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ORIGINAL PAPER

Association between habitual dietary flavonoid and lignan intake and colorectal cancer in a Spanish case–control study (the Bellvitge Colorectal Cancer Study)

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Abstract
Background Flavonoid-rich foods, such as fruits, vegetables, and tea, may have a protective effect upon colorectal cancer. However, current epidemiological evidence for a protective effect of flavonoid intake upon colorectal cancer is promising but not conclusive.
Objective To examine the relation between dietary flavonoid and lignan intakes and the risk of colorectal cancer within a Spanish population.
Design Data from the Bellvitge Colorectal Cancer Study, a case–control study (424 cases with incident colorectal cancer and 401 hospital-based controls), were used. A reproducible and validated food frequency questionnaire was administered in personal interviews. An ad hoc food composition database on flavonoids and lignans was compiled, mainly using data from the US Department of Agriculture and Phenol-Explorer databases. Adjusted odds ratios (ORs) and 95 % confidence intervals (CIs) were estimated using unconditional logistic regression models.
Results An inverse association was found between intake of total flavonoids (OR, 0.59; 95 % CI, 0.35–0.99 for the highest vs. the lowest quartile; p for trend = 0.04), lignans (OR, 0.59; 95 % CI, 0.34–0.99; p for trend = 0.03), and some individual flavonoid subgroups (flavones, proanthocyanidins) and the risk of colorectal cancer. Separate analyses by cancer site showed similar results.
Conclusions Intake of total dietary flavonoids (particularly certain flavonoid subgroups) and lignans was inversely associated with colorectal cancer risk in a Spanish population.

Keywords Flavonoids · Lignans · Colorectal cancer · Case–control study

Abbreviations

EPIC	European Prospective Investigation into Cancer and Nutrition
FCDB	Food composition database
PA	Proanthocyanidin
USDA	US Department of Agriculture

Introduction

Colorectal cancer is the third most common cancer in the developed world. In 2007, the World Cancer Research Fund and the American Institute for Cancer Research concluded that evidence for a protective effect of dietary

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fruit and vegetable intake upon colorectal cancer was considered as probable/limited [1]. Flavonoids and lignans are polyphenols that occur ubiquitously in plant-based foods, such as fruits, vegetables, cereals, tea, wine, and cocoa products [2]. These polyphenols are known to exhibit anti-carcinogenic bioactivity by means of antimutagenic, antiproliferative, anti-inflammatory, immunomodulatory, and antioxidant effects, as well as the ability to improve insulin sensitivity, modulate cell signaling, cell cycle regulation, and angiogenesis [3–7]. Consequently, flavonoids and lignans may play an important role in the apparent protection against colorectal cancer by fruits, vegetables, and tea [8].

To date, at least six case–control studies have investigated the relationship between flavonoid intake and colorectal cancer [9–14], even one in recurrent colorectal adenocarcinoma [15]. All of them have found inverse associations between colorectal cancer risk and intake of some subgroups and/or certain individual flavonoids. Regarding cohort studies, no significant relationships between flavonoid intake and colorectal cancer incidence have been found [16–22], with the exception of the Iowa Women's Health study [23]. Moreover, the European Prospective Investigation into Cancer and Nutrition (EPIC)—Norfolk Study suggested that intake of dietary phytoestrogens, particularly enterolignans, was inversely associated with colorectal cancer risk among women [24], although no associations were observed between risk and either urinary or plasma phytoestrogen biomarkers [25]. The assessment methods used in these studies have been quite heterogeneous, and some used old food composition databases (FCDB), which may explain some of these inconsistencies. Furthermore, flavonoid subclasses have large differences in bioavailability and may have different beneficial effects among subclasses [26]. Therefore, it is crucial to examine them separately.

The aim of this study was to investigate, using the most updated FCDBs, the relation between dietary flavonoid and lignan intakes and colorectal cancer risk by means of a case–control study in a Spanish population whose participants were likely to consume relatively large amounts of fruits and vegetables.

Subjects and methods

Study design

We conducted a hospital-based case–control study on colorectal cancer in Barcelona, Spain, from January 1996 to December 1998 [27]. The Bellvitge Colorectal Cancer Study identified 523 incident histologically confirmed cases of colorectal cancer diagnosed at the University Hospital of Bellvitge, Barcelona. Of these, 424 patients (81 % of eligible cases) agreed to participate. Of the remainder, 17 % could not be interviewed because they had either died, had mental or other impairments, or had been released without being approached and could not be traced, and 2 % refused to participate. These lost cases were similar to those included with respect to age, sex, socioeconomic status, educational level, tumor location, and extent.

The control group consisted of 470 patients without previous colorectal cancer who had been randomly selected among those admitted to the same hospital during the same period. To avoid selection bias, the criterion of inclusion in the control group was a new diagnosis. Twenty-two percent of controls were admitted for internal medicine, 19 % for acute surgery, 17 % for urology, 16 % for gastroenterology (hernia, peptic ulcer and cholecystitis), 15 % for traumatology, and 11 % for circulatory or respiratory conditions. Controls were frequency-matched to cases by sex and age (±5 years old). Of these, 401 (85 % of eligible controls) agreed to participate. Refusals were 9 %, while 6 % could not be interviewed because of mental or other impairments.

The Ethical Committee of the hospital approved the study protocol, and all participating subjects signed informed consent forms.

Dietary and lifestyle assessment

Participants were personally interviewed in the hospital by trained personnel using structured questionnaires designed to collect information on sociodemographic characteristics, medical history, lifetime smoking habits and alcohol consumption, leisure- and work-related physical activity, anthropometric measures, and detailed dietary information. The habitual diet over 12 months, one year prior to the diagnosis, including assessment of frequency and portion size, was recorded using a dietary history questionnaire, including more than 600 food and beverages and 150 recipes, which had been previously validated in the EPIC-Spanish cohort [28, 29]. Energy and alcohol intakes were estimated from the Spanish food composition table used for the EPIC study [30].

In order to estimate flavonoid and lignan intake, a FCDB was developed ad hoc and detailed elsewhere [31–34]. Briefly, data were mainly gathered from the most recent US Department of Agriculture (USDA) databases on flavonoids [35], isoflavones [36], and proanthocyanidins (PA) [37] and the French Phenol-Explorer database on polyphenols [38] as well as the UK Food Standards Agency database on phytoestrogens [24]. Furthermore, our database was expanded using retention factors, recipes, and estimations based on similar food or food group items and

logical zeros. The final database contained 1,877 food items and only 10 % of missing values.

Dietary intake was estimated for six flavonoid subgroups, lignans and all 40 individual compounds. Subgroups of compounds were as follows: anthocyanidins (cyanidin, delphinidin, malvidin, pelargonidin, peonidin, petunidin), flavanols (flavan-3-ol monomers [catechin, epigallocatechin, epicatechin, epicatechin 3-gallate, epigallocatechin 3-gallate, gallocatechin, catechin 3-gallate], PA [dimers, trimers, 4–6 monomers, 7–10 monomers, >10 monomers], theaflavins [theaflavin, theaflavin 3,3′-digallate, theaflavin 3′-gallate, theaflavin 3-gallate]), flavones (apigenin, luteolin), flavonols (isorhamnetin, kaempferol, myricetin, quercetin), flavanones (eriodictyol, hesperetin, naringenin), isoflavones (daidzein, genistein, glycetin, biochanin A, formononetin), and finally lignans (secoisolariciresinol, matairesinol, lariciresinol, pinoresinol).

Statistical analysis

Case and control demographic and lifestyle factors were summarized as numbers of subjects and percentages for categorical variables and means and standard deviations for continuous variables. Significance of differences between cases and controls was assessed using *t* tests, *U*-Mann–Whitney, and Chi-square tests where indicated.

As dietary flavonoid and lignan intake data were skewed (according to the Kolmogorov and Levene tests) and the natural logarithm could not be normalized, the medians (interquartile ranges) of the raw and energy-adjusted intakes were used to describe this variable for cases and controls prior to applying the nonparametric *U*-Mann–Whitney test. Intake of each flavonoid subgroup was also energy adjusted by dividing by total caloric intake (mg/1,000 kcal day), and subjects were then divided into quartiles of intake based on their distributions among controls. Using other energy-adjustment methods (e.g., the residual method [39]) did not significantly affect the results.

To investigate the relationship between colorectal cancer risk and intake of flavonoid and lignan subgroups by quartiles, the corresponding ORs and 95 % CIs were estimated using an unconditional logistic regression, because the controls were frequency-matched to cases. Flavonoids and lignans were included in the models as quartiles (categorically) based on the distributions among controls. To account for potential confounding and adjust slight differences in sex between cases and controls, models were adjusted for sex, age (years, continuous), body mass index (<18.5, 18.5–25, >25–30, >30 kg/m^2), energy intake (kcal/day, continuous), alcohol consumption (g/day, continuous), fiber intake (g/1,000 kcal day, continuous), red and processed meat consumption (g/1,000 kcal day, continuous), tobacco habits (former, current and never smoker), level of physical activity (no activity, low, high), regular drugs (aspirin, nonsteroidal anti-inflammatory drug (NSAID), both, none), and first-degree family history of colorectal cancer (yes, no). Tests for linear trend were performed by assigning the medians of each quartile as scores. Separate models were done for men and women, and the interaction between sex and flavonoid and lignan intakes was assessed with a likelihood-ratio test. Because there were no important differences between sexes in the risk of colorectal cancer by quartiles of flavonoid and lignan intakes, only the overall results are presented for the flavonoid and lignan intakes as a whole. We also performed analysis to assess the relationships between quartiles of fruit and vegetable consumption and colorectal cancer using the same models as for flavonoids.

The primary analysis was performed for all colorectal cancers combined; secondary analyses were carried out for colon and rectal cancers separately. The likelihood-ratio test was used to evaluate the association and heterogeneity between cancer sites. A two side p value of 0.05 was considered statistically significant.

Results

A total of 424 colorectal patients (cases) (265 and 159 with colon and rectal cancer, respectively) and 401 hospital-based control subjects were included in the current study (Table 1). The proportion of women was slightly higher for controls than cases. Cases and controls had similar age distributions. Both total energy and alcohol intakes were higher for cases than controls, while controls had higher BMIs and also higher consumptions of fruits and vegetables than cases. A higher percentage of cases reported a family history of colorectal cancer compared with controls.

Daily intake of total flavonoids, lignans, and all subgroups is shown in Table 2. Flavanols were the most important contributor (74 %) to total flavonoid intake (PA 65 %, flavan-3-ols monomers 9 %, theaflavins <0.1 %), followed by flavanones (10 %), flavonols (7 %), and anthocyanidins (7 %). Finally, the contributions of flavones (1 %) and isoflavones (<1 %) were minor. Controls and cases had similar raw flavonoid and lignan intakes, and isoflavones were the only flavonoid subgroup whose raw intake was significantly higher in cases than controls ($p = 0.04$). For energy-adjusted intakes, however (nutrient density), total flavonoids, flavanols, PA, flavonols, flavones, and lignans were significantly more consumed by controls than cases. The main food contributors to the subgroup and total flavonoid and lignan intake were also studied (Table 3). The richest source of most of the flavonoid and lignan subgroups was fruits, except for

Table 1 Demographic characteristics and diet variables of study participants

	Control $n = 401$	Case $n = 424$	p value
Demographic variables			
Age (*y*)[a]	65.1 (12.5)	66.2 (11.7)	0.22
Sex			
Female, *n* (%)	194 (48.4)	169 (39.9)	0.01
Male, *n* (%)	207 (51.6)	255 (60.1)	
BMI (kg/m^2)[a]	26.9 (4.8)	25.9 (4.2)	0.07
BMI			
<18.5, *n* (%)	9 (2.2)	17 (4.0)	0.01
18.5-<25, *n* (%)	142 (35.4)	172 (40.6)	
25-<30, *n* (%)	161 (40.1)	176 (41.5)	
>30, *n* (%)	89 (22.2)	59 (13.9)	
BMI 10 year prior to recruitment (kg/m^2)[a]	26.9 (5.1)	26.7 (3.9)	0.57
Tobacco			
Never smoker, *n* (%)	227 (56.6)	216 (50.9)	0.24
Former smoker, *n* (%)	115 (28.7)	133 (31.4)	
Current smoker, *n* (%)	59 (14.7)	75 (17.7)	
Physical activity			
Never, *n* (%)	66 (15.9)	74 (17.0)	0.98
Low, *n* (%)	140 (33.7)	137 (31.5)	
High, *n* (%)	210 (50.5)	224 (51.5)	
History of cancer			
No cancer, *n* (%)	226 (56.4)	200 (47.2)	<0.001
Colon, *n* (%)	20 (5.0)	65 (15.3)	
Other cancers, *n* (%)	155 (38.6)	159 (37.5)	
Diet variables			
Energy (kcal/day)[a]	1,968.9 (678.0)	2,174.7 (792.6)	<0.001
Alcohol (g/day)[a]	9.5 (36.4)	12.0 (37.6)	0.52
Vegetables (g/day)[a]	217.7 (155.3)	198.8 (162.0)	0.01
Fruit (g/day)[a]	281.7 (181)	262.9 (182)	0.06
Red meat (g/day)[a]	44.6 (35.2)	48.9 (42.7)	0.11
Processed meat (g/day)[a]	31.2 (31.5)	36.0 (41.7)	0.06
Tea (g/day)[a]	1.16 (11.1)	0.79 (16.2)	0.09
Calcium (mg/day)[a]	868.5 (482.3)	837.4 (402.0)	0.32
Folate (mcg/day)[a]	265.4 (120.9)	259.9 (109.4)	0.49
Fiber (g/day)[a]	24.1 (9.14)	25.3 (10.09)	0.13
Medications			
Aspirin and/or NSAID			
Aspirin, *n* (%)	54 (13.5)	63 (14.9)	<0.001
NSAID, *n* (%)	37 (9.2)	21 (5.0)	
Both, *n* (%)	21 (5.2)	3 (0.7)	
None, *n* (%)	289 (72.1)	337 (79.5)	

[a] Continuous variables are expressed as means (standard deviation)

p values for *t* tests, *U*-Mann–Whitney, and Chi-square tests where indicated

flavonols and phytoestrogens (vegetables), isoflavones (legumes), and theaflavins (tea).

Table 4 shows the multivariable-adjusted ORs and 95 % CIs of colorectal cancer for each quartile of energy-adjusted dietary flavonoid and lignan intakes. Significant inverse associations with colorectal cancer were observed for intake of flavones (OR, 0.59; 95 % CI, 0.37–0.93 for the highest versus the lowest quartile), PAs (OR, 0.58; 95 % CI, 0.35–0.96), total flavonoids (OR, 0.59; 95 % CI, 0.35–0.99), lignans (OR, 0.59; 95 % CI, 0.34–0.99), and phytoestrogens (OR, 0.53; 95 % CI, 0.30–0.93). No significant associations were observed between colorectal cancer risk and intake of isoflavones, anthocyanidins, flavanones, flavonols, flavanols, or flavan-3-ols monomers. Theaflavins were not considered because the consumption of tea, the only source of these compounds, was too limited in our population. We also performed similar analysis between fruit and vegetable intake and colorectal cancer risk. We observed a significant inverse association with the sum of fruits and vegetables (OR, 0.58; 95 % CI, 0.37-0.93), but not with fruit or vegetable intakes separately.

Table 5 illustrates the relation between intake of flavonoids and lignans and cancer of the colon and rectum separately. The estimated ORs did not differ substantially with respect to those of colorectal cancer, except for that of isoflavone intake, although the heterogeneity term was not significant. For total flavonoids (OR, 0.55; 95 % CI, 0.30–0.99), flavones (OR, 0.54; 95 % CI, 0.32–0.92), and lignans (OR, 0.52; 95 % CI, 0.28–0.96), statistically significant inverse associations between intake and colon cancer risk were observed. In the case of rectal cancer, we found significant inverse associations between risk and intake only for phytoestrogens (OR, 0.41; 95 % CI, 0.19–0.91), and borderline significant for PAs (OR, 0.54; 95 % CI, 0.27–1.09; *p* for trend 0.03).

Discussion

In the present Spanish case–control study, we examined the associations between colorectal cancer risk and dietary intake of total flavonoids, individual flavonoid subclasses, and lignans. In order to estimate the intake in participants, we developed a FCDB on flavonoids and lignans, which has enabled us to improve the accuracy and the precision of the flavonoid and lignan intake estimations in relation to previous studies.

To our knowledge, this is the first study that shows a significant inverse association between total flavonoid intake and colorectal cancer. After adjustment for standard colorectal cancer risk factors, we observed a reduction in the risk of colorectal, colon, and rectal cancers by 41, 45, and 36 %, respectively, for the highest compared to the

Table 2 Dietary flavonoid and lignan intakes of study participants

	Controls				Cases				*p value*	
	Raw data		Density data		Raw data		Density data		Raw data	Density data
	Median	IQR	Median	IQR	Median	IQR	Median	IQR		
Total flavonoids	199.6	128.9–298.4	109.6	69.1–167.9	196.4	119.8–308.4	97.8	62.9–148.8	0.87	0.02
Anthocyanidins	11.5	6.1–19.6	6.6	3.4–10.6	11.2	5.2–22.7	6.0	2.8–10.5	0.98	0.14
Flavanones	16.8	6.9–32.5	9.2	3.7–17.7	17.4	9.2–31.1	8.4	4.3-15.2	0.87	0.26
Flavonols	14.7	10.0–22.2	8.3	5.2–11.5	14.3	9.0–21.3	7.2	4.6–10.4	0.48	<0.01
Flavones	2.2	1.2–4.1	1.2	0.7–2.1	2.1	1.0–4.1	1.1	0.5–1.8	0.37	0.03
Flavanols	148.8	81.4–228.4	78.1	46.3–125.2	143.0	80.5–226.3	69.1	41.6–111.0	0.81	0.03
Flavan–3–ols	14.8	8.8–23.6	8.1	4.9–12.9	15.7	8.3–25.9	7.8	4.5–12.2	0.70	0.23
Proanthocyanidins	133.2	72.0–198.8	70.4	41.0–112.3	125.4	71.1–199.0	61.2	36.9–99.1	0.67	0.02
Isoflavones	0.2	0.1–0.3	0.1	0.1–0.2	0.2	0.1–0.4	0.1	0.1–0.2	0.04	0.66
Lignans	0.7	0.5–1.0	0.4	0.3–0.5	0.7	0.5–1.0	0.3	0.3–0.5	0.31	<0.01
Phytoestrogens	1.0	0.7–1.3	0.5	0.4–0.7	1.0	0.8–1.4	0.5	0.4–0.6	0.86	<0.01

Raw data were calculated as intake per day (mg/day); density data were calculated as intake divided by total caloric intake per day (mg/1,000 kcal day)

For theaflavins, all data were 0

Table 3 Percent contributions of food groups to the intake of flavonoid and lignan subgroups in the Bellvitge Colorectal Cancer Study

	Food sources (% contribution)
Total flavonoids	Fruits (65.1 %), wine (14.4 %), legumes (6.3 %), vegetables (4.2 %)
Anthocyanidins	Fruits (63.8 %), wine (30 %)
Flavanones	Fruits (83.6 %), fruit juices (8.0 %)
Flavonols	Vegetables (46.4 %), fruit (30.1 %), wine (9.9 %)
Flavones	Fruit (39.1 %), wine (20.7 %), vegetables (19.1 %), herbal tea (18.1 %)
Flavanols	Fruits (68 %), wine (14.5 %), legumes (8.3 %), chocolate products (4.2 %)
Flavan-3-ols	Fruits (52.4 %), wine (23.7 %), chocolate products (6.5 %), tea (5.8 %)
Proanthocyanidins	Fruit (70.1 %), wine (13.3 %), legumes (9.1 %)
Teaflavins	Tea (100 %)
Isoflavones	Legumes (44 %), soy products (33.6 %), cereal products (20.6 %)
Lignans	Fruit (32.3 %), vegetables (31.2 %), cereals products (10.7 %), wine (6.6 %)
Phytoestrogens	Vegetables (28.8 %), fruit (24.4 %), cereal products (13.1 %), legumes (11.5 %), wine (5.4 %)

lowest quartiles of total flavonoid intake. No previous studies had assessed the relationship between colorectal cancer risk and total flavonoid intake, where this refers not only to the sum of anthocyanidins, flavonols, flavones, flavanones, flavan-3-ol monomers, theaflavins, and isoflavones but also PAs. However, an Italian case–control study examined the protective effect of total flavonoids without PAs and individual flavonoid subgroups, across two separate studies. In the first study [11], they found significant inverse associations between colorectal cancer risk and intake of isoflavones, flavonols, flavones, and anthocyanidins, but no association with risk for total flavonoid intake, which did not include PAs. In addition, in the second study [12], they found a significant association between risk and intake of PAs, although a combined OR for total flavonoid and PAs was not calculated. Furthermore, a Scottish case–control study [10] also investigated the relation between colorectal cancer risk and total flavonoid intake, where all flavonoids (but not PAs) and procyanidins (dimers of PAs) were included. In this study, the authors showed an inverse relation between intake and colorectal cancer risk for flavonols, catechin, epicatechin, and procyanidins, but not for total dietary flavonoids. Other published case–control and cohort studies only examined certain flavonoid subclasses [9, 13–24].

Several cohort studies [16–22] did not find any significant relationships between colorectal cancer risk and intake of any flavonoid subclass, although inverse associations with certain flavonoid subclasses have been suggested in our and other case–control studies. Our results suggest that intake of flavones, PAs, lignans, and phytoestrogens (sum of isoflavones and lignans) is inversely related to colorectal cancer risk. Previous case–control studies also found similar inverse associations between risk and intake of PAs or procyanidins [10, 12], flavones [11], or lignans [14, 24]. Other case–control studies also reported inverse associations for intake of flavonols [10, 11], nontea flavonols [13],

Table 4 Association between flavonoid and lignan intake and risk of colorectal cancer in the Bellvitge Colorectal Cancer Study

	Upper cutoff (mg/1,000 kcal day)	Cases (*n*, %)		OR (95 % CI)		*p* for trend*
Total flavonoids						0.04
I	<68.9	128	30.2 %		1	
II	68.9–108.9	113	26.7 %	0.99	(0.66–1.49)	
III	109.0–167.9	111	26.2 %	0.88	(0.57–1.37)	
IV	>167.9	72	17.0 %	0.59	(0.35–0.99)	
Anthocyanidins						0.33
I	<3.3	133	31.7 %		1	
II	3.3–6.5	96	22.6 %	0.74	(0.49–1.12)	
III	6.6–10.6	93	21.9 %	0.75	(0.50–1.14)	
IV	>10.6	102	24.1 %	0.75	(0.47–1.20)	
Flavanones						0.85
I	<3.7	96	22.6 %		1	
II	3.7–9.1	138	32.5 %	1.46	(0.97–2.19)	
III	9.2–17.7	103	24.3 %	1.09	(0.71–1.66)	
IV	>17.7	87	20.5 %	1.19	(0.75–1.91)	
Flavonols						0.27
I	<5.1	127	30.0 %		1	
II	5.1–8.3	122	28.8 %	0.98	(0.65–1.47)	
III	8.4–11.5	89	21.0 %	0.78	(0.50–1.23)	
IV	>11.5	86	20.3 %	0.79	(0.46–1.33)	
Flavones						0.04
I	<0.7	139	32.8 %	1		
II	0.7–1.2	98	23.1 %	0.76	(0.51–1.15)	
III	1.3–2.1	101	23.1 %	0.79	(0.52–1.21)	
IV	>2.1	86	20.3 %	0.59	(0.37–0.93)	
Flavanols						0.04
I	<46.0	122	28.8 %		1	
II	46.0–77.7	116	27.4 %	1.09	(0.72–1.64)	
III	77.8–125.2	112	26.4 %	0.94	(0.62–1.44)	
IV	>125.2	74	17.5 %	0.63	(0.38–1.03)	
Flavan-3-ols						0.35
I	<4.9	113	26.7 %		1	
II	4.9–8.1	104	24.5 %	0.93	(0.61–1.40)	
III	8.2–12.9	111	26.2 %	1.05	(0.69–1.61)	
IV	>12.9	96	22.6 %	0.79	(0.49–1.28)	
Proanthocyanidins						0.02
I	<40.9	125	29.5 %		1	
II	40.9–70.3	122	28.8 %	1.11	(0.74–1.67)	
III	70.4–112.3	106	25.0 %	0.87	(0.57–1.34)	
IV	>112.3	71	16.7 %	0.58	(0.35–0.96)	
Isoflavones						0.24
I	<0.07	108	25.5 %		1	
II	0.07–0.11	102	24.1 %	0.98	(0.65–1.47)	
III	0.12–0.17	104	24.5 %	1.08	(0.72–1.63)	
IV	>0.17	110	25.9 %	1.25	(0.82–1.88)	
Lignans						0.07
I	<0.27	127	30.0 %		1	
II	0.27–0.36	105	24.8 %	0.72	(0.47–1.10)	

Table 4 continued

	Upper cutoff (mg/1,000 kcal day)	Cases (*n*, %)		OR (95 % CI)		*p* for trend*
III	0.37–0.50	101	23.8 %	0.61	(0.39–0.95)	
IV	>0.50	91	21.5 %	0.59	(0.34–0.99)	
Phytoestrogens						0.03
I	<0.39	123	29.0 %		1	
II	0.39–0.53	124	29.2 %	1.01	(0.67–1.51)	
III	0.54–0.74	118	27.8 %	1.03	(0.66–1.62)	
IV	>0.74	59	13.9 %	0.53	(0.30–0.93)	

ORs were estimated using a multiple logistic regression model adjusted for sex (men, women), age (years), BMI (<18.5, 18.5–25, >25–30, >30 kg/m^2), energy intake (kcal/day), alcohol (g/day) and fiber (g/1,000 kcal day) intake, red and processed meat intake (g/1,000 kcal day), tobacco consumption (former, current and never smoker), physical activity (no activity, low, high), regular drugs (aspirin, nonsteroidal anti-inflammatory drug (NSAID), both, none), and family history of colorectal cancer (yes/no)

* Tests for linear trend were performed by assigning the medians of each quartile as scores

Table 5 Association between flavonoid and lignan intake and risk of both colon and rectal cancers, separately, in the Bellvitge Colorectal Cancer Study

	Colon (265 cases)		Rectal (159 cases)		*p* for heterogeneity*
	OR 4 versus 1 quartile (95 % CI)	*p* trend†	OR 4 versus 1 quartile (95 % CI)	*p* trend†	
Total flavonoids	0.55 (0.30–0.99)	0.06	0.64 (0.32–1.29)	0.14	0.70
Anthocyanidins	0.66 (0.39–1.11)	0.21	1.02 (0.54–1.94)	0.87	0.39
Flavanones	1.17 (0.68–2.00)	0.85	1.20 (0.64–2.27)	0.97	0.97
Flavonols	0.65 (0.35–1.18)	0.08	1.00 (0.47–2.10)	0.96	0.40
Flavones	0.54 (0.32–0.92)	0.02	0.69 (0.37–1.28)	0.47	0.49
Flavanols	0.61 (0.35–1.09)	0.10	0.61 (0.31–1.22)	0.07	0.87
Flavan-3-ols	0.78 (0.45–1.34)	0.38	0.78 (0.39–1.54)	0.53	0.96
Proanthocyanidins	0.59 (0.33–1.05)	0.07	0.54 (0.27–1.09)	0.03	0.79
Isoflavones	1.50 (0.93–2.41)	0.04	0.83 (0.47–1.47)	0.49	0.19
Lignans	0.52 (0.28–0.96)	0.04	0.62 (0.30–1.29)	0.36	0.56
Phytoestrogens	0.55 (0.28–1.06)	0.06	0.41 (0.19–0.91)	0.05	0.31

ORs were estimated using a multiple logistic regression model adjusted for sex (men, women), age (years), BMI (<18.5, 18.5–25, >25–30, >30 kg/m^2), energy intake (kcal/day), alcohol (g/day) and fiber (g/1,000 kcal day) intake, red and processed meat intake (g/1,000 kcal day), tobacco consumption (former, current and never smoker), physical activity (no activity, low, high), regular drugs (aspirin, nonsteroidal anti-inflammatory drug (NSAID), both, none), and family history of colorectal cancer (yes/no)

† Tests for linear trend were performed by assigning the medians of each quartile as scores

* Between colon and rectal cancer

anthocyanidins [11], isoflavones [9, 11], and certain flavan-3-ol monomers [10], but these associations were not observed in our study. These differences could be partly explained by variability in flavonoid and lignan intakes among populations. For example, no evidence was found in the present study of a relationship between isoflavone intake and colorectal cancer risk, whereas Ahkter et al. [9] in a Japanese case–control study reported a significant inverse association with isoflavone intake. However, isoflavone intake in each study was very different, 39.75 mg/day in the Japanese study compared to only 0.21 mg/day in the present study.

Few previous studies have calculated ORs separately for colon and rectum tumors. Our data suggest that the protective effect exerted by flavonoid and lignan intake upon colon, rectal, and combined cancers is similar, in agreement with other studies [9–13]. It should be emphasized, however, that in the present study, OR confidence intervals were wider for rectal cancer than for colon cancer, due to the relatively small number of rectal cancer cases observed.

Numerous in vitro and animal studies have demonstrated the anticarcinogenic effects of flavonoids. Several possible mechanisms have been suggested, including inhibition of DNA oxidation, modulation of phase I and II

metabolic enzymes, regulation of cell proliferation and apoptosis, and modulation of inflammatory pathways [40]. Other suggested mechanisms of action are their local antioxidant, antiradical, and anti-inflammatory effects. Most flavonoids and lignans are poorly bioavailable [26] and therefore remain largely in the intestinal tract where they may act locally against carcinogens [41]. Moreover, they are partially biotransformed to phenolic acids by colonic microbiota, and then they may be absorbed and/or may exert their anticarcinogenic properties in situ [42]. In addition to exerting anticarcinogenic effects directly, phytoestrogens in particular may also protect against colorectal cancer through their estrogenic activities. Dietary supplementation with isoflavones may cause an up-regulation of the estrogen receptor β (ER-β) [43], decreasing the expression of proliferating cell nuclear antigen (PCNA), extracellular signal-regulated kinase (ERK)-1/2, AKT, and nuclear factor (NF)-κB, and up-regulating the expression of p21 [44, 45]. Therefore, phytoestrogens may also inhibit the development of colorectal cancer cells via apoptosis induction and modulation of the cell cycle progression.

The strengths and limitations of hospital-based case–control studies [46] should be considered when evaluating these data. Dietary habits can be influenced by a recent diagnosis of cancer or by the fact that the disease process could have been well underway during the reference period of the dietary history questionnaire. Another limitation is that our dietary history questionnaire was not developed specifically to estimate the intake of flavonoids and lignans, although it is a validated questionnaire in the Spanish population for specific foods [28] and nutrients [29], even using biomarkers [47]. Another possible drawback of our study is an underestimation of the real flavonoid and lignan intakes due to the consumption of food items with incomplete composition data. However, our FCDB was compiled from the most recent polyphenol composition data in the literature [24, 35–38] and expanded this with estimations, recipes, and retention factors. This expansion increased the amount of available food items ($n = 1{,}877$) and reduced the number of missing values (only 10 %) in comparison to FCDBs used in other studies. Finally, it is difficult to distinguish between the effects of fruit/vegetable versus total flavonoid intake because fruits and vegetables are major food sources for total flavonoids and these variables are highly correlated (rho Spearman = 0.694). Therefore, other compounds from fruits and vegetables could partially explain the effect of flavonoid and lignan intake on the reduction of colorectal cancer risk.

In conclusion, the present study suggests that high dietary flavonoid and phytoestrogen intake, particularly flavones, PAs, and lignans are associated with reduced risk of colorectal cancer. Similar results were observed after analyzing separately by cancer site, although reductions in risk for rectal cancer appeared to be slightly higher than for colon cancer. Confirmation of these findings is still required in large cohort studies.

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Conflict of interest The authors are not aware of any conflict of interest.

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