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REVIEW ARTICLE

Dietary flavonoid intake and colorectal cancer risk: evidence from human population studies

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Summary

Flavonoids are biologically active polyphenolic compounds widely distributed in plants. More than 5000 individual flavonoids have been identified, which are classified into at least 10 subgroups according to their chemical structure. Flavonoids of 6 principal subgroups– flavonols, flavones, anthocyanidins, catechins, flavanones, and isoflavones- are relatively common in human diets. Flavonoids are a large and diverse group of phytochemicals and research into their anti-carcinogenic potential with animal and cellular model systems supports a protective role. Whether dietary intake of flavonoids is protective against colorectal cancer in humans cannot be easily extrapolated from cell line and animal findings. Epidemiological assessment of the relationship between dietary flavonoid intake and colorectal cancer is limited, with different case-control and cohort study design investigating different combinations of flavonoids. Epidemiologic studies on flavonoid intake and colorectal cancer risk that were conducted yielded inconsistent results, with positive, inverse, and null associations. Because only a very limited number of epidemiological studies have been conducted to examine the associations of dietary intake of flavonoids with colorectal cancer risk, it is premature to make public health recommendations at this time. However, the data to date are promising and emphasize the need for further investigation of these important bioactive plant compounds. This review summarises the epidemiological evidence from case-control and cohort studies on the associations of dietary flavonoid intake with the risk for colorectal cancer. The difficulties in investigating this topic and possibilities for further research are then discussed.

Key words: flavonoids, colorectal cancer risk, human studies

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Introduction

Critical assessment of potential risk factors suggests that diets rich in plant-based foods, such as fruit and vegetables, may reduce the risk of developing colorectal cancer [1,2]. Though the mechanism by which these foods exert a protective effect is unclear, one hypothesis is the presence of high levels of potentially anti-carcinogenic phytochemicals [3].

Flavonoids are a large and diverse group of phytochemicals and research into their anti-carcinogenic potential with animal and cellular model systems supports a protective role [4,5]. Structurally distinct subclasses of flavonoids have varying capacities to modulate the progression of colorectal cancer, acting as antioxidants [6,7], anti-inflammatory agents [8-11], anti-proliferative agents [12-14] or as regulators of signal transduction pathways [15,16]. Of all the tissues in the human body, the large intestine may be exposed to higher flavonoid concentrations than other tissues [17].

Flavonoids are a group of potentially chemoprotective compounds widely distributed in fruit, vegetables, and beverages of plant origin and have similar structures that consist of two phenolic benzene rings linked to a heterocyclic pyre or pyrone [18]. More than 5000 individual flavonoids have been identified, which are classified into at least 10 subgroups according to their chemical structure [19,20].

Flavonoids of 6 principal subgroups –flavonols, flavones, anthocyanidins, catechins, flavanones, and isoflavones- are relatively common in human diets [18]. Proanthocyanidins are another important but often overlooked polyphenol subclass [21].

This review summarises the epidemiological evidence from case-control and cohort studies on the associations of dietary flavonoid intake with the risk for colorectal cancer. The difficulties in investigating this topic and possibilities for further research are then discussed.

Food sources of flavonoids

Flavonoids are ubiquitous in the plant food supply, but the subclasses do not seem to be uniformly distributed [19]. For many plants, the skins of the fruit or the outer edge of the vegetable as well as the leaves contain the most concentrated sources of flavonoids. In addition, flavonoid content is influenced by factors such as season, sunlight, climate, and food preparation and processing [18].

Main dietary sources of flavonoids vary between the subgroups [22,23]. Flavonols, such as quercetin, kaempferol, and myricetin are the most abundant flavonoids in plant foods and are mainly present in leafy vegetables, apples, onions, broccoli, and berries. Flavones (e.g., apigenin and luteolin) and anthocyanidins are present in relatively low quantities in grains, leafy vegetables, and herbs. Catechins (flavan-3-ols), such as catechin and epicatechin, are abundant in tea, apples, grapes, chocolate, and red wine. Flavanones, such as naringenin and hesperetin, known also as citrus flavonoids, are predominantly contained in citrus fruits and their juices. Isoflavones (e.g. daidzein and genistein) are mainly found in soya beans and soy-based products, and together with lignans, whose precursors are present in a wide variety of plant foods, form the group of phytoestrogens [24]. The phytoestrogen class lignans is widespread in a large range of such foods as cereals, fruit, vegetables, nuts, seeds, coffee, and tea [25].

Studying the role of those flavonoid subclasses, which are considered to be relevant to the daily intake, has been difficult because of incomplete databases [26]. Efforts to update databases are constantly made. In 2003, United States Department of Agriculture (USDA) published a new food composition data which contained 5 subclasses (flavones, flavonols, flavan-3-ols, flavanones and anthocyanidins), a total of 26 flavonoids [27]. This resource greatly facilitates future quantitative studies of flavonoids and risk of cancer in humans [22]. In 2006, USDA published a new flavonoid database [28].

Bioavailability of certain flavonoids

Individual flavonoid compounds and their dietary sources have differing relative bioavailability [29]. Consequently, when assessing several flavonoid subclasses in relation to disease, the sources and types of flavonoids could have different potential relationships with colorectal cancer [30].

Most flavonoids present in foods are in the form

of esters, glycosides, or polymers that cannot be absorbed in their native form [31]. They are usually absorbed by passive diffusion after being converted to aglycons in the gastrointestinal tract [32,33]. 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 % for tea catechins to 20 % for quercetin and isoflavones [34,35]. While recent studies have suggested that the bioavailability of certain flavonoids from food (e.g., onions) may be higher than expected [36], 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 [33]. Furthermore, the microorganisms in the colon act as enzymes in catalyzing flavonoids into an array of metabolites [37]. Interindividual variation in the possession of colonic microbial flora and the variable influences of foods on microbial metabolite production add further complexity [38].

Epidemiological evidence on the association of flavonoids intake and risk of colorectal cancer

Epidemiological assessment of the relationship between dietary flavonoid intake and colorectal cancer is limited, with different case-control and cohort study design investigating different combinations of flavonoids. Epidemiological studies on flavonoid intake and colorectal cancer risk that were conducted yielded inconsistent results. Previous epidemiological studies have concentrated mainly on 2 subclasses- flavonols and flavones - while the role of other subclasses, such as flavan-3-ols, has been only evaluated in a few studies.

Case-control studies

A multicentric Italian case-control study [39], including 1,953 cases of colorectal cancers (1,225 colon cancers and 728 rectal cancers) and 4,154 cancer-free hospital controls, found reduced risk of developing colorectal cancer with increasing intake of isoflavones (odds ratio/OR=0.76, for the highest vs the lowest quintile; p_{trend}=0.001), anthocyanidins (OR=0.67; p_{trend}=0.001), flavones (OR=0.78; p_{trend}=0.004), and flavonols (OR=0.64; p_{trend}<0.001), but not with catechin (OR=0.98), flavanones (OR=0.96) or total flavonoids (OR=0.97).

Data from this multicentric Italian case-control study was used to investigate whether proanthocyanidins are related to colorectal cancer risk [40]. A trend of decreasing risk with increasing intake of proanthocyanidins was found for all classes except monomers. The OR for the highest vs the lowest quintile of intake was 0.82 for monomers and dimers combined, 0.88 for monomers, 0.75 for dimers, 0.74 for all polymers with 3 or more mers, 0.84 for trimers, 0.80 for 4-6 mers, 0.79 for 7-10 mers, 0.69 for more than 10 mers, and 0.74 for total proanthocyanidins. The associations were apparently stronger for rectal than for colon cancer, in the absence of significant heterogenity.

A large Scottish case-control study [41], including 1,456 incident colorectal cancer cases and 1,456 population-based controls, also observed reduced colorectal cancer risk with higher intakes of flavonols, catechins and procyanidins type B1-B4, though not with flavanones or phyto-oestrogens. After energy adjustment, reductions in colorectal cancer risk associated with the highest quartiles of intake (vs the lowest quartile) were 27% for flavonols (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). No risk reducations were associated with intake of flavones (p_{trend}=0.54), flavanones (p_{trend}=0.22), and phytoestrogens (p_{trend}=0.26).

Simons et al. [42] used case-control approach to examine the association of dietary flavonol, flavone and catechin intake with colorectal cancer endpoints within the Netherlands Cohort Study (NLCS). After 13.3 years of follow up, 1,444 male and 1,041 female incident colorectal cancer cases and 2,191 male and 2,247 female subcohort members were available for analysis. No association of total flavonol and flavone intake and total catechin intake with colorectal cancer endpoints was observed. In men with a body mass index (BMI) ≥ 25kg/m^2, there was a significant inverse association of total catechin intake with rectal cancer (quintile 4 vs 1, hazard ratios/ HR=0.52, 95%

CI=0.29-0.94; quintile 5 vs 1, HR=0.63, 95%CI=0.36-1.08; p_{trend}=0.04). In this group, a significant inverse trend in HRs for rectal cancer was also observed for intake of the individual catechins - catechin and epicatechin. Contrary to men, a significant inverse trend in the association of total catechin intake with colon cancer was observed in women with BMI < 25kg/m^2 (quintile 5 vs 1, HR=0.62, 95% CI=0.43-0.91; p_{trend}=0.04). Significant inverse trends in HRs for colon cancer in this group were also observed for intake of myricetin, gallocatechin (GC), epigallocatechin (EGC) and epigallocatechin gallate (EGCG). For kaempferol intake and intake of other individual catechins, there was an indication of an inverse trend in HRs for colon cancer.

In those two case-control studies that observed a significant inverse trend in the association of flavonoid intake with colorectal cancer risk [39,41], consumption in the highest quintile was >28.5 mg/day [39] and 36.7 mg/day [41] relative to <13.2 mg/day and <16.0 mg/day, respectively, in the lowest quintile. In the study of Simons et al. [42], total flavonol and flavone intake was comparable with intake in these two studies [39,41], i.e. >36.0 mg/day in men and >38.3 mg/day in women in the highest quintile relative to <16.0 mg/day and 18.4 mg/day, respectively, in the lowest quintile. Therefore, detection of an association of total flavonol and flavone intake with colorectal cancer endpoints in the study of Simons et al. [42] does not seem to have been limited by a relatively small contrast of intake, which is most importantly determined by the reference category. Conversely, there may have been a relatively small contrast in catechin intake in the study of Simons et al. [42], namely >84.3 mg/day in men and >95.9 mg/day in women in the highest quintile relative to <24.2 and 36.2 mg/day respectively, in the lowest quintile.

Ward et al. [43] examined the risk of colorectal cancers relative to phytoestrogen intake provided on the basis of a comprehensive database. Between 1993 and 2006, 221 cases of colorectal cancer cases (125 male and 96 female cases) were diagnosed in the EPIC-Norfolk cohort, with a mean follow-up length of 9 years. OR for 221 colorectal cases and 886 controls were calculated relative to phytoestrogen intake. For men, colorectal cancer risk was not associated with the intake of any of the phytoestrogens under study. Among women, colorectal cancer risk was inversely associated with enterolactone (OR=0.33, 95% CI=0.14-0.74; p=0.008) and total enterolignans (OR=0.32, 95% CI=0.13-0.79; p=0.013), with a positive trend detected for secoisolariciresinol (OR=1.60, 95% CI=0.96-2.69; p=0.074).

In the North East of Scotland a population-based case-control study [30] including 261 colorectal cancer cases (186 colon cancers and 75 rectal cancers) and 408 controls was carried out to evaluate any independent association of total dietary and non-tea intake of 4 flavonoid subclasses (flavonols, catechins (flavon-3-ols), procyanidins and flavanones) and the risk of developing colorectal cancer. No association between total dietary flavonol, procyanidin or flavon-3-ol intake and risk of developing colorectal cancer was observed. There was a significant trend (p_{trend}=0.04) towards increased risk of colorectal cancer with higher levels of flavanone intake. Stratification by cancer site strengthened this observation, with a significant trend apparent for colon cancer (multivariate OR=1.3, 95% CI=0.7-2.4; highest vs lowest quartile; p_{trend}<0.01). Analysis of non-tea flavonoid intake indicated a significant inverse association between non-tea flavonol intake and risk of colorectal cancer (p_{trend} <0.05), but not for non-tea procyanidin or flavon-3-ol intake. Separate analyses of colon and rectal cancer cases demonstrated that non-tea flavonol intake was significantly associated with a reduced risk of developing colon (OR=0.5, 95% CI=0.3-0.8; highest vs lowest quartile; p_{trend}<0.01), but not rectal cancer in the adjusted model. Further assessment of the relationship with intake of individual non-tea flavonol compounds highlighted an association between quercetin (highest vs lowest quartile multivariate adjusted OR=0.4, 95% CI=0.2-0.8; p_{trend}<0.01) and colon cancer. In this study [30], researchers did not observe a trend in risk with total flavonols, catechin or epicatechin intakes and colorectal cancer. One explanation may be that their findings may reflect the smaller sample size or the different age distribution of subjects. Alternatively it may be that the examined population drank more tea and consumed less fruit

and vegetables, providing relatively lower non-tea sources of flavonoids [30].

The findings of the Fukuoka Colorectal Cancer Study [44], including 816 incident colorectal cancer cases and 815 community controls, add to epidemiologic evidence for protective effects of soy foods and isoflavones in colorectal carcinogenesis. Budhathoki et al. [44] observed that energy-adjusted intakes of soy foods (dry weight) and isoflavones were inversely associated with colorectal cancer risk in men and postmenopausal women, but not in premenopausal women. The multivariate-adjusted OR for the highest vs lowest quintile was 0.65 (95% CI=0.41-1.03; p_{trend}=0.03) for soy foods and 0.68 (95% CI=0.42-1.10; p_{trend}=0.051) for isoflavones in men. The corresponding values for postmenopausal women were 0.60 (95% CI=0.29-1.25; p_{trend}=0.053) and 0.68 (95% CI=0.33-1.40; p_{trend}=0.049). The site-specific analysis showed inverse associations of soy foods (p_{trend}=0.007) and isoflavones (p_{trend}=0.02) with rectal cancer in men.

Cohort studies

In the Seven Countries Study, which comprised 16 cohorts, flavonoid intake was not related to colorectal cancer mortality during 25 years of follow-up [45].

In the Finnish α-Tocopherol, β-Carotene Study (ATBC) [46] cohort of 27,110 male smokers, including 133 colorectal cancer, there was borderline direct association with intake of the sum of flavonols and flavones (OR=1.70, 95% CI=1.00-2.70; p_{trend}=0.10). Men in the ATBC study were followed from enrollment in 1985-1988 to the end of the intervention trial in 1993.

No significant associations were found in a Dutch case-cohort study [47] of 3,726 subjects, including 603 colorectal cancer cases, with respect to flavonols and the flavone luteolin (OR=0.97, 95% CI=0.71-1.32; p_{trend}=0.92).

Similarly, in the Finish Mobile Clinic Health Examination Survey, one of the largest and most comprehensive cohort studies, a cohort of about 10,000 men and women, examined at two different times [48,49], with 72 and 90 colorectal cancers, respectively, found no association with single compounds of flavonols and flavanones (OR=0.74, 95% CI=0.32-1.68) [48] and total flavonoids (computed as the sum of flavonols, flavanones and flavones; OR=0.84, 95% CI=0.43-1.64) [49].

The Iowa Women's Health study [50], on a cohort of 34,651 postmenopausal women, including 132 rectal and 635 colon cancers, found an inverse association between intake of catechin and rectal cancer incidence (OR=0.55, 95% CI=0.32-0.95; p_{trend}=0.002, for the higher vs the lowest quintile) but not for colon cancer (OR=1.10, 95% CI=0.85-1.44; p_{trend}=0.63) after 13 years of follow-up. Another study within the Iowa Women's Health study found no association of catechin intake with rectal or colon cancer risk [51].

In the study that observed a significant inverse association of total catechin intake with rectal cancer risk [50], the mean intake in subsequent quintiles was 3.6, 8.7, 14.8, 24.7 and 75.1 mg/day. However, another study in which there was a similarly high contrast in catechin intake (i.e.>134.8 mg/day in the highest quintile relative to <6.7 mg/day in the lowest quintile) but a longer follow-up time, found no association of total catechin intake with rectal cancer risk [51].

In the USA cohort [38] of 107,401 subjects (71,976 women from the Nurses' Health Study and 35,425 men from the Health Professionals Follow-up Study), including 878 incident colorectal cancers (498 in women and 380 in men) documented between 1990 and 2000, the total flavonoid intake was not inversely associated with colorectal cancer risk among women (relative risk/RR=1.13, 95% CI=0.83-1.52; p_{trend}=0.42), men (RR=1.28, 95% CI=0.89-1.83; p_{trend}=0.21), and among women and men combined (RR=1.19, 95% CI=0.94-1.49; p_{trend}=0.15). Higher intakes of individual flavonols, including quercetin, myricetin, and kaempferol, were also not related to a lower risk for colorectal cancer. Intakes of primary food sources of flavonoids were also not significantly associated with risk of colorectal cancer.

In a population study consisting of 2590 middle-aged eastern Finnish men of the prospective population-based Kuopio Ischaemic Heart Disease Risk Factor Study (KIHDRFS) [26], the mean intake of flavonoids was 131.0 ± 214.7 mg/day. During a mean follow-up time of 16.2 years, 55 colorectal cancers occurred. Flavonoid intake was not associated with the

risk of colorectal cancer (RR=1.16, 95% CI=0.58-2.34; p_{trend}=0.831). No association between intake of flavonols (RR=1.53, 95% CI=0.72-3.23; p_{trend}=0.585), flavones (RR=0.71, 95% CI=0.30-1.65; p_{trend}=0.561), flavanones (RR=0.90, 95% CI=0.37-2.20; p_{trend}=0.518), flavan-3-oils (RR=1.37, 95% CI=0.65-2.89; p_{trend}=0.820) and anthocyanidins (RR=0.59, 95% CI=0.24-1.41; p_{trend}=0.974), and risk of colorectal cancer was found.

In the Japan Public Health Center (JPHC)-based prospective study [52] in a cohort of 83,063 subjects (39,069 men and 43,994 women), including 886 colorectal cancer cases (291 proximal colon, 286 distal colon, and 277 rectum), the intake of isoflavones, miso soup, and soy food was not associated with colorectal cancer in either men or women. By colorectal cancer subsite, the risk of proximal colon cancer in men decreased with increasing consumption of isoflavones, miso soup, and soy food (compared with men in the lowest quartiles of intake, the HR in the highest quartiles were 0.55, 95% CI=0.33-0.92 and 0.51, 95% CI=0.30-0.87). The results showed no association for distal colon and rectal cancer in men or for subsites of colorectal cancer in women.

Clinical research

In addition, to investigate biological prevention with flavonoids the recurrence risk of neoplasia was studied in patients with resected colorectal cancer and after adenoma polypectomy [53]. Eighty-seven patients, 36 with resected colon cancer and 51 after polypectomy, were divided into 2 groups: one group (n=31) was treated with a flavonoid mixture (daily standard dose 20 mg apigenin and 20 mg EGCG and compared with a matched control group (n=56). Both groups were observed for 3-4 years by surveillance colonoscopy and by questionnaire. Of 87 patients enrolled in this study, 36 had resected colon cancer and 29 of these patients had surveillance colonoscopy. Among the flavonoid-treated patients with resected colon cancer (n=14), there was no cancer recurrence and one adenoma developed. In contrast, the cancer recurrence rate of the 15 matched untreated controls was 20% (3 of 15) and adenomas evolved in 4 of those patients (27%). The combined recurrence rate for neoplasia was 7% (1 of 14) in the treated patients and 47% (7 of 15) in the controls (p=0.027). Therefore, the results of this nonrandomized trial suggested that sustained long-term treatment with flavonoid mixture could reduce the recurrence rate of colon neoplasia in patients with resected colon cancer.

Other evidence for a potential effect of dietary flavonoids comes from the Polyp Prevention Trial (PPT) which examined the effectiveness of a 4-year low-fat, high-fiber, high-fruit and high-vegetable diet on adenoma recurrence [54]. In this randomized trial, total flavonoid intake was not associated with any or advanced adenoma recurrence, while greater flavonols consumption was associated with a decreased risk of advanced adenoma recurrence (4th vs 1st quartile during the trial, OR=0.24, 95% CI=0.11-0.53; p_{trend}=0.0006). Similar inverse associations were observed to a smaller extent for isoflavonoids (OR=0.46, 95% CI=0.22-0.95; p_{trend}=0.01), the flavonols kaempferol (OR=0.44, 95% CI=0.22-0.89; p_{trend}=0.03) and isorhamnetin (OR=0.44, 95% CI=0.21-0.90; p_{trend}=0.07), and the isoflavonoids genistein (OR=0.38, 95% CI=0.19-0.76; p_{trend}=0.003) and formononetin (OR=0.49, 95% CI=0.26-0.95; p_{trend}=0.02). Bobe et al. suggested that 5 prospective cohort studies [38,46-49] used older and smaller databases [55-59] and evaluated the effect of flavonols over an intake range similar or narrower than that of their participants at baseline (median, 14.9 mg/day; interquartile range/IQR, 1.10-21.2 mg/day), for which they also did not observe a protective association. In comparison, the median flavonol intake of intervention group during PPT was 29.5 mg/day (IQR, 20.7-39.8 mg/day). In support of their findings, other studies have reported a minimum of 5 servings of vegetables per day must be consumed before a reduction in rectal cancer risk can be observed [60]. Therefore, the results of the PPT suggested that the protective association between flavonols and colorectal cancer might be achieved only at flavonol intake levels that are higher than what is commonly consumed by Western populations [61,62].

It is important to note that 25 colorectal cancer patients scheduled to undergo resection of the primary tumor or liver metastases received mirtocyan, an anthocyanin-rich standardized bilberry extract,

1.4, 2.8 or 5.6 g (containing 0.5-2.0 g anthocyanins) daily for 7 days before surgery [63]. Mirtocyan anthocyanins and methyl and glucuronide metabolites were identified in plasma, colorectal tissue, and urine, but not in the liver. Anthocyanin concentrations in plasma and urine were roughly dose-dependent, reaching approximately 179 ng/g in tumor tissue at the highest dose. In tumor tissue from all patients on mirtocyanin, proliferation was decreased by 7% compared with preintervention values. Some authors have reported that doses containing <0.5 g bilberry anthocyanins [63] are necessary to conclude whether they may be appropriate for development as colorectal cancer chemopreventive agents.

In a presurgical model, 25 colon cancer patients having not received prior therapy consumed 60g/day (20g/x3/day) of an anthocyanin-rich black raspberry powder daily for 2-4 weeks [64]. Biopsies of normal-appearing and tumor tissues were taken before and after berry treatment. The berries reduced the proliferation rates and increased apoptosis in colon tumors but not in normal-appearing crypts. The number of CD 105 stained blood vessels was also reduced in berry-treated colon tumors, suggesting an antiangiogenic effect of short-term berry treatment [64].

Commentary

Whether dietary intake of flavonoids is protective against colorectal cancer in humans cannot be easily extrapolated from cell line and animal findings. One important caveat, however, is that these *in vitro* and animal model studies tested isolated flavonoids, which may not accurately represent the action of the compound in the context of the food matrix in a mixed diet. Epidemiologic studies on the association of flavonoid intake with colorectal cancer risk are important, yet to date such studies have been limited [41].

For colorectal cancer and flavonoid intake, data from cohort and case-control studies are inconsistent, with positive, inverse, and null associations. It is important to note that, all studies in nutritional epidemiology are limited by the fact that bioactive compounds in foods are highly correlated. The influence of any one nutrient or compound is not completely independent of other nutrients [65]. The possibility cannot be ruled out that the protective associations observed for flavonoids are simply either markers of unmeasured constituents of plants or markers of a generally healthy lifestyle [30].

Moreover, the variability in findings across the studies reviewed may be due to differences in study design and analysis [22]. For example, food-frequency questionnaires were used in some studies [38-40,46], whereas diet histories were used in others [48,49]. Estimates of dietary intake using different assessment methods are not necessarily comparable. In addition, some of the studies were small, with less than 200 cases, and thus had very limited power to detect an association [26,46,48,49]. The studies also used different nutrient databases to obtain flavonoid values, which could contribute to the inconsistencies in results. Two recent case-control studies, both based on larger, updated, and tested flavonoid databases [66,67], suggested an inverse association between high flavonoid consumption, in particular flavonols, and colorectal cancer risk in humans [39-41]. Prospective cohort studies generally observed no association between flavonoid consumption and colorectal cancer risk [38,46-49]; however, only a small subset of flavonoids was evaluated, the intake ranges were limited, and the databases only contained a partial list of flavonoids from the major foods. It is also important to note that databases are not able to capture all of the variability in plant flavonoid content that may be attributable to factors such as sunlight and heat.

In addition, the intake of flavonoids may vary between different seasons, being highest in summer and autumn, when vegetables are consumed in high amounts. Seasonal variation may have caused some misclassification of subjects and therefore underestimation in the relation between flavonoid intake and the risk of cancer [26].

Another potential limitation is that, whereas ATBC [46] and the Iowa Women's Health study [50] assessed total flavonoids or one class of flavonoids (catechin), the other cohorts and most of the case-control studies assessed exposure to very specific flavonoids [39-41]. These differences in the exposure may have contributed to inconsistencies in study findings.

Finally, studies did not uniformly adjust relative risks or odds ratios for potential confounding factors. As with

all observational investigations, residual confounding can still occur despite statistical adjustments, which can bias estimates of risk [22]. For these reasons, caution should be exercised in the interpretation of the results of the observational studies reviewed.

Case-control studies may suffer for several drawbacks that make them less suitable for studying the effects of diet on the risk of disease. Recall bias, with misclassification of subjects because case subjects remember their diet differently, compared with control subjects, if this is assessed with questionnaires after diagnosis of the disease, is one hazard. Because significant associations were reported mainly for the case-control studies, these biases might have influenced the flavonoid data reported to date. Therefore, more prospective studies should be conducted on the flavonoid-cancer association before any conclusions are drawn. Strengths of these studies, for example, include the prospective design and high completeness of follow up of cancer incidence, which minimize the probability of recall bias and selection bias to occur. In addition, the large number of colorectal cancer cases provided sufficient power to detect associations. The assessment of many potential risk factors at baseline furthermore enabled investigators to adjust for risk factors for colorectal cancer that could potentially confound the studied associations. Still, despite the fact that investigators adjusted for a number of potential confounders, the possibility that residual confounding may have affected their results has to be discussed.

Further investigation of the effect flavonoid from different fruit and vegetables and their processed products is required to determine whether the observed association is due to flavonoid *per se* or to other as yet unidentified components of fruit and vegetables which are co-associated with flavonoids [68,69]. Intervention studies comparing the effects of individual flavonoids with flavonoid-rich diets may be required to elucidate whether the main protective effects are actually due to these phytochemicals. Future studies in human populations may benefit most from cohort designs, which can assess diet over a prolonged period of time and capture dietary exposures that influence early carcinogenic events.

Conclusion

Dietary change, both feasible and safe, represents a viable strategy for preventing colorectal cancer. Because only a very limited number of epidemiological studies have been conducted to examine the associations of dietary intake of flavonoids with colorectal cancer risk, it is premature to make public health recommendations at this time. However, the data to date are promising and emphasize the need for further investigation of these important bioactive plant compounds. These kinds of data are important for formulating focused published health recommendations, both for the general population and for those who are at increased risk of disease due to family history or lifestyle behaviors.

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