (0 23–1 16)	0 12

*Tea: low = 0–250 mL, middle = 251–500 mL, high = > 500 mL daily.
Apples: low = 0–18 g, middle = 19–110 g, high = > 110 g daily.
†History of myocardial infarction in 1985, intake of total energy, saturated fatty acids, cholesterol, coffee, alcohol, vitamin C, vitamin E, beta-carotene, and dietary fibre.
‡History of myocardial infarction in 1985, intake of total energy, and saturated fatty acids, physical activity, body-mass index, smoking, serum total and HDL cholesterol, and systolic blood pressure.

Table 3: **Crude and adjusted relative risks of mortality from coronary heart disease according to intake of tea and apples**

All-cause mortality	Flavonoid intake (mg daily)			p for trend
	0–19 0	19 1–29 9	29 9	
Number of men	268	268	269	
Deaths	70	60	55	
Mortality rate (per 1000 person-years)	59 0	47 7	42 9	
Crude RR (95% CI)	1 00	0 81 (0 57–1 14)	0 72 (0 51–1 03)	0 071
RR adjusted for age and diet*	1 00	0 68 (0 47–0 96)	0 67 (0 45–0 98)	0 039
RR adjusted for age, diet and risk factors†	1 00	0 75 (0 52–1 07)	0 72 (0 50–1 05)	0 084

*Prevalence of chronic diseases (yes/no), intake of total energy, saturated fatty acids, cholesterol, coffee, alcohol, vitamin C, vitamin E, beta-carotene, and dietary fibre.
†Prevalence of chronic diseases (yes/no), physical activity, body-mass index, smoking, serum total and HDL cholesterol, systolic blood pressure, intake of total energy, and saturated fatty acids.

Table 4: **Crude and adjusted relative risks of all-cause mortality by tertile of flavonoid intake**

myocardial infarction at baseline. For the highest versus the lowest tertile of flavonoid intake the crude relative risk was 0·31 (95% CI 0·10–0·99) and the risk after adjustment for age, diet, and risk factors was 0·29 (0·09–0·93).

Mortality rates from coronary heart disease decreased from the lowest to the highest tertile of tea consumption; the trend showed borderline significance ($p = 0·08$, table 3). Tea consumption was inversely related to coffee consumption ($r_s = -0·27$). However, coffee drinking was not associated with coronary heart disease mortality (p for trend = 0·79), and the inverse association between tea consumption and coronary heart disease mortality was more pronounced after adjustment for potential confounders including coffee (p for trend 0·033, table 3). Apple consumption was also inversely related to mortality from coronary heart disease but not significantly so (table 3). Men who ate onions had an adjusted relative risk of 0·85 (0·46–1·61) of mortality from coronary heart disease compared with those who did not. Incidence of a first myocardial infarction was not related to the consumption of tea (p for trend = 0·58), apples (p for trend = 0·39), or onions ($p = 0·28$).

All-cause mortality rates decreased with increasing flavonoid intake (table 4). The significance of this decrease was borderline and persisted after adjustment for potential confounders (p for trend = 0·084).

Discussion

In these elderly men, high intakes of flavonoids predicted lower mortality from coronary heart disease and, less strongly, lower incidence of myocardial infarction. Although the power of the study is limited owing to the small number of deaths and incident cases, the associations with coronary heart disease mortality were highly significant. The predominant flavonoid in foods is quercetin; tertiles of quercetin intake gave essentially the same relative risks as tertiles of total flavonoid intake.

The decrease in coronary heart disease mortality was not accompanied by increases in the rates of death from other causes; the highest tertile of flavonoid intake had 12 fewer men dying from coronary heart disease than the lowest tertile, and 15 fewer dying of all causes. The difference in total mortality failed to reach statistical significance in the final multivariate model, because the contrast due to coronary heart disease mortality was diluted by a constant, but larger, number of deaths from other causes.

Flavonoid intake was not significantly associated with a history of myocardial infarction at baseline, possibly because of higher mortality in the lowest tertile of flavonoid intake. Adjustment for baseline history of myocardial infarction did not attenuate the inverse relation between flavonoid intake and coronary heart disease mortality—in fact, the relation became more pronounced, and the relative risk estimates were similar to the results when participants with a baseline history of myocardial infarction were excluded from the analysis.

It is possible that a high flavonoid intake is merely an indicator of a healthy lifestyle or of a diet high in vegetables and fruits and low in fat. However, adjustment for dietary variables and for non-dietary risk factors such as physical activity, blood lipids, or obesity did not attenuate the relation between flavonoid intake and coronary heart disease. Also, the major source of flavonoids in this population is not fruits or vegetables but tea, which provided about 61%.

Flavonoid intake and tea consumption were highly correlated and both were inversely associated with mortality from coronary heart disease. The inverse association between flavonoid intake and death from coronary heart disease might therefore be due to substances in tea other than flavonoids, such as the antioxidant tea polyphenols (-)epicatechin gallate, and (-)epigallocatechin gallate.[23,24] However, the observed effects of total flavonoids on coronary heart disease mortality in our study were stronger than those of tea itself. Onion and apple consumption were less strongly related to coronary heart disease mortality than was flavonoid intake. In addition, intakes of tea, apples, and onions were not related to incidence of myocardial infarction. This finding suggests that quercetin and other flavonoids themselves rather than unknown substances in these foods lead to the lower coronary heart disease mortality rates. Coffee consumption has been associated with coronary heart disease,[25] and there was an inverse relation between coffee consumption and both flavonoid and tea intake in our population. However, coffee consumption was not related to coronary heart disease, and adjustment for coffee consumption did not change the association of flavonoid intake or tea consumption with coronary heart disease mortality.

The average flavonoid intake of the Zutphen elderly men (26 mg daily) is similar to the 24 mg we found previously for Dutch men aged 60 years and over.[2] These values are much lower than the previous estimation by Kühnau,[1] who reported that in the US the daily intake of all flavonoids combined was 1 g (expressed as glycoside), of which about 100 mg (expressed as aglycons) consisted of the flavonols and flavones we investigated. Kühnau's estimation was based on food analysis techniques now considered inappropriate, which presumably led to an overestimate of their flavonoid content. Also the results are based on analyses of whole foods, in contrast to our analyses of the edible parts, and on food disappearance data, which overestimate the real intake of foods. In the Dutch diet, tea, onions, and apples contributed about 84% to total flavonoid intake.[2] In other cultures (eg, Mediterranean countries) red wine, which contains 10–20 mg/L combined flavonoids,[12] could also be an important source of flavonoids. The polyphenolic flavonoids present in wines may partly

explain the reduced risk of coronary heart disease of wine drinkers (the "French paradox").[26]

There is evidence that free-radical oxidation of LDL plays an important part in atherogenesis.[9] Flavonoids are scavengers of free radicals such as superoxide anions and lipid peroxy radicals,[2-6] and will thus interrupt radical chain reactions. Some flavonoids, including quercetin, inhibit the oxidative modification of LDL by macrophages in vitro, probably by inhibiting the generation of hydroperoxides and by protecting α-tocopherol present in lipoproteins from oxidation.[7] Quercetin also inhibits the cytotoxicity of oxidised LDL in vitro.[8] It is possible that quercetin and other flavonoids reduce the rate of formation of oxidised LDL and thus inhibit the growth of atherosclerotic plaques. In addition, flavonoids inhibit cyclo-oxygenases, which may reduce thrombosis.[10] Flavonoids possibly act through both mechanisms; the combination could explain why the association between flavonoid intake and coronary heart disease mortality was stronger than the association with incidence of myocardial infarction. Data on the absorption and metabolism of flavonoids are scarce and inconclusive,[27-29] and more studies are needed to elucidate the mechanisms.

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