Flavonoid Intake and Colorectal Cancer Risk in Men and Women

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Dietary flavonoids can inhibit cancer development by protecting tissues against free oxygen radicals and inhibiting cell proliferation, but observational studies of flavonoid intake and colorectal cancer incidence are sparse. The authors prospectively evaluated the association between intake of flavonoids and colorectal cancer incidence in 71,976 women from the Nurses' Health Study and 35,425 men from the Health Professionals Follow-Up Study. Dietary intake was assessed in 1990, 1994, and 1998 by means of a food frequency questionnaire. The authors used Cox proportional hazards models with time-varying variables to estimate relative risks of colorectal cancer. Between 1990 and 2000, the authors documented 878 incident cases of colorectal cancer (498 in women and 380 in men). Total flavonoid intake was not inversely associated with colorectal cancer risk among women and men combined. The combined relative risk for the highest quintile of total flavonoid intake compared with the lowest was 1.19 (95% confidence interval: 0.94, 1.49; \( p \) for trend = 0.15). Higher intakes of individual flavonols, including quercetin, myricetin, and kaempferol, were also not related to a lower risk of colorectal cancer. These data provide little support for the hypothesis of an association between flavonoid intake and colorectal cancer risk, at least within the ranges of intakes consumed in the populations studied.

colorectal neoplasms; flavones; flavonoids; flavonols

Abbreviations: FFQ, food frequency questionnaire; HPFS, Health Professionals Follow-up Study; NHS, Nurses' Health Study; SD, standard deviation.
Arts et al. (8) observed an inverse association between certain flavonoid subgroups and risk of rectal cancer. However, in three other cohort studies, investigators did not observe a lower risk of colorectal cancer with flavonoid intake (9–11). The relation of colorectal cancer to flavonoids from food sources, such as tea and apples, has also been examined in many studies, but findings have been inconclusive (9–28).

In this analysis, we prospectively examined the relation between intakes of flavonoids and flavonoid subgroups and colorectal cancer risk in men and women from two large prospective cohort studies, the Nurses’ Health Study (NHS) and the Health Professionals Follow-up Study (HPFS). We additionally examined the associations between major food sources of flavonoids and colorectal cancer risk in the NHS and HPFS cohorts.

MATERIALS AND METHODS

The study population

The NHS was established in 1976 when 121,700 female registered nurses aged 30–55 years from 11 US states were enrolled. The HPFS was established in 1986 when 51,529 US male dentists, podiatrists, pharmacists, optometrists, osteopaths, and veterinarians aged 40–75 years were enrolled. Every 2 years, participants in both cohorts have completed a mailed questionnaire asking for information on various risk factors and the occurrence of diseases, including colorectal cancer.

In the present analysis, we excluded participants who reported a daily energy intake outside the plausible range of 600–3,500 kcal/day (for women) or 800–4,200 kcal/day (for men) on the dietary questionnaire, as well as those who reported having had a diagnosis of cancer (except nonmelanoma skin cancer). These exclusions left a total of 71,976 women and 35,425 men for the analyses.

Dietary assessment

Dietary intake data were collected from the NHS and HPFS participants in 1990, 1994, and 1998 using a 131-item food frequency questionnaire (FFQ) (29, 30). For each food or beverage item on the FFQ, participants reported their average consumption during the past year. Participants chose from nine answers ranging from “never” or “less than one serving per month” to “six or more servings per day.” Individual nutrient intakes were calculated by multiplying the frequency of each food consumed by the nutrient content of the specified portion size. This information was obtained from the US Department of Agriculture (31) and was supplemented by information supplied by food manufacturers.

We created our flavonoid database by first analyzing foods known to be important dietary sources of flavonoids, including apples, apple juice, onions, tea, red wine, avocado, cantaloupe, watermelon, blueberries, green beans, corn, alfalfa sprouts, yellow squash, green pepper, and tofu (30). For foods that were not analyzed, we utilized values from a previous analysis of Dutch foods (32). We also imputed values for 46 foods using values from related foods; half of these foods were listed on the questionnaire and others were written in by participants (30). Intakes of flavonols and flavones, including quercetin, kaempferol, myricetin, apigenin, and luteolin, were calculated as the sums of the products of frequency and flavonoid content (frequency of consumption of each food × flavonoid content for the specified serving size) (33). The measure “total flavonoids” represents the sum of these five compounds.

The reproducibility and validity of the dietary questionnaire were assessed previously by comparing responses from the FFQ with responses from two 1-week dietary records in women (29). Although the reproducibility and validity of values for flavonoid intake could not be directly tested, we examined correlations between the questionnaire and the dietary records for the major food sources of flavonoids. The Pearson correlation coefficients were 0.90 for tea, 0.66 for apples, 0.49 for broccoli, and 0.44 for tomatoes (29).

Case ascertainment

On each biennial questionnaire, we asked participants whether they had been diagnosed with colon or rectal cancer in the prior 2 years. We sought permission to obtain hospital records and pathology reports for persons who reported a diagnosis of colorectal cancer and persons who were deceased. Physicians who were blinded to exposure data reviewed and extracted information on histology, anatomic location, and stage of cancer. Between 1990 and 2000, we documented 878 incident cases of colorectal cancer—498 in women and 380 in men. Specifically, 408 women and 293 men had a primary tumor of the colon and 90 women and 87 men had rectal cancer.

Data analysis

In the present study, we did not analyze intakes of two flavonols, luteolin and apigenin, which contributed very minor amounts to total flavonoid intakes in both cohorts (<0.1 mg/day). We first grouped intakes of flavonoids and individual flavonols (i.e., quercetin, myricetin, and kaempferol) in both cohorts into quintiles. Intakes of the major food sources of flavonoids, including tea, onions, broccoli, apples, and tomatoes, were categorized a priori. Intake of onionsincluded onion consumed as a garnish or as a vegetable. Apple sources included apple juice or cider and fresh apple. Tomato sources included tomato juice, tomato sauce, and fresh tomato. We evaluated the long-term intakes of flavonoids, individual flavonols, and major food items from the baseline FFQ (1990) and follow-up FFQs (1994 and 1998) and computed cumulative average intakes.

We calculated person-years of observation for each participant from the date of return of the baseline questionnaire (June 1990 for the NHS and January 1990 for the HPFS) to the date of a report of colorectal cancer, other cancer, or death or the end of follow-up (June 2000 for the NHS and January 2000 for the HPFS). We first analyzed the NHS and HPFS cohorts separately. We used Cox proportional hazards models with time-varying variables to model the relative risks of colorectal cancer, comparing higher quintiles with
the lowest quintile (referent). In the multivariable models, results were adjusted for age (in 5-year categories) and potential risk factors for colorectal cancer, including: body mass index (weight (kg)/height (m)$^2$); <23, 23–<25, 25–<27, 27–<30, or ≥30 kg/m$^2$); physical activity (metabolic equivalents/week, in quintiles); history of colorectal cancer in a first-degree relative; previous colorectal polyps (yes/no); prior colorectal polyps (percentile); prior screening sigmoidoscopy or colonoscopy (yes/no); alcohol consumption (g/day); smoking status (never, past, or current); current smoking (percent), current aspirin use (percent); current postmenopausal hormone replacement therapy (percent); current multivitamin supplement use (percent); total caloric intake (kcal/day); red meat intake (servings/day); total folate intake (μg/day); total fiber intake (g/day); and total calcium intake (mg/day). We additionally adjusted for postmenopausal hormone therapy (never, past, or current) in the NHS cohort. Covariates were assessed repeatedly and updated throughout the analysis.

We performed tests for trend by using the nutrient intake values to construct continuous variables (for nutrient analysis) or by using the frequency responses in servings per day or per week (for food analysis). All $p$ values were two-sided.

We also combined relative risk estimates from the NHS and HPFS cohorts using a random-effects model developed by DerSimonian and Laird (34).

### RESULTS

The baseline mean intakes of flavonoids were similar in the NHS and HPFS cohorts: 21.7 mg/day (standard deviation (SD), 15.1) and 22.4 mg/day (SD, 14.5) in women and men, respectively. Of the individual flavonols, quercetins (15.9 mg/day (SD, 10.2) in women, 16.8 mg/day (SD, 10.3) in men) were the major contributor to intake of flavonoids, followed by kaempferol (4.8 mg/day (SD, 5.2) in women, 4.3 mg/day (SD, 4.8) in men) and myricetin (0.98 mg/day (SD, 1.1) in women, 1.1 mg/day (SD, 1.0) in men). The major food sources of flavonoids in both cohorts were tea (35 percent in women and 25 percent in men), onions (23 percent in women, 25 percent in men), apples (8 percent in women, 10 percent in men), broccoli (8 percent in women, 7 percent in men), and tomatoes (6 percent in women, 7 percent in men). Together, these accounted for approximately 80 percent of food sources of flavonoids in both women and men.

Table 1 presents the baseline distributions of risk factors for colorectal cancer according to quintiles of flavonoid intake in the NHS and HPFS cohorts. Participants who consumed greater amounts of flavonoids were less likely to be current smokers, consumed less alcohol, and were more likely to be physically active. Persons reporting a higher

### TABLE 1. Age-adjusted data on risk factors for colorectal cancer according to quintiles* (Q) of flavonoid intake at baseline among participants in the Nurses’ Health Study and the Health Professionals Follow-up Study, 1990

<table>
<thead>
<tr>
<th></th>
<th>Nurses’ Health Study (women)</th>
<th>Health Professionals Follow-up Study (men)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Q1</td>
<td>Q2</td>
</tr>
<tr>
<td>No. of participants</td>
<td>14,611</td>
<td>14,222</td>
</tr>
<tr>
<td>Age (years)</td>
<td>55.5</td>
<td>56.0</td>
</tr>
<tr>
<td>Body mass index† (kg/m$^2$)</td>
<td>25.6</td>
<td>25.7</td>
</tr>
<tr>
<td>Family history of colorectal cancer† (%)</td>
<td>10.2</td>
<td>10.1</td>
</tr>
<tr>
<td>Prior colorectal polyps (%)</td>
<td>1.3</td>
<td>1.2</td>
</tr>
<tr>
<td>Prior screening sigmoidoscopy or colonoscopy (%)</td>
<td>9.8</td>
<td>9.8</td>
</tr>
<tr>
<td>Current smoking (%)</td>
<td>23.4</td>
<td>16.9</td>
</tr>
<tr>
<td>Alcohol consumption (g/day)</td>
<td>6.0</td>
<td>5.4</td>
</tr>
<tr>
<td>Regular physical activity (metabolic equivalents/week)</td>
<td>13.2</td>
<td>15.7</td>
</tr>
<tr>
<td>Current postmenopausal hormone replacement therapy (%)</td>
<td>27.4</td>
<td>28.3</td>
</tr>
<tr>
<td>Current aspirin use (%)</td>
<td>44.0</td>
<td>46.4</td>
</tr>
<tr>
<td>Current multivitamin supplement use (%)</td>
<td>35.9</td>
<td>38.1</td>
</tr>
<tr>
<td>Total caloric intake (kcal/day)</td>
<td>1,706</td>
<td>1,752</td>
</tr>
<tr>
<td>Red meat intake (servings/day)</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Total folate intake (μg/day)</td>
<td>385</td>
<td>418</td>
</tr>
<tr>
<td>Total fiber intake (g/day)</td>
<td>14.9</td>
<td>17.5</td>
</tr>
<tr>
<td>Total calcium intake (mg/day)</td>
<td>968</td>
<td>994</td>
</tr>
</tbody>
</table>

* Ranges of flavonoid intakes in the quintiles were 0–9.6, 9.6–13.7, >13.7–19.5, >19.5–31.1, and >31.1 mg/day for the Nurses’ Health Study cohort and 0–10.7, >10.7–14.9, >14.9–20.5, >20.5–30.5, and >30.5 mg/day for the Health Professionals Follow-up Study cohort.

† Weight (kg)/height (m)$^2$.

‡ History of colorectal cancer in a first-degree relative.
intake of flavonoids also reported higher intakes of total folate and total fiber.

Total flavonoid intake was not significantly associated with risk of colorectal cancer in the NHS cohort; the age-adjusted relative risks in the higher quintiles of flavonoid intake relative to the lowest quintile were 0.88, 0.83, 0.83, and 0.98 (p for trend = 0.93). The respective values among men were 1.12, 1.03, 1.06, and 1.24 (p for trend = 0.33). Additional adjustment for risk factors for colorectal cancer did not substantially alter the results; the multivariable relative risks comparing the highest category of flavonoid intake with the lowest were 1.13 (95 percent confidence interval: 0.83, 1.52; p for trend = 0.42) in women and 1.28 (95 percent confidence interval: 0.89, 1.83; p for trend = 0.21) in men. Accordingly, we report multivariable results from each cohort and from both cohorts combined for the relation of flavonoid intake to colorectal cancer risk.

Intakes of individual flavonols were also not appreciably associated with colorectal cancer risk. In the pooled analysis combining the two cohorts, no evidence for inverse associations was observed between intakes of individual flavonoids, including quercetin, kaempferol, and myricetin, and risk of colorectal cancer (table 2).

We further examined the primary food sources of flavonoids in relation to risk of colorectal cancer in both men and women. Intakes of tea, onions, broccoli, and tomatoes were not significantly associated with risk of colorectal cancer in either women or men (table 3). Higher apple consumption was weakly associated with a lower risk of colorectal cancer in men; the relative risk in the highest intake category (≥2 servings/day) relative to the lowest (<2 servings/week) was 0.82 (95 percent confidence interval: 0.51, 1.30; p for trend = 0.06). However, no overall linear trend was noted in men and women combined (p = 0.11) (table 3).

In analyses carried out using intake at baseline (i.e., 1990) without cumulative updating, results (data not shown) were similar to the findings obtained using cumulative updating. Additional analyses carried out according to tumor site also revealed no significant associations between intakes of

### TABLE 2. Relative risk of colorectal cancer according to quintiles of intake of flavonoids and their subclasses (updated cumulatively) in the Nurses’ Health Study and the Health Professionals Follow-up Study, 1990–2000

<table>
<thead>
<tr>
<th>Quintile of intake</th>
<th>1 (referent)</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>( P_{\text{trend}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total flavonoids</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NHS* (women)†</td>
<td>1.00</td>
<td>0.92</td>
<td>0.68, 1.24</td>
<td>0.95</td>
<td>0.70, 1.28</td>
<td>0.95</td>
</tr>
<tr>
<td>HPFS* (men)†</td>
<td>1.00</td>
<td>1.08</td>
<td>0.75, 1.53</td>
<td>1.07</td>
<td>0.75, 1.54</td>
<td>1.10</td>
</tr>
<tr>
<td>Pooled</td>
<td>1.00</td>
<td>0.99</td>
<td>0.78, 1.23</td>
<td>1.00</td>
<td>0.79, 1.26</td>
<td>1.01</td>
</tr>
<tr>
<td>Total no. of cases</td>
<td>171</td>
<td>170</td>
<td>165</td>
<td>172</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Subclasses</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quercetin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NHS (women)†</td>
<td>1.00</td>
<td>0.72</td>
<td>0.53, 0.98</td>
<td>0.86</td>
<td>0.64, 1.15</td>
<td>0.80</td>
</tr>
<tr>
<td>HPFS (men)†</td>
<td>1.00</td>
<td>1.22</td>
<td>0.86, 1.74</td>
<td>1.06</td>
<td>0.74, 1.54</td>
<td>1.29</td>
</tr>
<tr>
<td>Pooled</td>
<td>1.00</td>
<td>0.93</td>
<td>0.55, 1.57†</td>
<td>0.93</td>
<td>0.74, 1.18</td>
<td>1.01</td>
</tr>
<tr>
<td>Total no. of cases</td>
<td>178</td>
<td>105</td>
<td>165</td>
<td>175</td>
<td>195</td>
<td></td>
</tr>
<tr>
<td>Kaempferol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NHS (women)†</td>
<td>1.00</td>
<td>1.07</td>
<td>0.81, 1.43</td>
<td>0.88</td>
<td>0.65, 1.19</td>
<td>0.78</td>
</tr>
<tr>
<td>HPFS (men)†</td>
<td>1.00</td>
<td>0.79</td>
<td>0.55, 1.13</td>
<td>0.99</td>
<td>0.71, 1.39</td>
<td>1.01</td>
</tr>
<tr>
<td>Pooled</td>
<td>1.00</td>
<td>0.94</td>
<td>0.70, 1.26</td>
<td>0.93</td>
<td>0.74, 1.16</td>
<td>0.88</td>
</tr>
<tr>
<td>Total no. of cases</td>
<td>183</td>
<td>170</td>
<td>168</td>
<td>158</td>
<td>199</td>
<td></td>
</tr>
<tr>
<td>Myricetin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NHS (women)†</td>
<td>1.00</td>
<td>0.74</td>
<td>0.55, 0.99</td>
<td>0.69</td>
<td>0.52, 0.93</td>
<td>0.84</td>
</tr>
<tr>
<td>HPFS (men)†</td>
<td>1.00</td>
<td>1.10</td>
<td>0.77, 1.79</td>
<td>1.22</td>
<td>0.86, 1.73</td>
<td>1.10</td>
</tr>
<tr>
<td>Pooled</td>
<td>1.00</td>
<td>0.89</td>
<td>0.60, 1.31</td>
<td>0.91</td>
<td>0.53, 1.58‡</td>
<td>0.94</td>
</tr>
<tr>
<td>Total no. of cases</td>
<td>183</td>
<td>164</td>
<td>165</td>
<td>173</td>
<td>193</td>
<td></td>
</tr>
</tbody>
</table>

* RR, relative risk; CI, confidence interval; NHS, Nurses’ Health Study; HPFS, Health Professionals Follow-up Study.
† In multivariable models, results were adjusted for age, body mass index, family history of colorectal cancer (first-degree relative), history of colorectal polyps, prior sigmoidoscopy screening, physical activity, smoking status, red meat intake, alcohol consumption, total energy intake, total calcium intake, total folate intake, total fiber intake, aspirin use, and multivitamin use. Among women, multivariate models also included adjustment for postmenopausal hormone replacement therapy. See table 1 for quintile cutpoints for total flavonoids.
‡ Test for heterogeneity by sex was statistically significant.
flavonoids or individual flavonols and colon or rectal cancer (data not shown).

**DISCUSSION**

In this prospective study, we observed no associations between intakes of flavonoids or individual flavonols, including quercetin, kaempferol, and myricetin, and risk of colorectal cancer. Intakes of primary food sources of flavonoids were also not significantly associated with risk of colorectal cancer.

Observational studies on the association between flavonoids and colorectal cancer risk have been limited. Similar to our findings, the findings of most of these studies have not supported the hypothesis of an inverse association with colorectal cancer risk or mortality (9–11). In the Seven Countries Study, which comprised 16 cohorts, flavonoid intake was not related to colorectal cancer mortality during 25 years of follow-up (11). In the Finnish Mobile Clinic Health Examination Survey, a cohort study of 5,309 men and 4,745 women, Knekt et al. (9) also observed a null association between flavonoid intake and incidence of colorectal cancer, although investigators from a previous small study reported...
a suggestive inverse association (20). Moreover, in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study, intakes of total flavonols and flavones were weakly positively associated with risk of colorectal cancer among 27,110 male smokers (10). To our knowledge, only one cohort study of women has observed an inverse association with intake of catechins, the flavonoid subclass, but the inverse association was confined to rectal cancer (8). The overall observations suggest a lack of inverse association between flavonoid intake and colorectal cancer, raising the question of whether the protective effects obtained in in vitro studies can be achieved in humans.

Findings relating foods rich in flavonoids to colorectal cancer risk have been inconclusive. Results from the Finnish Mobile Clinic Health Examination Survey suggested that apple consumption may explain an inverse association observed with total cancer incidence (9, 20), while our study and another cohort study (21) revealed no significant association. Intake of broccoli has been linked to reduced risk of colorectal cancer in some studies (22, 23) but not in our study or the Iowa Women’s Health Study (24). In addition, some (25, 26) but not all (21, 27) studies have reported an inverse association between tomato intake and colorectal cancer risk. In our study, tomato consumption did not appear to be protective against colorectal cancer. Studies investigating the association between onion intake and colorectal cancer risk have been limited; in our study and in several others (10, 20, 28), no association with onion intake was observed.

Tea was another major food source of flavonoids in our cohorts. Investigators in at least two observational studies have reported a lower risk of colorectal cancer among participants who drank tea frequently than in those who never drank tea (12, 13). However, our study and several others (14–19) did not reveal an inverse association of tea intake with risk of colorectal cancer. On the basis of findings from seven cohort studies and 12 case-control studies, Tavani and La Vecchia (19) concluded that there is no overall association between tea intake and risk of colon or rectal cancer. Their review provides little support for the hypothesis that tea may be a potent protective agent against colorectal carcinogenesis.

Most flavonoids present in foods are in the form of esters, glycosides, or polymers that cannot be absorbed in their native form (35). They are usually absorbed by passive diffusion after being converted to aglycons in the gastrointestinal tract (36, 37). It has been shown that a large fraction of flavonoids remains unabsorbed; the amount that is bioavailable is only a small proportion of the ingested amount, ranging from 0.2–0.9 percent for tea catechins to 20 percent for quercetin and isoflavones (38, 39). While recent studies have suggested that the bioavailability of certain flavonoids from food (e.g., onions) may be higher than expected (40), it remains unclear whether the beneficial effects of antiproliferation and antioxidation from in vitro studies would also be present in humans, since these effects were often obtained with much greater concentrations than can be achieved in humans through diet (37). Furthermore, the microorganisms in the colon act as enzymes in catalyzing flavonoids into an array of metabolites (41). Interindiv-

In conclusion, our data provide little support for an association between flavonoid intake and risk of colorectal cancer, at least within the ranges of intakes consumed in our population. We were also unable to confirm inverse associations between the major food sources of flavonoids consumed in our cohorts and colorectal cancer risk.

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REFERENCES


Am J Epidemiol 2006;164:644–651
41. Spencer JP. Metabolism of tea flavonoids in the gastrointestinal tract. J Nutr 2003;133(suppl):325S–61S.