Dietary Flavonoids and the Risk of Colorectal Cancer

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Abstract

***In vitro* and *in vivo* laboratory data point to chemoprotective effects of flavonoids on colorectal cancer. However, there has been limited epidemiologic research on the dietary intake of flavonoids and risk of colorectal cancer. Recent expansions of dietary databases to include flavonoid data now make such studies feasible. Association between the six main classes of flavonoids and the risk of colorectal cancer was examined using data from a national prospective case-control study in Scotland, including 1,456 incident cases and 1,456 population-based controls matched on age, sex, and residence area. Dietary, including flavonoid data, were obtained from a validated, self-administered food frequency questionnaire. Risk of colorectal cancer was estimated using conditional logistic regression models in the whole sample and stratified by sex, smoking status, and cancer site and adjusted for established and putative risk factors. After energy adjustment, reductions in colorectal cancer risk associated with the highest quartiles of intake (versus the lowest quartile) were 27% for flavonols [odds ratio (OR), 0.73; P_{trend} = 0.012], 32% for quercetin (OR, 0.68; P_{trend} = 0.001), 32% for catechin (OR, 0.68; P_{trend} < 0.0005); 26% for epicatechin (OR, 0.74; P_{trend} = 0.019), and 22% for procyanidins (OR, 0.78; P_{trend} = 0.031). The significant dose-dependent reductions in colorectal cancer risk that were associated with increased consumption of flavonols, quercetin, catechin, and epicatechin remained robust after controlling for overall fruit and vegetable consumption or for other flavonoid intake. The risk reductions were greater among nonsmokers, but no interaction beyond a multiplicative effect was present. Sex-specific or cancer-type differences were not observed. No risk reductions were associated with intake of flavones (P_{trend} = 0.64), flavonones (P_{trend} = 0.22), and phytoestrogens (P_{trend} = 0.26). This was the first of several a priori hypotheses to be tested in this large study and showed strong and linear inverse associations of flavonoids with colorectal cancer risk.** (Cancer Epidemiol Biomarkers Prev 2007;16(4):684–93)

Introduction

Colorectal cancer is the third most common cancer in both men (14.7% of cases) and women (11.3% of cases) in Scotland and the second most frequent cancer-related cause of death for men (11% of cancer related deaths) and the third for women (9% of cancer-related deaths; ref. 1). Colorectal cancer incidence rates are ~10-fold higher in developed than developing countries, and differences in diet and lifestyle are likely to explain most of this difference (2, 3). The World Cancer Research Fund report 1997 (4) concluded that there is enough evidence to support an inverse association between dietary fruit and vegetable intake and several cancers including colorectal cancer. Results from more recent cohort studies are inconclusive. Some cohort studies reported no or little effect of vegetable or fruit intake on colorectal cancer risk (5-7). However, in the Nurses' Health study, an inverse association between fruit consumption and colorectal adenomas was identified (8), and in a population-based prospective study of women in central Sweden, individuals who consumed very low amounts of fruit and vegetables had the greatest risk of colorectal cancer (9). Various compounds found in plant foods have been suggested as candidates for the observed protected effects with the most important to date being fiber and folate. The most recent studies have reported a 40% reduction in risk associated with the highest versus the lowest quartile of fiber in food (10, 11) and a 30% reduction in risk associated with the highest versus the lowest quartile of folate in food (12).

Flavonoids are biologically active polyphenolic compounds widely distributed in plants. More than 6,000 plant flavonoids have been described, and they have been classified into at least 10 chemical groups according to structural patterns (13). However, laboratory and epidemiologic studies have focused on six flavonoid subgroups: flavones, flavonols, flavan-3-ols (catechins), procyanidins, flavanones, and isoflavones. The main dietary sources of these flavonoids differ widely among subgroups (14-17). Flavonols, such as quercetin, kaempferol, and myricetin, are mainly present in leafy vegetables, apples, onions, and berries, and these are the most abundant flavonoids in foods. Flavones (e.g., apigenin and luteolin) and procyanidins are in low quantities in some vegetables and wine, respectively. Flavan-3-ols (catechins) are found in green tea, black tea, grapes, apples, chocolate, and red wine. Flavanones, such as naringenin and hesperetin, known also as citrus flavonoids, are found in citrus fruits and their juices (18). The last group, isoflavones, can be found in soya beans and together with lignans, whose precursors are present in a wide variety of plant foods, form the group of phytoestrogens (19).

Colon-specific *in vitro* cell line and *in vivo* animal studies have reported anticarcinogenic properties associated with flavonoids, including free radical scavenging, modifying or inactivating enzymes that activate or detoxify carcinogens, inhibiting the induction of transcription factors (such as activator protein-1 activity), and inducing apoptosis (20, 21). A few observational studies have reported associations between flavonoid intake and incidence of different types of cancer (breast, lung, stomach, prostate, urothelial, bladder, and colorectal; refs. 16, 17, 22-25). We have identified seven studies

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that have examined the association with colorectal cancer and have summarized these in Table 1. Flavonoid measurements were made with either a dietary history method (17, 26) or with a food frequency questionnaire (23, 27-29). Three of the six studies were small, with less than 200 cases, and thus had very limited power to detect an association (17, 23, 26), and the three larger cohort studies did not investigate all six subgroups of flavonoids (27, 29, 30). In the Italian case-control study, the effect of the main six flavonoid subgroups was examined, and the authors have reported a significant inverse association for isoflavones, anthocyanidins, flavones, and flavonols (28).

The objective of the present case-control study was to examine associations between dietary intake of the six major flavonoid subgroups [flavonols, flavones, flavan-3-ols (catechins), procyanidins, flavanones, and phytoestrogens] as well as of the most abundant individual flavonoid compounds (quercetin, catechin, epicatechin, naringenin, and hesperitin) on colorectal cancer risk. To our knowledge, this is the largest case-control study to investigate the effects of both the major individual dietary flavonoids and all six main subclasses on colorectal cancer risk.

Patients and Methods

Study Population. Our study included 1,456 cases and 1,456 matched controls from an epidemiologic case-control study of colorectal cancer (Study of Colorectal Cancer in Scotland). We aimed to recruit prospectively all incident cases of adenocarcinoma of colon or rectum in patients aged 16 to 79 years presenting to surgical units in Scottish hospitals. The main exclusions were as follows: patient death before ascertainment; patient too ill to participate; case was a recurrence of colorectal cancer; or patient unable to give informed consent due to learning difficulties or other medical conditions. We sought to minimize ascertainment bias from loss due to death on ward or soon after diagnosis by basing research staff in the main surgical centers throughout Scotland so that ascertainment occurred as soon after admission as possible and clinically appropriate. Recruitment took place typically within 2 to 3 months of diagnosis to limit survival bias. We ascertained and approached about 80% of all incident cases in Scotland (with reference to other sources of data such as the Scottish Cancer Registry and audit data). During the same period, controls were drawn at random from a population-based register

Table 1. Colorectal cancer risk and flavonoid intake (results from published studies)

Study	Type of study	Country	Population	Flavonoid	Comparison (high vs low)	Outcome	No. cases	Adjusted RR (95% CI)	P_{trend}
Knekt et al. (26)	Cohort	Finland	9959 MF	Flavonols, flavones	M >4.8 vs <2.1 F >5.5 vs <2.4	Colorectal	72	0.74 (0.32-1.68)	—
Goldbohm et al. (30)	Cohort	Netherlands	3726 MF	Flavonols, luteolin	43.5 vs 12.7	Colorectal	603	0.97 (0.71-1.32)	0.92
Hirvonen et al. (23)	Cohort	Finland	27110 M	Flavonols, flavones	16.3 vs 4.2	Colorectal	133	1.70 (1.00-2.70)	0.10
Knekt et al. (17)	Cohort	Finland	9865 MF	Quercetin	M >3.9 vs <1.5 F >4.7 vs <1.8	Colorectal	90	0.62 (0.33-1.17)	0.22
				Kaempferol	M >0.8 vs <0.1 F >0.9 vs <0.1		90	1.13 (0.60-2.12)	0.96
				Myricetin	M >0.1 vs <0.06 F >0.2 vs <0.03		90	1.31 (0.71-2.43)	0.39
				Hesperetin	M >15.4 vs 0 F >26.8 vs <3.2		90	0.97 (0.50-1.90)	0.84
				Naringenin	M >4.7 vs <4.7 F >7.7 vs <0.9		90	0.93 (0.48-1.82)	1.00
				Total	M >26.9 vs <4.3 F >39.5 vs <8.5		90	0.84 (0.43-1.64)	0.95
Arts et al. (27)	Cohort	USA	34651 F	Catechins	>75.1 vs <3.6	Colon	635	1.10 (0.85-1.44)	0.63
						Proximal c	352	1.18 (0.84-1.66)	0.11
						Distal c	268	1.04 (0.67-1.62)	0.37
					>24.7 vs <3.6	Rectal	132	0.55 (0.32-0.95)	0.002
				Catechin and epicatechin	>24.3 vs <3.2	Colon	635	1.04 (0.71-1.29)	0.90
						Proximal c	352	1.06 (0.70-1.61)	0.39
						Distal c	268	1.09 (0.65-1.80)	0.42
					>15.7 vs <3.2	Rectal	132	0.92 (0.50-1.71)	0.75
				Gallates	>50.8 vs <0.4	Colon	635	0.95 (0.72-1.25)	0.44
						Proximal c	352	1.18 (0.81-1.72)	0.25
						Distal c	268	0.71 (0.46-1.09)	0.76
					>8.9 vs <0.4	Rectal	132	0.39 (0.22-0.71)	0.02
Lin et al. (29)	Cohort	USA	107401 MF	Total flavonoids	M >30.5 vs <10.7 F >31.1 vs <0.96	Colorectal	380 498	1.28 (0.89-1.83) 1.13 (0.83-1.52)	0.21 0.42
				Quercetin			380 498	1.16 (0.80-1.68) 1.01 (0.75-1.35)	0.40 0.40
				Kaempferol			380 498	1.09 (0.78-1.52) 1.14 (0.85-1.52)	0.29 0.55
				Myricetin			380 498	1.33 (0.93-1.89) 0.89 (0.67-1.18)	0.43 0.96
Rossi et al. (28)	Case-control	Italy	6107 MF	Isoflavones	>33.9 vs <14.4	Colorectal	1,953	0.76 (0.63-0.91)	0.001
				Anthocyanidins	>31.7 vs <5.3		1,953	0.67 (0.54-0.82)	<0.001
				Flavan-3-ols	>88.5 vs <20.8		1,953	0.98 (0.82-1.18)	0.736
				Flavanones	>67.0 vs <12.5		1,953	0.96 (0.81-1.15)	0.430
				Flavones	>0.7 vs <0.3		1,953	0.78 (0.65-0.93)	0.004
				Flavonols	>28.5 vs <13.2		1,953	0.64 (0.54-0.77)	<0.001
				Total flavonoids	>191.1 vs <75.3		1,953	0.97 (0.81-1.16)	0.500

Abbreviations: M, male; F, female.

(community health index) and invited to participate. Cases and controls were matched on age (±1 years), gender, and residence region. More than 99% of the study participants were White Caucasians. We were unable to approach some cases to take part in the study. The main reasons were patients dying shortly after diagnosis or too ill to be given the study information. Participation rates among those approached were ~58% for cases and an estimated 57% for population-based controls. Of these, questionnaires were completed to a sufficiently high level of completeness to permit valid analysis by 82% of cases and 97% of controls recruited. The lower completion rate in cases is likely to be due to cases being readmitted to hospital or otherwise too ill to cooperate fully in the study. Ethical approval was obtained from the MultiCentre Research Ethics committee for Scotland, 18 Local Research Ethics committees, 18 Caldicott guardians, and 16 NHS Trust management committees, and all participants provided written informed consent.

Lifestyle and Dietary Data. The subjects were asked to complete one questionnaire with lifestyle and cancer information reporting their status 1 year before the diagnosis (for cases) or the recruitment (for controls). All participants were asked about their general medical history, physical activity (both occupational and leisure), and smoking status. Additionally, subjects were asked to report any regular intake of aspirin and non-steroidal anti-inflammatory drugs. Reported height, weight, and waist circumference were recorded. Participants were also asked to report some demographic, socioeconomic, and race/ethnicity data. Finally, women were asked about their menstrual and reproductive history as well as about the type of hormone replacement therapy and hormonal contraception, if taken.

In addition, a semiquantitative food frequency questionnaire (Scottish Collaborative Group FFQ, version 6.41) was completed by participants.[5] This questionnaire was developed for use in a wide range of studies of diet and health in Scotland, and the validity for ranking macronutrients and micronutrients in younger adults have been previously described (31). In particular, it has been validated against serum phytoestrogen concentrations (ref. 32; weighted kappa statistic, 0.16; $P = 0.002$) and for the estimation of dietary flavonoid intakes against 4-day weighted diet records in a Scottish population (33). The correlation coefficients (Spearman rank) between weighted diet records and food frequency questionnaire intakes for flavonols, procyanidins, and flavan-3-ols (catechins) were >0.7 ($r = 0.70$, 0.73, and 0.94, respectively; $P < 0.001$), supporting our use of this food frequency questionnaire to yield valid ranks of individual intakes of these flavonoids. However, correlation coefficients for flavone and flavanone intakes were low ($r = 0.12$ and 0.33, respectively); thus, the observed associations with these particular subgroups should be treated with caution (33). The food frequency questionnaire consisted of a list of 150 foods, and the individuals were asked to describe the amount and frequency of each food on the list they have eaten a year before diagnosis or recruitment. The sources of the six subgroups of the flavonoids that were included in the questionnaire were leafy vegetables, onions, apples, berries, grapes, citrus fruit and their juices, red wine, tea (green and black), chocolate, and soya products. Two additional fields were included: one for the participants to report any other foods that were eaten regularly and not included in the 150 food list and one to report any vitamin, mineral, or food supplements taken. Frequencies of consumption of the specified measures of each food were converted into nutrients using an in-house calculation program based on the weights of these measures and the nutrient composition of representative foods derived from the U.K. food composition tables (*McCance and Widdowson's The Composition of Foods*, 6th summary edition). Nutrient information on supplements was collected from the manufacturer's product information, by contacting the company or from the Internet, and then added in the daily nutrient intake. A small number of the study participants reported intake of herbal remedies (a source of flavonoids) as food supplements. However, data regarding the exact nutrients of herbal remedies were not available and therefore were not included in the daily nutrient intake.

The quality of completion of both questionnaires was checked weekly soon after receipt. Forms with more than a maximum acceptable number of blank or incorrect entries were returned to participants for additional information (with a follow-up phone call where necessary). If these were returned with no additional data, then these forms were not included in the analysis.

Flavonoid Data. Data were obtained from a nutrient database for flavonoids by Kyle and Duthie (34) for the subgroups of flavones, flavonols, flavan-3-ols (catechins), procyanidins, and flavanones and also for the flavonoids quercetin, kaempferol, myricetin, apigenin, luteolin, catechin, epicatechin, epigallocatechin, epicatechin-3 gallate, epigallocatechin-3 gallate, gallocatechin, naringenin, and hesperetin. Phytoestrogen values were derived from a database derived by Ritchie et al. (35). The final list of flavonoids included in the study was determined in advance of the analysis after an investigation of their distributions in this study population and the correlation coefficients among the various compounds and was based also on the quality of the compositional information available for that compound. We elected not to attempt to study apigenin, luteolin, and gallates (epigallocatechin, epicatechin-3 gallate, epigallocatechin-3 gallate, and gallocatechin) because their distributions were not suitable for analysis (perhaps due to limited compositional information). In addition, gallates (epigallocatechin, epicatechin-3 gallate, epigallocatechin-3 gallate, and gallocatechin) were highly correlated with each other and with flavan-3-ols (catechins) and thus could not be studied separately. Therefore, we elected to study the following subclasses: flavonols (summary measurement of quercetin, kaempferol, and myricetin), flavones (summary measurement of apigenin and luteolin), flavan-3-ols (catechins; summary measurement of catechin, epicatechin, and gallates), procyanidins (summary measurement of procyanidin type BI-IV), flavanones (summary measurement of naringenin and hesperetin), and phytoestrogens (summary measurement of isoflavones and lignans) and the following individual compounds: quercetin, catechin, epicatechin, naringenin, and hesperetin.

Statistical Analysis. The statistical packages used were Intercooled STATA, version 7.2 (Stata Corp., College Station, TX) and SPSS, version 13.0 (SPSS, Inc., Chicago, IL). Initially, the distribution of the variables was checked, and any variable showing a skewed distribution was normalized using either log or square root transformation. For non-normally distributed variables after transformation, nonparametric tests were used. The Spearman rank correlation coefficient was used to test the correlation between each individual flavonoid as well as between each flavonoid and fruit/vegetable intake. The Pearson χ^2 test was used to test the difference between cases and controls in terms of sex, smoking status, non-steroidal anti-inflammatory drug intake, herb intake, physical activity, and Carstairs Deprivation Index (DEPCAT). The t test was used to test differences in mean age, total energy, total fiber and alcohol intake. The Wilcoxon rank-sum test was used to test for differences in crude vegetable and fruit intake and in energy-adjusted flavonoid intake. Conditional logistic

[5] http://www.foodfrequency.org

regression models were used to estimate the strength of association between flavonoid categories and colorectal cancer risk. Flavonoid intake was adjusted for total energy intake by using either the residual method, as determined by Willet and Stampfer (ref. 36; for the normal distributed flavonoid variables) or the standard method including the total energy variable as a covariate in the regression model (for the non-normal distributed variables). The core statistical model (model 0) was corrected for total energy. Model I was corrected for family history of colorectal cancer (low, medium, and high risk), smoking (yes versus no), body mass index (in kg/m^2, continuously), physical activity (total hours of cycling and any other sport activities; four categories), total fiber intake (g/d, continuously), alcohol intake (g/d, continuously), and regular non-steroidal anti-inflammatory drug intake (yes versus no). The associations were tested in two additional models (models II and III). In model II, in addition to the confounding factors of model I, fruit and vegetable intake (measures per day, continuously) was included. In model III, the associations were further adjusted mutually between flavonoid categories. In addition to the whole sample analysis, odds ratios (OR) and 95% confidence intervals (95% CI) were calculated in stratified subgroups according to cancer type (colon and rectal cancer), smoking status (smokers and nonsmokers), and gender (men and women).

Results

Table 2 shows demographic, dietary, and lifestyle characteristics for the 1,456 cases and 1,456 controls in the study population. There were no significant differences between the cases and the controls in terms of sex, body mass index, daily fiber intake, smoking, physical activity, and area deprivation index, but there was a significant difference in age ($P = 0.031$). Control individuals reported a significant lower total daily

Table 2. Demographic characteristics and lifestyle factors for the study population

Variables	Cases* (n = 1,456)	Controls* (n = 1,456)	P †
Age (y)	63.9 (9.64)	64.7 (9.53)	0.031
Gender			
Men	846 (58.1)	844 (58.1)	0.992
Women	610 (41.9)	609 (41.9)	
Energy intake (kJ/d)	11,215 (4245)	10,696 (3959)	0.001
Fiber intake (g/d)	22.49 (9.61)	22.54 (9.98)	0.887
BMI (kg/m^2)	26.56 (4.37)	26.64 (4.51)	0.627
Smoking			
No	601 (55.4)	619 (42.7)	0.508
Yes ‡	846 (44.6)	829 (57.3)	
Frequent NSAID intake			
No	900 (61.8)	807 (55.5)	0.001
Yes§	556 (38.2)	646 (44.5)	
Physical activity (cycling and other sport), h/d			
0	830 (60.2)	779 (55.4)	0.455
0-3.5	322 (23.3)	346 (24.6)	
3.5-7	144 (10.4)	173 (12.3)	
>7	84 (6.1)	107 (7.7)	
Herb intake			
No	1,419 (97.4)	1,398 (96.0)	0.039
Yes	38 (2.6)	58 (4.0)	
Type of cancer			
Colon cancer	822 (57.8)	—	—
Rectal cancer	606 (42.2)	—	
Deprivation‖			
1	148 (10.2)	148 (10.2)	—
2	293 (20.2)	293 (20.2)	
3	358 (24.6)	358 (24.6)	
4	355 (24.5)	355 (24.5)	
5	155 (10.6)	155 (10.6)	
6	111 (7.6)	111 (7.6)	
7	34 (2.3)	34 (2.3)	
Fruit and vegetable intake (measures per day)	7.2 (5-10.4)	7.8 (5.2-11.2)	0.007
Flavonols (mg/d)¶	26.8 (15.3-36.1)	28.0 (16.7-37.2)	0.01
Quercetin (mg/d)¶	17.3 (11.2-22.4)	18.1 (12.1-23.5)	0.002
Flavones (mg/d)**	1.1 (0.5-2)	1 (0.5-1.8)	0.13
Flavan-3-ols (mg/d)**	115.6 (42-160.3)	115.2 (43.4-164.85)	0.55
Catechin (mg/d)¶	7 (4.6-9.1)	7.5 (5.0-9.7)	0.0006
Epicatechin (mg/d)¶	23.7 (12.8-32)	24.5 (13.7-33.6)	0.03
Procyanidins (mg/d)¶	32.1 (16.0-44.2)	33.5 (17.5-46.1)	0.05
Flavanones (mg/d)**	20.2 (8.1-39.7)	20.6 (6.95-42.35)	0.64
Naringenin (mg/d)**	9.9 (3.3-21)	9.9 (4.1-19)	0.67
Hesperetin (mg/d)**	10.5 (4.1-20.5)	10.6 (3.4-21.7)	0.64
Phytoestrogens (μg/d)¶	573.3 (405.9-835.3)	594.8 (386.6-888.8)	0.38

Abbreviations: BMI, body mass index; NSAID, nonsteroidal anti-inflammatory drugs.
*Mean (SD) for quantitative variables; number of subjects (%) for categorical variables; median (interquartile range) for flavonoid and fruit and vegetable variables.
†P from the Pearson χ^2 for categorical variables, from t test for continuous variables, and from Wilcoxon rank-sum test for flavonoid and fruit and vegetable intake variables.
‡Smokers were defined as individuals who have smoked at least one cigarette per day.
§Frequent use was defined as an intake of at least 4 d per week for at least 1 mo.
‖Locally based deprivation index (Carstairs Deprivation Index) based on the 2001 Census data: seven categories ranging from very low deprivation (DEPCAT = 1) to very high deprivation (DEPCAT = 7).
¶Energy-adjusted variables (residual method).
**Energy-adjusted (amount per mJ/d).

Table 3. Dietary sources of flavonoids in our population

Flavonoids	Main sources
Flavonols	Tea (64.3%), onions (9.1%), soups: home made (6.3%)
Flavones	Soups: home made (78.2%), other salad vegetables (10.9%), meat or chicken pies, pasties, sausage roll (4.3%)
Flavan-3-ols	Tea (89.3%), apples (3.1%), red wine (2.1%)
Procyanidins	Tea (74.2%), apples (11.2%), red wine (8.4%)
Flavanones	Oranges, satsumas, or grapefruits (69.3%); pure fruit juice (29.1%); red wine (1.2%)
Phytoestrogens	Soya milk (26.3%), whole meal bread (including toast and sandwiches; 18.0%), soya beans, TVP, tofu, or soya meat substitute (13.4%)
Quercetin	Tea (52.1%), onions (13.7%), soups: home made (9.2%)
Catechin	Tea (45.6%), red wine (16.3%), other fruits (9.7%)
Epicatechin	Tea (68.1%), apples (11.7%), chocolate (6.0%)
Naringenin	Oranges, satsumas, or grapefruits (70.8%); pure fruit juice (26.5%); red wine (2.2%)
Hesperitin	Oranges, satsumas, or grapefruits (68.0%); pure fruit juice (31.2%); red wine (0.6%)

energy intake (P = 0.001). Because this could distort or confound the relationship between flavonoid intake and colorectal cancer, we adjusted flavonoid intake for total energy intake. For the normal distributed variables, the residual method of Willet and Stampfer (36) was used. However, greatly skewed variables were adjusted by using the standard multivariate method. Control individuals also reported taking non-steroidal anti-inflammatory drugs (P = 0.001) and herb supplements (P = 0.039) regularly more often than cases. Wilcoxon rank test showed that the consumption of fruit and vegetables, flavonols, and procyanidins and of the individual compounds quercetin, catechin, and epicatechin differ significantly between cases and controls (Table 2). A number of different foods contributed to the intake of the flavonoid subclasses (Table 3). Table 4 presents results of the four multiple logistic regression models on the relationship between quartiles of flavonoid intake and risk of colorectal cancer as OR, 95% CI, and P_{trend} for colorectal cancer for each of the six flavonoid subgroups and five compounds studied. In model 0 (adjusted only for total energy intake), flavonols, procyanidins, quercetin, catechin, and epicatechin were significantly inverse associated with colorectal cancer, and this association was also dose dependent [for high versus low quartile, OR (95%): 0.73 (0.59-0.90), 0.78 (0.63-0.96), 0.68 (0.55-0.84), 0.68 (0.55-0.83), and 0.74 (0.60-0.90), respectively]. In model I, the confounding variables included are family history of colorectal cancer, total energy intake, total fiber intake, alcohol intake, nonsteroidal anti-inflammatory drug intake, smoking, body mass index, and physical activity. Flavonols, quercetin, catechin, and epicatechin show a strong inverse and dose-dependent effect on colorectal cancer risk (P_{trend}: 0.015, 0.002, <0.0005, and 0.031, respectively) with a ~30% reduction in risk for those of high versus those of low intake [OR (95% CI): 0.73 (0.59-0.91), 0.70 (0.56-0.87), 0.69 (0.55-0.87), and 0.74 (0.60-0.92), respectively]. After applying the Bonferroni correction for six independent tests ($P \leq 0.0083$), the inverse associations of quercetin and catechin remained significant. Because these associations could be confounded by other compounds present in fruit and vegetables or by the intake of other flavonoids, we explored these relationships further in models II and III. In model II, the ORs were adjusted for fruit and vegetable intake, in addition to the confounding factors of model I. The observed associations with quercetin, catechin, and epicatechin remained significant [for high versus low quartile, OR (95% CI): 0.77 (0.61-0.97), 0.75 (0.59-0.94), and 0.74 (0.60-0.92), respectively]. Finally in model III, the ORs were adjusted mutually for each flavonoid variable in addition to the confounding factors of the first model. The observed associations with flavonols, quercetin, catechin, epicatechin, and procyanidins became stronger and remained significant after the Bonferroni correction [for high versus low quartile, OR (95% CI): 0.23 (0.13-0.40), 0.38 (0.22-0.63), 0.46 (0.32-0.65), and 0.28 (0.15-0.50), respectively]. In distinct contrast, there were no associations between flavones, flavonones, and phytoestrogens and colorectal cancer risk (P_{trend}: 0.60, 0.37, and 0.68, respectively in model I). The observed association with quartiles of procyanidin intake was not statistically significant in models I and II but was of borderline significance in model III (P = 0.005) after allowing for multiple tests.

Ninety-six participants reported consumption of herbal remedies as food supplements that contain flavonoids or/and phytoestrogens. Because we were not able to identify the exact nutrient composition of herbal remedies, we adjusted for herbal remedy intake by adding this covariate in a fifth model (data not shown), which had no effect on the direction and strength of the associations.

In Table 5, OR, 95% CI, and P_{trend} for colorectal cancer risk are presented as before for groups stratified by cancer site (colon or rectal), sex, and smoking status. The covariates that were included in these models are the same as in model I. In general, flavonols, procyanidins, quercetin, catechin, and epicatechin seem to have a strong inverse and dose-dependent effect on colorectal cancer risk. Observed associations did not vary by cancer site, but the effects of flavonols, quercetin, catechin, and phytoestrogens seemed to be slightly stronger among males [OR (95% CI): 0.72 (0.53-0.96), 0.68 (0.50-0.92), 0.64 (0.47-0.85), and 0.75 (0.57-1.01), respectively]. However, there is not enough evidence to support a gender effect. Inverse associations with colorectal cancer risk were stronger in the nonsmoking group [e.g., OR, 0.52 (95% CI, 0.30-0.90) versus OR, 0.78 (95% CI, 0.54-1.13) for flavonols in nonsmokers compared with smokers]. However, there was no evidence of any interaction effect, and the findings were consistent with a multiplicative effect model (data not shown).

Discussion

The recent increase in published data on flavonoid content of foods has enabled the development of databases, which can be linked to food frequency questionnaires. In this study, the 150 foods listed in the food frequency questionnaire, and for which we had nutrient information, included all the most important sources of flavonoids, and we were able to identify any participants that used herbal remedies as food supplements. The estimates of this food frequency questionnaire for flavonols, flavan-3-ols (catechins), and procyanidin dietary intake (for which we have shown inverse associations with colorectal cancer risk) have been shown to be strongly correlated (r > 0.7) with 4-day weighed record estimates in the Scottish population. These provided us with the opportunity to investigate the chemoprotective effects of these compounds, which have been reported *in vitro* and animal *in vivo* studies.

Table 4. Flavonoids and risk of colorectal cancer: adjusted ORs and 95% CI for flavonoid intake in whole sample from multiple logistic regression models (cases and controls matched on age, gender, and area of residence)

Flavonoids	Quartiles of flavonoid variables	Whole sample		Whole sample, OR (95% CI)			
		Cases	Controls	Model 0*	Model I†	Model II‡	Model III§
Flavonols‖							
1	0-16.00	386	341	1.00	1.00	1.00	1.00
2	16.00-27.40	360	367	0.86 (0.70-1.06)	0.84 (0.67-1.04)	0.87 (0.70-1.09)	0.57 (0.43-0.76)
3	27.40-36.75	379	348	0.96 (0.78-1.18)	0.93 (0.75-1.16)	0.96 (0.77-1.20)	0.41 (0.27-0.63)
4	>36.75	330	396	0.73 (0.59-0.90)	0.73 (0.59-0.91)	0.77 (0.62-0.97)	0.23 (0.13-0.40)
P				0.012¶	0.015¶	0.062	<0.0005**
Quercetin‖							
1	0-11.67	387	340	1.00	1.00	1.00	1.00
2	11.67-17.71	374	353	0.92 (0.75-1.14)	0.92 (0.74-1.14)	0.97 (0.78-1.20)	0.75 (0.57-0.99)
3	17.71-22.86	374	353	0.92 (0.75-1.14)	0.87 (0.70-1.09)	0.91 (0.73-1.14)	0.55 (0.37-0.81)
4	>22.86	320	406	0.68 (0.55-0.84)	0.70 (0.56-0.87)	0.77 (0.61-0.97)	0.38 (0.22-0.63)
P				0.001**	0.002**	0.022¶	<0.0005**
Flavones ††							
1	0-0.5	413	420	1.00	1.00	1.00	1.00
2	0.5-1.1	335	336	0.99 (0.80-1.21)	1.01 (0.81-1.25)	1.05 (0.85-1.30)	1.05 (0.85-1.31)
3	1.1-1.9	344	374	0.89 (0.73-1.09)	0.94 (0.76-1.16)	0.95 (0.76-1.18)	1.01 (0.81-1.26)
4	>1.9	363	325	0.99 (0.80-1.23)	1.11 (0.88-1.40)	1.11 (0.88-1.41)	1.30 (1.01-1.68)
P				0.679	0.602	0.598	0.641
Flavan-3-ols (catechins) ††							
1	0-42.6	371	357	1.00	1.00	1.00	1.00
2	42.6-115.25	355	372	0.92 (0.74-1.14)	0.92 (0.73-1.15)	0.92 (0.74-1.15)	1.10 (0.81-1.49)
3	115.25-162.1	385	343	1.07 (0.87-1.33)	1.07 (0.86-1.34)	1.08 (0.86-1.35)	1.56 (0.98-2.50)
4	>162.1	344	383	0.80 (0.65-0.99)	0.81 (0.65-1.01)	0.80 (0.64-1.01)	1.37 (0.73-2.57)
P				0.139	0.076	0.168	0.301
Catechin‖							
1	0-4.85	395	332	1.00	1.00	1.00	1.00
2	4.85-7.23	379	348	0.91 (0.74-1.12)	0.92 (0.74-1.15)	0.93 (0.75-1.16)	0.75 (0.58-0.97)
3	7.23-9.40	358	369	0.82 (0.66-1.01)	0.78 (0.63-0.97)	0.80 (0.64-0.99)	0.56 (0.41-0.76)
4	>9.40	323	403	0.68 (0.55-0.83)	0.69 (0.55-0.87)	0.75 (0.59-0.94)	0.46 (0.32-0.65)
P				<0.0005**	<0.0005**	0.004**	<0.0005**
Epicatechin‖							
1	0-13.29	382	345	1.00	1.00	1.00	1.00
2	13.29-24.24	360	367	0.90 (0.73-1.10)	0.90 (0.72-1.12)	0.91 (0.73-1.14)	0.64 (0.47-0.86)
3	24.24-32.61	388	339	1.05 (0.85-1.29)	1.04 (0.83-1.30)	1.08 (0.86-1.35)	0.52 (0.33-0.82)
4	>32.61	325	401	0.74 (0.60-0.90)	0.74 (0.60-0.92)	0.74 (0.60-0.92)	0.28 (0.15-0.50)
P				0.019¶	0.031¶	0.036¶	<0.0005**
Procyanidins‖							
1	0-16.67	380	347	1.00	1.00	1.00	1.00
2	16.67-32.65	368	359	0.93 (0.75-1.15)	0.94 (0.75-1.17)	0.95 (0.76-1.20)	0.77 (0.57-1.04)
3	32.65-45.16	372	355	0.95 (0.77-1.17)	0.96 (0.76-1.19)	0.98 (0.79-1.22)	0.64 (0.42-0.99)
4	>45.16	335	391	0.78 (0.63-0.96)	0.81 (0.65-1.00)	0.82 (0.66-1.02)	0.46 (0.27-0.81)
P				0.031¶	0.076	0.111	0.005**
Flavanones ††							
1	0-16.67	380	347	1.00	1.00	1.00	1.00
2	16.67-32.65	368	359	1.30 (1.06-1.61)	1.42 (1.14-1.77)	1.44 (1.15-1.80)	1.43 (1.15-1.80)
3	32.65-45.16	372	355	1.21 (0.99-1.49)	1.32 (1.08-1.69)	1.37 (1.10-1.72)	1.35 (1.08-1.70)
4	>45.16	335	391	0.93 (0.75-1.15)	1.13 (0.89-1.43)	1.20 (0.94-1.53)	1.18 (0.93-1.50)
P				0.449	0.367	0.164	0.219
Naringenin ††							
1	0-3.81	348	379	1.00	1.00	1.00	1.00
2	3.81-9.98	393	334	1.29 (1.05-1.59)	1.38 (1.11-1.73)	1.40 (1.12-1.74)	1.41 (1.12-1.77)
3	9.98-19.73	380	347	1.28 (1.04-1.59)	1.43 (1.14-1.80)	1.46 (1.16-1.82)	1.46 (1.13-1.89)
4	>19.73	334	392	0.91 (0.73-1.12)	1.09 (0.86-1.39)	1.16 (0.91-1.49)	1.19 (0.81-1.76)
P				0.421	0.381	0.161	0.049¶
Hesperetin ††							
1	0-3.95	345	382	1.00	1.00	1.00	1.00
2	3.95-10.66	398	329	1.30 (1.05-1.60)	1.41 (1.13-1.76)	1.42 (1.14-1.78)	1.43 (1.13-1.80)
3	10.66-21.13	372	355	1.19 (0.97-1.46)	1.33 (1.07-1.67)	1.35 (1.08-1.70)	1.36 (1.06-1.75)
4	>21.13	340	386	0.92 (0.74-1.14)	1.11 (0.87-1.40)	1.18 (0.92-1.50)	1.21 (0.83-1.77)
P				0.364	0.460	0.220	0.092
Phytoestrogens‖							
1	0-402.68	355	373	1.00	1.00	1.00	1.00
2	402.68-583.54	392	335	1.22 (0.99-1.51)	1.23 (0.99-1.54)	1.17 (0.94-1.47)	1.21 (0.97-1.51)
3	583.54-857.55	370	358	1.09 (0.88-1.34)	1.14 (0.92-1.42)	1.04 (0.84-1.30)	1.11 (0.89-1.38)
4	>857.55	340	387	0.92 (0.75-1.13)	0.93 (0.74-1.15)	0.81 (0.64-1.01)	0.90 (0.72-1.13)
P				0.241	0.686	0.036¶	0.264

Abbreviations: BMI, body mass index; NSAID, nonsteroidal anti-inflammatory drugs.
*Model 0: adjusted for total energy intake.
†Model I: adjusted for family history of colorectal cancer, total energy intake, total fiber intake, alcohol intake, NSAID intake, smoking, BMI, and physical activity.
‡Model II: adjusted as in model I and for fruit/vegetable intake.
§Model III: adjusted as in model I and mutually between flavonoid categories.
‖Energy adjustment: residual model (normally distributed variables).
¶Significant at the 0.05 level.
**Significant at the 0.05 level and after Bonferroni correction ($P \leq 0.0083$).
†† Energy adjustment: multiple regression model (non-normally distributed variables).

Table 5. Adjusted ORs for flavonoid intake in our sample stratified for type of cancer (colon and rectal cancer), smoking, and gender

Flavonoid variables	Whole sample, OR* (95% CI)	Type of cancer, OR* (95% CI)		Smoking, OR* (95% CI)		Gender, OR* (95% CI)	
		Colon	Rectal	No	Yes	Male	Female
Flavonols†							
1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
2	0.84 (0.67-1.04)	0.95 (0.71-1.27)	0.72 (0.51-1.01)	0.79 (0.47-1.33)	0.75 (0.51-1.09)	0.89 (0.60-1.06)	0.88 (0.62-1.25)
3	0.93 (0.75-1.16)	0.92 (0.68-1.23)	0.94 (0.67-1.33)	0.85 (0.48-1.48)	0.94 (0.65-1.37)	0.79 (0.60-1.06)	1.16 (0.83-1.60)
4	0.73 (0.59-0.91)	0.79 (0.59-1.06)	0.69 (0.49-0.98)	0.52 (0.30-0.90)	0.78 (0.54-1.13)	0.72 (0.53-0.96)	0.72 (0.72-1.01)
P	0.015‡	0.123	0.150	0.030‡	0.374	0.037‡	0.199
Quercetin†							
1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
2	0.92 (0.74-1.14)	0.94 (0.70-1.26)	0.88 (0.63-1.23)	1.18 (0.69-2.03)	0.77 (0.53-1.12)	0.89 (0.67-1.19)	0.94 (0.67-1.33)
3	0.87 (0.70-1.09)	0.82 (0.62-1.11)	0.95 (0.67-1.35)	0.76 (0.43-1.34)	0.85 (0.58-1.24)	0.73 (0.55-0.98)	1.15 (0.81-1.63)
4	0.70 (0.56-0.87)	0.76 (0.56-1.02)	0.68 (0.48-0.98)	0.63 (0.36-1.11)	0.70 (0.47-1.03)	0.68 (0.50-0.92)	0.71 (0.51-1.00)
P	0.002§	0.046‡	0.071	0.044‡	0.117	0.005§	0.120
Flavones‖							
1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
2	1.01 (0.81-1.25)	0.92 (0.68-1.25)	1.10 (0.79-1.52)	1.19 (0.74-1.92)	1.03 (0.71-1.49)	0.88 (0.67-1.17)	1.18 (0.85-1.66)
3	0.94 (0.76-1.16)	0.89 (0.67-1.19)	0.91 (0.65-1.27)	1.32 (0.75-2.32)	0.97 (0.67-1.41)	0.81 (0.62-1.07)	1.14 (0.80-1.63)
4	1.11 (0.88-1.40)	1.07 (0.79-1.46)	1.03 (0.70-1.50)	1.00 (0.55-1.79)	1.28 (0.87-1.89)	1.08 (0.80-1.45)	1.11 (0.76-1.64)
P	0.602	0.758	0.873	0.891	0.318	0.986	0.624
Flavan-3-ols (catechins)‖							
1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
2	0.92 (0.73-1.15)	0.88 (0.65-1.19)	0.95 (0.67-1.37)	0.98 (0.56-1.71)	0.76 (0.50-1.14)	0.87 (0.65-1.17)	0.92 (0.73-1.15)
3	1.07 (0.86-1.34)	1.15 (0.85-1.54)	1.04 (0.73-1.48)	0.83 (0.49-1.41)	1.05 (0.71-1.55)	0.99 (0.73-1.34)	1.07 (0.86-1.34)
4	0.81 (0.65-1.01)	0.79 (0.59-1.07)	0.87 (0.62-1.24)	0.63 (0.36-1.10)	0.84 (0.57-1.23)	0.83 (0.62-1.13)	0.81 (0.65-1.01)
P	0.076	0.393	0.56	0.092	0.731	0.395	0.291
Catechin†							
1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
2	0.92 (0.74-1.15)	0.77 (0.57-1.04)	1.19 (0.84-1.67)	0.94 (0.53-1.67)	0.91 (0.63-1.33)	0.81 (0.61-1.10)	1.16 (0.81-1.60)
3	0.78 (0.63-0.97)	0.71 (0.53-0.96)	0.93 (0.67-1.31)	0.66 (0.38-1.12)	0.80 (0.55-1.16)	0.72 (0.54-0.96)	0.91 (0.65-1.28)
4	0.69 (0.55-0.87)	0.66 (0.49-0.89)	0.77 (0.54-1.10)	0.57 (0.32-1.00)	0.70 (0.48-1.03)	0.64 (0.47-0.85)	0.82 (0.57-1.17)
P	<0.0005§	0.006§	0.063	0.014‡	0.052	0.002§	0.121
Epicatechin†							
1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
2	0.90 (0.72-1.12)	0.98 (0.73-1.31)	0.77 (0.55-1.08)	0.97 (0.56-1.70)	0.68 (0.46-1.00)	0.91 (0.69-1.20)	0.85 (0.60-1.22)
3	1.04 (0.83-1.30)	1.12 (0.83-1.51)	0.96 (0.69-1.35)	0.99 (0.57-1.72)	0.99 (0.68-1.42)	0.97 (0.72-1.29)	1.13 (0.80-1.60)
4	0.74 (0.60-0.92)	0.77 (0.58-1.04)	0.75 (0.53-1.05)	0.59 (0.35-1.00)	0.70 (0.48-1.02)	0.77 (0.58-1.03)	0.70 (0.50-0.99)
P	0.031‡	0.149	0.215	0.052	0.261	0.123	0.129
Procyanidins†							
1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
2	0.94 (0.75-1.17)	1.09 (0.81-1.48)	0.72 (0.51-1.03)	0.58 (0.49-1.49)	0.75 (0.50-1.12)	0.94 (0.70-1.26)	0.93 (0.66-1.33)
3	0.96 (0.76-1.19)	1.04 (0.78-1.39)	0.84 (0.60-1.18)	0.89 (0.54-1.48)	0.91 (0.63-1.33)	0.99 (0.75-1.33)	0.90 (0.64-1.26)
4	0.81 (0.65-1.00)	0.90 (0.67-1.20)	0.74 (0.53-1.05)	0.61 (0.35-1.06)	0.75 (0.51-1.09)	0.83 (0.62-1.10)	0.79 (0.56-1.10)
P	0.076	0.403	0.127	0.113	0.272	0.266	0.163
Flavanones‖							
1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
2	1.42 (1.14-1.77)	1.56 (1.16-2.08)	1.15 (0.81-1.65)	1.72 (1.00-2.98)	1.60 (1.10-2.32)	1.45 (1.10-1.92)	1.30 (0.90-1.87)
3	1.32 (1.08-1.69)	1.44 (1.06-1.95)	1.23 (0.87-1.73)	1.35 (0.76-2.39)	1.31 (0.88-1.94)	1.33 (0.99-1.78)	1.37 (0.96-1.96)
4	1.13 (0.89-1.43)	1.19 (0.87-1.63)	0.98 (0.66-1.44)	1.39 (0.79-2.45)	0.80 (0.52-1.21)	1.16 (0.84-1.59)	1.07 (0.74-1.55)
P	0.367	0.423	0.851	0.446	0.310	0.355	0.732
Naringenin‖							
1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
2	1.38 (1.11-1.73)	1.55 (1.16-2.09)	1.09 (0.76-1.55)	1.37 (0.79-2.38)	1.67 (1.15-2.44)	1.39 (1.05-1.84)	1.34 (0.92-1.93)
3	1.43 (1.14-1.80)	1.54 (1.13-2.11)	1.29 (0.91-1.83)	1.51 (0.83-2.73)	1.33 (0.90-1.98)	1.35 (1.01-1.82)	1.55 (1.07-2.23)
4	1.09 (0.86-1.39)	1.18 (0.86-1.61)	0.94 (0.64-1.38)	1.28 (0.73-2.26)	0.75 (0.49-1.15)	1.16 (0.84-1.60)	1.03 (0.71-1.49)
P	0.381	0.427	0.880	0.431	0.214	0.315	0.824
Hesperetin‖							
1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
2	1.41 (1.13-1.76)	1.41 (1.05-1.89)	1.33 (0.92-1.91)	1.67 (0.96-2.92)	1.53 (1.05-2.24)	1.48 (1.11-1.97)	1.21 (0.73-1.74)
3	1.33 (1.07-1.67)	1.34 (0.99-1.83)	1.33 (0.94-1.88)	1.19 (0.68-2.10)	1.25 (0.84-1.84)	1.31 (0.98-1.76)	1.32 (0.92-1.90)
4	1.11 (0.87-1.40)	1.12 (0.82-1.53)	1.05 (0.72-1.54)	1.40 (0.79-2.45)	0.76 (0.49-1.17)	1.15 (0.84-1.58)	1.03 (0.72-1.49)
P	0.460	0.613	0.655	0.476	0.236	0.424	0.837
Phytoestrogens†							
1	1.00	1.00	1.00	1.00	1.00	1.00	1.00
2	1.23 (0.99-1.54)	1.00 (0.75-1.34)	1.55 (1.10-2.20)	1.48 (0.85-2.56)	1.14 (0.78-1.65)	1.16 (0.87-1.54)	1.42 (0.99-2.02)
3	1.14 (0.92-1.42)	1.01 (0.76-1.34)	1.30 (0.91-1.85)	1.85 (1.04-3.28)	1.17 (0.82-1.67)	0.87 (0.66-1.15)	1.65 (1.16-2.36)
4	0.93 (0.74-1.15)	0.77 (0.57-1.03)	1.27 (0.89-1.81)	0.95 (0.53-1.71)	1.06 (0.73-1.54)	0.75 (0.57-1.01)	1.24 (0.88-1.76)
P	0.686	0.104	0.420	0.818	0.674	0.015‡	0.96

Abbreviations: BMI, body mass index; NSAID, nonsteroidal anti-inflammatory drugs.

*Data matched on age, gender, and residence area (adjusted for family history of colorectal cancer, total energy intake, total fiber intake, alcohol intake, NSAID intake, smoking, BMI, and physical activity).

† Energy adjustment: residual model (normal distributed variables).

‡ Significant at the 0.05 level.

§Significant at the 0.05 level and after Bonferroni correction ($P \leq 0.0083$).

‖Energy adjustment: multiple regression model (non-normal distributed variables).

We were able to show that a number of different foods contribute to the intake of the six flavonoid subgroups under study so that results are not determined by one major food category, and that there was a wide variation in flavonoid intake in this study population (Table 3). According to results of a study assessing the ability of the food frequency questionnaire to estimate flavonoid intake, dietary sources of flavonols, flavan-3-ols (catechins), and procyanidins were diverse, but the main single source in Scotland was black tea, similar to other populations (22, 27, 37-41). In particular, the main sources of flavonols were black tea (46% of the intake), onions (14%), and apples (10%). The main sources of flavones were sweet peppers (24% of the intake), lettuce (18%), and pizza (12%). The main sources of flavan-3-ols were black tea (64% of the intake), apples (11%), and white wine (5%). The main sources of flavanones were orange juice (38% of the intake), white wine (19%), and red wine (19%). The main sources of procyanidins were black tea (50% of the intake), apples (21%), and red wine (9%).[6]

We estimated the median and range of flavonoid intake in the Scottish population from 4-day weighted record data in a study of 81 individuals,[6] and most of the flavonoid subgroups were broadly similar to other populations. The average daily intake of flavonols and flavones in our population was 19 mg/d (range, 1.9-58 mg/d). This intake is similar to intakes of four other populations (39-42) but approximately thrice the average intake of a Spanish (22) and a Finish population (43). The average daily intake of flavan-3-ols (catechins) was 58 mg/d (range, 1.8-263.3 mg/d), similar to a Dutch population (37) but much higher than the median intake of an American study (27). Flavanones and procyanidins median intake was 1.2 mg/d (range, 0-238.6 mg/d) and 22.5 mg/d (range, 0-144.5 mg/d), respectively.

Our main findings were that patients with colorectal cancer consumed lower amounts of the flavonol and procyanidin subclass and the individual flavonoids quercetin, catechin, and epicatechin than matched control individuals. These associations persisted after controlling for overall energy intake and a number of known and putative confounding factors including overall fruit and vegetable intake in multivariate logistic regression models. There was a dose-response relationship with reduction in risk associated with each increasing quartile of consumption with trend tests being highly significant even after adjustment for multiple tests. The effects of the flavonoid subclasses and individual compounds in model 0, which was only adjusted for energy intake, were very similar to the effects of the flavonoid subclasses and individual compounds in model I and II. Therefore, possibly, the strong associations seen in models I and II were not due to an artifact of modeling. We investigated the existence of collinearity effects by correcting for overall fruit and vegetable intake and for intakes of other individual flavonoids and showing that the observed effects became more clearly defined. According to our results, the direction of the effect of flavonols, procyanidins, quercetin, catechin, and epicatechin remains similar in all four models, although the size of effect changes. It is difficult to be certain which is the true sized effect among the models because there is limited knowledge on the biological mechanism of flavonoids. Therefore, it might be possible that this very large effect reported in model III is due to instability because of the highly correlated variables. However, for those flavonoid subclasses (flavonols and procyanidins) and individual compounds (quercetin, catechin, and epicatechin) that the direction of the effect remains constant in all three models, we can assume that there is a true association with colorectal cancer.

We then explored associations between the intakes of the three compounds that seem to account for most flavonol intake in Scotland and colorectal cancer risk. The comparison of highest versus lowest quartile intakes of these foods showed ORs for colorectal cancer risk of 0.79 (95% CI, 0.63-0.98; P_{trend} = 0.053) for tea, 0.96 (95% CI, 0.62-1.5; P_{trend} = 0.90) for apples, and 0.91 (95% CI, 0.65-1.26; P_{trend} = 0.91) for onions. Thus, there is evidence in favor of an inverse association, but this is less well defined than in the analysis of the association of flavonol, quercetin, or catechin intake and colorectal cancer risk, which supports our interpretation of the data.

The highest quartiles of intake of the flavonol subgroup and the main flavonol compound quercetin, of the flavan-3-ols (catechins) compounds catechin and epicatechin, and of procyanidins showed moderately strong inverse associations in the multivariate models consistent with protective effects ranging from 54% to 74% (assuming the associations to be causal). The association with catechin and epicatechin but the lack of association with the flavan-3-ol subgroup (comprising catechin, epicatechin, and gallates) may be explained by our inability to study the other main representatives of flavan-3-ols (catechins) gallates (epigallocatechin, epicatechin-3 gallate, epigallocatechin-3 gallate, and gallocatechin) as described in Materials and Methods. There were no cancer type differences, but the associations with flavonols, quercetin, catechin, and epicatechin were stronger among the nonsmokers than smokers. Additionally, the effects of flavonols, quercetin, catechin, and phytoestrogens seem slightly more significant in the male subgroup, but there is not enough evidence to support a gender effect.

In marked contrast, we showed no associations between intake of the other three of six flavonoid subclasses studied (flavones, flavonones, and phytoestrogens) and colorectal cancer risk. The reasons for these differences are not clear. They could be explained by different biological action of these flavonoid subgroups, limited dietary sources (celery and herbs for flavones, citrus fruit for flavanones, and soya products for phytoestrogens), low levels of dietary intake of these subgroups in Scotland across all population groups (e.g., soya and soya products are not commonly consumed in Scotland) leading to insufficient variation in intake across the population to permit their study, or less complete nutritional database information on these subgroups leading to greater misclassification and loss of study power. In addition, a validation study of the food frequency questionnaire showed that estimated intakes for flavones and flavonones did not correlate closely (r = 0.12 and 0.33, respectively) with results from 4-day weighed records (33); thus, interpretation of the finding for these compounds is problematic, and results may represent false-negative findings.

This study, with 1,456 cases and 1,456 matched controls, is the largest study (in terms of numbers of cases) investigating the association between colorectal cancer risk and both the six main flavonoid subgroups and the main individual flavonoid compounds. Most of previous epidemiologic studies were much smaller in scale and thus had limited power to detect associations with dietary flavonoids (Table 1). The Iowa Women's Health study, which was of similar size, was limited to the investigation of the flavan-3-ols (catechins), catechin, and epicatechin only and was restricted to postmenopausal women. This exploratory study, which investigated many cancers, reported an inverse association with rectal cancer but did not correct statistical significance levels to account for the many tests done. The authors concluded that the role of flavonoid intake in colorectal cancer should be studied further (27). In the large case-control study conducted in Italy, the authors reported that dietary intake of flavonols and anthocyanidins significantly decreases the risk of colorectal cancer, results that are in accordance with those from our population. However, an inverse association for flavones and isoflavones

[6] Kyle et al., unpublished data.

was found, which did not replicate from our study (28). This may be due to the lower validity of our questionnaire for flavones (see above) and the fact that we studied phytoestrogens rather than isoflavones, which represent a subgroup of phytoestrogens. Our study differs from the Italian study by studying closely matched population-based rather than hospital-based controls. It also employed a dietary analysis approach using a food frequency questionnaire that yielded results for flavonoids in close correlation with those obtained with 4-day weighed records and based on a nutrient database developed for this study population.

We compared basic information on age, gender, and place of residence of our cases with data aggregated over a 5-year period (1999-2003) from the Scottish Cancer Registry. There was a slight overrepresentation of male cases, but the distribution among the 15 boards of Scotland was similar to the one from the Cancer Registry. One limitation of this study is the possibility of limited external validity due to the underrepresentation of cases that were very ill when presented to the hospital and therefore could not take part in the study. In addition, there are some already recognized limitations of case-control studies employing food frequency questionnaires, including recall bias, misclassification bias due to imprecise measures of dietary intake, and residual confounding after attempts to control for known confounders. We attempted to limit these problems by close matching of and adoption of identical study procedures in cases and controls, adoption of a recall period 1 year before cancer diagnosis for cases and recruitment date for controls to attempt to reduce recall bias, use of a food frequency questionnaire that had been validated in Scotland against 4-day weighted diet records (31, 44) and against serum flavonoid concentrations (32), and use of images of portion sizes and careful instructions to improve accuracy of reporting diet. Kristal et al. has recently reported that one of the most important limitations of the food frequency questionnaire design is its low validity with correlation between food frequency questionnaire and recall derived nutrients often lower than 0.4 (45). However, the food frequency questionnaire used in this study has shown high correlation with 4-day weighted diet records (correlation coefficients >0.7) for the flavonoids subgroups that were found to be significantly associated with colorectal cancer (33).

Several animal and cell line studies have reported chemoprotective effects of flavonoids. Several possible mechanisms have been suggested, including inhibition of DNA oxidation (46, 47); alteration of phase I and II drug-metabolizing enzymes (47-49); inhibition of protein kinases; blocking of receptor-mediated functions; alteration of cell cycle checkpoint apoptosis; inhibition of angiogenesis, invasion, and metastasis; and epigenetic changes in promoter methylation and chromatin remodeling (50). An alternative theory for the protective effect of flavonoids is through their regulation of the gene for the *COX-2* gene. Increased expression of cyclooxygenase-2 enzyme provides survival advantage to cancer cells through increased cell proliferation and angiogenesis. Results from recent laboratory and mechanistic studies show that flavonoids inhibit the expression of cyclooxygenase-2 both on mRNA and protein levels by inhibit signaling of the extracellular signal-regulated kinase and Akt pathways (51). Quercetin, in particular, which is the major representative of flavonols in diet, has been found in several animal and cell line studies to have anticarcinogenic effects. However, the exact molecular pathways that are responsible for these effects are to be established. Activation of the β-catenin/Tcf pathway by accumulation of β-catenin in the nucleus has been shown to be important in human carcinogenesis (52). Hoon Park et al. have recently shown that the anticarcinogenic effects of quercetin might be due to its ability of inhibiting the β-catenin/Tcf signaling via the decrease of nuclear β-catenin/Tcf-4 proteins (52). In other studies, quercetin has been found to inhibit cell growth and to induce apoptosis in colon cancer cells by down-regulating the Akt pathway and ErbB2/ ErbB3 (receptor tyrosine kinases) signaling (53, 54).

It has been shown that flavonoids can cross the intestinal barrier and reach concentrations in the blood that have shown to have effects in some *in vitro* studies (55). Absorption is accompanied by conjugation and metabolism including forms different to those found in foods. Metabolism of flavonoids by the intestinal microflora may result in a proportion of the flavonoids that reach the colon being subject to further breakdown from the original polyphenol structure into metabolites before exerting any direct beneficial action. Some of these metabolites have differing biological activity to the original polyphenols. Many of the published *in vitro* studies have not investigated these issues of bioavailability and metabolism (56). In addition, it is likely that individuals vary in their ability to metabolize flavonoids, and this may also modulate flavonoid action (56).

In conclusion, this large case-control study investigated an a priori hypothesis that flavonoid intake is associated with reduced risk of colorectal cancer. Given the evidence of chemoprotective effects from *in vitro* and animal *in vivo* studies, this was given high priority and explored as the first analysis in this data set to minimize problems with multiple testing. Moderately strong inverse associations that showed dose-response relationships were found in multivariate logistic regression models between colorectal cancer risk and the intake of the flavonol subgroup and the main flavonol compound quercetin and of catechin and epicatechin. This was in marked contrast with lack of association with other flavonoid subgroups. These data provide support for the limited but growing epidemiologic evidence that certain flavonoids are associated with a decreased risk of colorectal cancer, and that therefore flavonoid intake might be an important dietary determinant of colorectal cancer risk within and across populations. However, confirmation of these findings in still required in further large-scale studies.

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