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Dietary intake of fiber, whole grains and risk of colorectal cancer: An updated analysis according to food sources, tumor location and molecular subtypes in two large US cohorts

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Epidemiologic evidence relating fiber intake to colorectal cancer (CRC) remains inconclusive and data are limited on different food sources of fiber and heterogeneity by tumor subsite and molecular profile. We prospectively followed for CRC incidence 90,869 women from the Nurses' Health Study (1980–2012) and 47,924 men from the Health Professionals Follow-up Study (1986–2012), who completed a validated food frequency questionnaire every 4 years. Cox proportional hazards regression was used to examine the associations with CRC risk for total, cereal, fruit and vegetable fiber and whole grains. We also assessed the associations according to tumor subsites (proximal colon, distal colon and rectum) and molecular markers (microsatellite

Key words: fiber, whole grains, colorectal cancer, molecular epidemiology

Abbreviations: AICR: American Institute for Cancer Research; BMI: body mass index; CI: confidence interval; CIMP: CpG island methylator phenotype; CRC: colorectal cancer; EPIC: European Prospective Investigation into Cancer and Nutrition; FFPE: formalin-fixed paraffin-embedded; FFQ: food frequency questionnaire; GI: glycemic index; GL: glycemic load; HPFS: Health Professionals Follow-up Study; HR: hazard ratio; IPW: inverse probability weighting; MET: metabolic equivalent of task; MSI: microsatellite instability; NHS: Nurses' Health Study
Additional Supporting Information may be found in the online version of this article.

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instability, *BRAF* mutation, CpG island methylator phenotype and *KRAS* mutation). We documented 3,178 CRC cases during 3,685,903 person-years of follow-up in the NHS and HPFS. Intake of total dietary fiber was not associated with CRC risk after multivariable adjustment in either women (hazard ratio [HR] comparing extreme deciles, 1.17; 95% CI, 0.92–1.48, p_{trend} = 0.55) or men (HR, 0.90; 95% CI, 0.67–1.21, p_{trend} = 0.47). Higher intake of cereal fiber and whole grains was associated with lower CRC risk in men with an HR of 0.75 (95% CI, 0.57–1.00) and 0.72 (95% CI, 0.54–0.96), respectively. No heterogeneity was detected by tumor subsite or molecular markers ($p_{heterogeneity}$ › 0.05). Higher intake of total dietary fiber within the range of a typical American diet is unlikely to substantially reduce CRC risk. The potential benefit of cereal fiber and whole grains in men warrants further confirmation.

What's new?

Epidemiologic evidence relating fiber intake to colorectal cancer (CRC) remains inconclusive and data are still limited on different food sources and heterogeneity by tumor subsite and molecular profile. Here, total dietary fiber intake within the range of a typical American diet was not found to be associated with CRC risk after adjusting for other dietary and lifestyle factors. Higher intake of cereal fiber and whole grains was associated with lower CRC risk in men. Associations of dietary fiber and CRC did not vary by tumor subsites and molecular markers (microsatellite instability, BRAF mutation, CpG island methylator phenotype, and KRAS mutation).

Introduction

The long-standing hypothesis that higher intake of dietary fiber reduces the risk of colorectal cancer (CRC) originates from the observation of low incidence of CRC among rural Africans who consume a diet with high fiber content.[1] Although studies in rodent animal models provide support for the hypothesis, epidemiologic data on the relationship between dietary fiber intake and CRC risk have been inconclusive, with conflicting results reported by numerous prospective studies.[2–4] A pooled analysis of 13 largely US-based prospective cohorts conducted in 2005 showed that intake of dietary fiber was associated with lower risk of CRC in the age-adjusted model, but the inverse association was attenuated to null after adjusting for other risk factors for CRC, particularly those dietary factors that are correlated with fiber and CRC, such as folate, red meat and alcohol intake.[5] The recent expert report by the World Cancer Research Fund/American Institute for Cancer Research (AICR) concludes that there is probable evidence supporting a protective effect of fiber on CRC while noting the substantial heterogeneity between studies and no significant association in the dose–response meta-analysis of 21 prospective studies.[6]

Beyond total fiber, data remain relatively limited for different food sources of fiber in relation to CRC risk. Some studies have reported that fiber consumed from cereals is more strongly associated with lower risk of colorectal neoplasia than that from fruits and vegetables.[3,4,7–9] Whole grains are an important source of dietary fiber, and in contrary to refined grains, whole grains are rich in a variety of micronutrients and bioactive substances, including vitamins, minerals, antioxidants, phytoestrogens and other phytochemicals.[10] So far, only eight prospective studies have examined the association of whole grains with risk of CRC and three studies reported a beneficial association.[4,11–17]

Moreover, increasing data suggest that CRC is a heterogeneous disease, with a potentially different etiology for tumors arising from different subsites. For example, obesity and metabolic factors have been more strongly associated with increased risk of distal colon cancer than proximal colon or rectal cancer, whereas smoking has been predominantly related to higher risk of proximal colon cancer.[18,19] For fiber, some,[4,5] but not all[2,9] studies found that the inverse association was stronger for rectal cancer than colon cancer. Moreover, limited evidence suggests that fiber intake is not differentially associated with CRC risk by molecular markers (e.g., *KRAS* and *BRAF* mutations).[20–22] However, these studies are limited by insufficient control for confounding and the retrospective case–control study design.[20–22]

Therefore, to extend our knowledge, we performed a comprehensive assessment of intake of total dietary fiber, different sources of fiber and whole grains in relation to risk of overall CRC and different CRC subtypes according to subsite and molecular markers. We used data from two large ongoing prospective studies, the Nurses' Health Study (NHS) and Health Professionals Follow-up Study (HPFS), in which detailed dietary data have been repeatedly collected using validated food frequency questionnaire (FFQ) and CRC diagnosis documented with standardized pathological and molecular characterization.

Methods

Study population

The NHS enrolled 121,700 female nurses aged 30–55 at time of study entry in 1976. The HPFS included 51,529 male health professionals aged 40–75 at enrollment in 1986. Participants have returned questionnaires every 2 years with greater than 90% follow-up and provided information about lifestyle and dietary factors, medication use and diagnoses of CRC and other

diseases. At baseline, we excluded participants with implausibly high or low caloric intakes (i.e., <600 or >3,500 kcal/day for women; <800 or >4,200 kcal/day for men), a high number of blank items on their FFQs (>60 for the NHS; >70 for the HPFS), missing data on fiber intake, and a history of cancer (except for nonmelanoma skin cancer) prior to baseline (1980 for the NHS and 1986 for the HPFS). A total of 138,793 participants were included in the current study (90,869 from the NHS and 47,924 from the HPFS). The study was approved by the institutional review board at the Brigham and Women's Hospital and Harvard T.H. Chan School of Public Health.

Assessment of fiber and whole grains

We obtained dietary information through FFQs that were administered in 1980, 1984, 1986 and every 4 years thereafter in the NHS, and in 1986 and every 4 years thereafter in the HPFS. On each FFQ, participants were asked how often, on average, they consumed each food of a standard portion size during the previous 4 years. The content of fiber in foods was derived using the Association of Official Analytical Chemists method.[23] We calculated the daily intake for each nutrient by multiplying the reported frequency of consumption of each item by its nutrient content and then summing across all foods. Total daily nutrient intake including nutrient supplement was adjusted for total energy intake using the residual method. In addition to total fiber, we separately assessed fiber intake from major food sources, including cereals, vegetables and fruits. We also assessed whole grain consumption from all grain-containing foods (rice, bread, pasta and breakfast cereals) according to the dry weight of whole grain ingredients in each food, as previously described.[24] Because intake of whole grains was not assessed until 1984 in the NHS, we considered 1984 as the baseline of follow-up for the whole grain analysis in women. To capture long-term exposure, we calculated the cumulative average intake from all preceding questionnaires up to the current cycle.

The validity of FFQs for assessment of fiber intake has been previously documented in the two cohorts.[25,26] The Spearman correlation coefficients (adjusted for random within-person variation) of dietary fiber intake assessed by FFQ and two 1-week diet records were 0.63 in women, and 0.65 in men, respectively.[25,26]

Ascertainment of CRC cases

On each biennial follow-up questionnaire, participants were asked whether they had a diagnosis of CRC in the previous 2 years. For participants who reported a positive diagnosis of CRC, we asked for their written informed consent to acquire medical records and pathologic reports. Study physicians, blinded to exposure data, reviewed all records to confirm CRC diagnosis and extract data on histological type and anatomic location. Tumors in the cecum, ascending colon, hepatic flexure, transverse colon or splenic flexure were classified as proximal; tumors in the descending or sigmoid colon as distal, and those in the rectum or rectosigmoid junction as rectal. Participants who died from CRC and were not captured by our regular follow-up questionnaires were identified and confirmed through information from various sources, including next-of-kin, the National Death Index, and death certificates. The 9th version of the International Classification of Diseases was used (code 153 and 154).

Tumor molecular marker assessment

We collected formalin-fixed paraffin-embedded (FFPE) tissue blocks from the hospitals throughout the US where participants with CRC had undergone surgery.[27] Normal and tumor sections from all CRC cases were reviewed by a pathologist (S.O.) and used for DNA extraction. Details about molecular marker assessment have been described elsewhere. Briefly, we performed real-time polymerase chain reaction (PCR) and pyrosequencing targeted for *KRAS* codons 12, 13, 61 and 147.[28] Microsatellite instability (MSI) status was determined using 10 microsatellite markers (D17S250, D18S55, D18S56, D18S67, D18S487, D2S123, D5S346, BAT25, BAT26 and BAT40), and tumors were classified as MSI-high if 30% or more of the markers demonstrated instability.[29] We quantified DNA methylation using PCR in eight CpG island methylator phenotype (CIMP)-specific promoters [*MLH1, NEUROG1, RUNX3, CACNA1G, CDKN2A (p16), CRABP1, IGF2* and *SOCS1*], and classified tumors as CIMP-high if six or more promoters were methylated, and as CIMP-low/negative if 0–5 promoters were methylated.[29] Finally, we performed PCR and targeted pyrosequencing toward the *BRAF* codon 600 mutation.[29]

Covariate assessment

In the baseline and biennial follow-up questionnaires, we assessed a variety of CRC risk factors, including family history of CRC, history of lower gastrointestinal endoscopy, pack-years of smoking before age of 30, smoking status, body mass index (BMI), physical activity, regular use of aspirin and regular multivitamin use. Participants were defined as having a positive family history of CRC if at least one of their parents and siblings had been diagnosed with CRC. For physical activity, weekly energy expenditure was estimated by multiplying the typical intensity expressed in metabolic equivalent of task (MET; the ratio of metabolic rate during the activity to metabolic rate at rest) by the reported hours spent per week.[30] Consistent with our prior analyses, regular aspirin use was defined as use of at least two standard tablets (325 mg) of aspirin per week.[31] Dietary risk factors, including alcohol, processed red meat, folate, calcium and vitamin D, were assessed through FFQs as previously described.[32] To account for the amount of carbohydrate in a typical serving, we derived a global dietary glycemic load (GL) score by multiplying the amount of carbohydrates in the diet by the average glycemic index (GI).[33,34] The GI values for single food items on the FFQ were derived from available databases and publications.[35]

Statistical analysis

For each participant, we calculated follow-up time (in months) from the age at which the baseline questionnaire was returned until the age at the date of death, CRC diagnosis, loss to follow-

up or end of follow-up (June 1, 2012, for the NHS, January 31, 2012, for the HPFS), whichever came first. We computed hazard ratio (HR) and 95% confidence intervals (CI) of CRC in relation to deciles of fiber intake using Cox proportional hazards model with age as the time scale. We also stratified the model by calendar time to account for any period effect. Test for trend was performed using the median intake of each decile as a continuous variable. In multivariable analysis, we further adjusted for several risk factors for CRC, including family history of CRC (yes or no), history of lower gastrointestinal endoscopy (yes or no), pack-years of smoking before age of 30 (for women: no, <5 years, ≥5 years; for men: no, <10 years, ≥10 years), smoking status (never, past, current smokers with 1–14 pack-years, current smokers with 15–24 pack-years and current smokers with ≥25 pack-years), BMI (kg/m^2, continuous), physical activity (for women: <5, 5–11.4, 11.5–21.9, ≥22 MET-hr/week; for men: <10, 10–22.5, 22.5–41.5, ≥41.5 METs/week), alcohol intake (for women: 0.0–0.14, 0.15–1.9, 2.0–7.4, ≥7.5 g/day; for men: 0.0–0.9, 1.0–5.9, 6.0–14.9, ≥15 g/day), regular aspirin use (yes or no), regular multivitamin use (yes or no) and menopausal hormone use (only for women: premenopausal, menopausal hormone therapy use no/past/current). We also adjusted for other dietary factors, including intake of processed red meat, total folate, calcium and vitamin D and GL; median intake in each of the quartiles was used as a continuous variable in the model. We tested the between-cohort difference by using the random-effects meta-analysis approach for the HRs for continuous fiber intake, and such test can be interpreted as $p_{\text{heterogeneity}}$ by sex, because each of the two cohorts comprised women and men only.

To examine the potential lagged effect of fiber intake on CRC, we also performed a latency analysis using fiber intake assessed at varying time points prior to CRC diagnosis.[36] For example, in the HPFS, for a latency of 4–8 years before diagnosis, we used dietary fiber intake in 1986 for the person-years accumulated from 1990 through 1994, intake in 1990 for person-years from 1994 through 1998 and so forth. For these latency analyses, covariates were not lagged.

To examine whether the association between dietary fiber intake and CRC risk varies according to anatomic location and molecular subtypes, we used Cox proportional hazards regression models with a duplication method for competing risks data and tested for the heterogeneity using a one degree-of-freedom likelihood ratio test, comparing a model that allows for separate associations of fiber intake with CRC according to tumor subtypes with a model that assumes a common association.[37] Since only a fraction of CRC cases in the two cohorts had tissues available for molecular marker assessment, we first compared the basic characteristics of patients with and without tissue specimens, and then performed a sensitivity analysis using the inverse probability weighting (IPW) in the Cox regression to minimize any potential bias due to unavailability of tumor tissue (Supporting Information).[38]

We used SAS software version 9.4 (SAS Institute Inc., Cary, NC). All statistical tests were two-sided. A p value of <0.05 was considered statistically significant.

Results

We documented 1,902 CRC cases during 2,640,101 person-years of follow-up of women in the NHS and 1,276 cases during 1,045,802 person-years of follow-up of men in the HPFS. Among CRC cases with available data on anatomic location, we identified 1,321 proximal colon cancers, 921 distal colon cancers and 683 rectal cancers. Participants with higher fiber intake tended to have a healthier lifestyle (Table 1). For example, they were more likely to be physically active, use multivitamins and consume more folate, calcium and vitamin D; and were less likely to smoke, drank less alcohol and consumed less processed red meat.

We first examined the association between total fiber intake and overall incidence of CRC (Table 2). In the age-adjusted model, higher intake of total dietary fiber was associated with lower risk of CRC, with the HR comparing the highest to the lowest deciles of 0.90 (95% CI, 0.73–1.11) in women and 0.66 (95% CI, 0.51–0.85) in men. For total dietary fiber, HR for the trend was 0.93 (95% CI, 0.88–0.99; $p_{\text{trend}} = 0.01$) in women and 0.90 (95% CI, 0.86–0.95; $p_{\text{trend}} < 0.001$) in men. However, the associations were attenuated to null after adjusting for other dietary and lifestyle factors, with an HR of 1.02 (95% CI, 0.96–1.09) for women and 0.98 (95% CI, 0.92–1.04) for men ($p_{\text{trend}} > 0.05$). This attenuation seemed to be largely driven by a few confounding factors, including history of endoscopy, physical activity and consumption of alcohol, calcium and processed red meat (Supporting Information Fig. S1).

In the latency analysis, we did not find any association between total fiber intake and CRC risk after multivariable adjustment regardless of the length of the latency period (Supporting Information Table S1).

We then examined the association of fiber from different food sources and whole grains with risk of CRC (Table 2). Higher intake of cereal fiber appeared to be associated with lower risk of CRC in men even after multivariable adjustment (HR comparing extreme deciles, 0.75, 95% CI, 0.57–1.00; HR for the trend, 0.86, 95% CI, 0.76–0.98, $p_{\text{trend}} = 0.02$), whereas no association was found in women (HR comparing extreme deciles, 1.15; 95% CI, 0.92–1.43; HR for the trend, 1.07, 95% CI, 0.91–1.25, $p_{\text{trend}} = 0.43$). Similar results were found for whole grains (HR comparing extreme deciles in men, 0.72, 95% CI, 0.54–0.96; HR for the trend in men, 0.90, 95% CI, 0.82–0.98, $p_{\text{trend}} = 0.01$; HR comparing extreme deciles in women, 1.08, 95% CI, 0.85–1.38; HR for the trend in women, 1.02, 95% CI, 0.91–1.14, $p_{\text{trend}} = 0.73$). On the other hand, no statistically significant association was observed for fruit or vegetable fiber in either men or women.

We then examined the associations of dietary fiber and whole grains with CRC risk by anatomic subsite (Table 3). No statistically significant heterogeneity was observed in either

Table 1. Basic characteristics of study participants according to deciles of total fiber intake in women (NHS, 1980–2012) and men (HPFS, 1986–2012)

	Women			Men		
	Decile 1	Decile 5	Decile 10	Decile 1	Decile 5	Decile 10
Age, years	58.1 (11.1)	59.9 (11.2)	63.1 (11.2)	61.2 (10.5)	63.3 (11.1)	66.4 (11.3)
Body mass index, kg/m^2	24.8 (4.7)	25.1 (4.4)	24.5 (4.1)	25.8 (4.1)	25.7 (3.8)	24.7 (4.1)
Physical activity, MET-hr/week[1]	10.9 (14.3)	15.3 (16.4)	22.8 (24.2)	19.0 (20.7)	25.1 (21.6)	33.6 (29.0)
Pack-years of smoking before age of 30	7.7 (5.3)	6.8 (5.3)	6.9 (5.8)	12.0 (6.7)	10.8 (6.5)	10.6 (6.9)
Current smoking, %	32	15	9	12	4	1
Family history of colorectal cancer, %	14	17	17	12	14	15
History of lower gastrointestinal endoscopy, %	13	18	18	19	25	25
Regular multivitamin use, %	39	49	52	37	47	53
Regular aspirin use, %[2]	33	43	37	43	49	43
Postmenopausal, %	70	74	75			
Current postmenopausal hormone use, %[3]	45	46	48	-	-	-
Dietary intake						
Total fiber, g/day	9.39 (2.13)	15.3 (1.50)	25.8 (3.94)	12.9 (1.91)	20.5 (0.90)	35.3 (5.73)
Cereal fiber, g/day	2.51 (1.14)	3.85 (1.49)	5.52 (3.05)	3.72 (1.41)	6.12 (2.06)	10.6 (6.22)
Fruit fiber, g/day	1.71 (1.03)	3.69 (1.41)	7.65 (3.41)	1.89 (1.12)	4.22 (1.75)	8.90 (4.18)
Vegetable fiber, g/day	3.41 (1.15)	5.41 (1.42)	9.15 (3.53)	4.18 (1.43)	6.68 (1.89)	11.9 (4.72)
Whole grain, g/day	8.10 (6.76)	15.8 (9.36)	29.1 (16.9)	10.5 (7.82)	24.0 (12.6)	47.7 (25.4)
Processed and red meat, serving/week	8.35 (4.40)	6.87 (3.32)	4.18 (3.00)	8.93 (5.45)	6.83 (4.03)	2.86 (2.79)
Total folate, μg/day	315 (205)	412 (190)	554 (248)	406 (224)	524 (224)	713 (290)
Calcium, mg/day	782 (353)	920 (333)	1,059 (379)	852 (409)	914 (340)	1,056 (429)
Vitamin D, IU/day	308 (230)	347 (208)	420 (268)	372 (252)	421 (247)	522 (316)
Alcohol, g/day	10.3 (14.6)	5.95 (8.58)	3.61 (6.05)	18.7 (21.3)	10.9 (12.7)	5.87 (8.22)
Glycemic load	88.5 (25.7)	95.9 (17.3)	107 (18.4)	111 (26.5)	127 (18.9)	152 (22.6)

Cumulative average values are presented. Mean (SD) is presented for continuous variables and percentage for categorical variables. All variables are adjusted for age except for age itself.
[1]Physical activity is represented by the product sum of the METS of each specific recreational activity and hours spent on that activity per week.
[2]A standard tablet contains 325 mg aspirin and regular users were defined as those who used at least two standard tablets per week.
[3]The percentage of current hormone use was only calculated among postmenopausal women only.
Abbreviations: NHS, the Nurses' Health Study; HPFS, the Health Professionals Follow-up Study; MET, metabolic equivalent task.

women or men, although cereal fiber showed a suggestively inverse association with rectal cancer (HR per 5-g/day increment: 0.77 [95% CI, 0.66–0.99] in men and 0.84 [95% CI, 0.62–1.15] in women) but not with proximal or distal colon cancer.

Finally, we examined whether the association of fiber with CRC differed by tumor molecular subtypes. In the two cohorts (NHS/HPFS), we documented 1,337 cases with data for *KRAS* mutation, 1,316 for MSI, 1,281 for CIMP and 1,333 for *BRAF* mutation. The basic characteristics of these patients did not show any substantial difference from those with missing tumor marker data (Supporting Information Table S2). As shown in Table 4, we did not find any statistically significant evidence of heterogeneity according to the studied molecular subtypes for total fiber, fiber from different sources and whole grains, except for *KRAS*-mutant CRC with whole grains in women ($p_{heterogeneity}$ = 0.03) (Table 4). The IPW-adjusted Cox regression analyses yielded similar results (Supporting Information Table S3).

Discussion

The results from these two large prospective cohort studies did not support the hypothesis that higher intake of total dietary fiber within the range of a typical American diet decreases CRC risk. When fiber from different food sources was examined, higher intake of cereal fiber and whole grains was associated with a lower risk for CRC in men. No heterogeneity was observed according to tumor sublocation or molecular markers.

The present study confirmed the results of an earlier pooled analysis of 13 prospective cohort studies that fiber had an inverse association with risk of CRC in the age-adjusted model, but the association was attenuated to null after adjusting for other CRC risk factors.[5] Similar findings have also been reported in our earlier analysis of the NHS/HPFS cohorts based on a limited number of CRC cases and relatively short duration of follow-up, as well as other US studies, including the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial[3] and the Women's Health Study.[39] In contrast,

Table 2. Association between intake of total fiber, fiber from different food sources and whole grains[2] and colorectal cancer risk in women (NHS, 1980–2012) and men (HPFS, 1986–2012)

	Decile 1	Decile 2	Decile 3	Decile 4	Decile 5	Decile 6	Decile 7	Decile 8	Decile 9	Decile 10	HR (95% CI) per 5 g/day [1]	p_{trend}	$p_{heterogeneity}$ by sex
Total fiber													
Women													
Median, g/day	9.56	12.1	13.5	14.6	15.6	16.6	17.8	19.1	21.0	24.8			
No. of cases	157	184	191	185	182	191	196	214	193	209			
Age-adjusted HR (95% CI)	1 (ref)	1.12 (0.90–1.38)	1.13 (0.92–1.40)	1.04 (0.84–1.29)	0.99 (0.80–1.23)	1.00 (0.81–1.24)	0.97 (0.79–1.20)	1.02 (0.83–1.25)	0.88 (0.71–1.09)	0.90 (0.73–1.11)	0.93 (0.88–0.99)	0.01	0.37
Multivariable HR (95% CI)	1 (ref)	1.17 (0.95–1.45)	1.23 (0.99–1.52)	1.15 (0.93–1.43)	1.13 (0.90–1.40)	1.16 (0.93–1.45)	1.15 (0.92–1.44)	1.23 (0.98–1.53)	1.10 (0.87–1.38)	1.17 (0.92–1.48)	1.02 (0.95–1.09)	0.56	0.35
Men													
Median, g/day	13.1	15.9	17.6	19.1	20.5	22.0	23.6	25.5	28.2	33.7			
No. of cases	126	128	151	109	110	128	123	139	138	124			
Age-adjusted HR (95% CI)	1 (ref)	0.96 (0.75–1.23)	1.11 (0.88–1.41)	0.75 (0.57–0.96)	0.72 (0.56–0.93)	0.80 (0.63–1.03)	0.74 (0.58–0.95)	0.80 (0.62–1.02)	0.77 (0.60–0.98)	0.66 (0.51–0.85)	0.90 (0.86–0.95)	<0.001	
Multivariable HR (95% CI)	1 (ref)	1.00 (0.78–1.29)	1.23 (0.96–1.57)	0.84 (0.65–1.10)	0.83 (0.63–1.08)	0.96 (0.74–1.24)	0.91 (0.70–1.18)	1.02 (0.78–1.32)	1.01 (0.77–1.32)	0.91 (0.68–1.21)	0.98 (0.92–1.04)	0.47	
Cereal fiber													
Women													
Median, g/day	1.60	2.40	2.89	3.26	3.65	3.98	4.44	4.93	5.75	7.43			
No. of cases	172	205	195	184	176	176	198	197	200	199			
Age-adjusted HR (95% CI)	1 (ref)	1.15 (0.94–1.41)	1.10 (0.90–1.35)	1.02 (0.83–1.26)	0.96 (0.78–1.19)	0.94 (0.76–1.16)	1.03 (0.84–1.27)	0.99 (0.81–1.22)	0.96 (0.78–1.18)	0.89 (0.73–1.10)	0.86 (0.75–0.99)	0.03	0.12
Multivariable HR (95% CI)	1 (ref)	1.20 (0.98–1.48)	1.18 (0.96–1.45)	1.11 (0.90–1.37)	1.07 (0.86–1.33)	1.06 (0.86–1.32)	1.19 (0.96–1.48)	1.17 (0.94–1.45)	1.18 (0.95–1.46)	1.15 (0.92–1.43)	1.07 (0.91–1.25)	0.43	0.04
Men													
Median, g/day	2.58	3.71	4.51	5.17	5.79	6.46	7.20	8.07	9.34	12.0			
No. of cases	144	142	137	122	140	126	119	132	115	99			
Age-adjusted HR (95% CI)	1 (ref)	0.96 (0.76–1.21)	0.96 (0.76–1.22)	0.87 (0.68–1.11)	0.93 (0.74–1.18)	0.82 (0.64–1.04)	0.75 (0.59–0.96)	0.80 (0.63–1.02)	0.69 (0.54–0.89)	0.58 (0.45–0.75)	0.75 (0.67–0.83)	<0.001	
Multivariable HR (95% CI)	1 (ref)	1.00 (0.79–1.27)	1.03 (0.81–1.31)	0.96 (0.75–1.23)	1.06 (0.83–1.35)	0.95 (0.74–1.22)	0.89 (0.69–1.15)	0.98 (0.76–1.27)	0.87 (0.67–1.13)	0.75 (0.57–1.00)	0.86 (0.76–0.98)	0.02	
Fruit fiber													
Women													
Median, g/day	1.03	1.85	2.44	2.96	3.49	4.01	4.60	5.33	6.32	8.50			
No. of cases	186	170	175	187	172	209	205	194	212	192			
Age-adjusted HR (95% CI)	1 (ref)	0.90 (0.73–1.11)	0.88 (0.72–1.08)	0.89 (0.72–1.09)	0.79 (0.64–0.97)	0.93 (0.76–1.14)	0.88 (0.72–1.07)	0.79 (0.65–0.97)	0.81 (0.66–0.99)	0.70 (0.57–0.86)	0.83 (0.74–0.92)	0.001	0.96
Multivariable HR (95% CI)	1 (ref)	0.92 (0.75–1.14)	0.94 (0.76–1.15)	0.96 (0.78–1.18)	0.87 (0.70–1.08)	1.03 (0.84–1.26)	0.98 (0.80–1.21)	0.90 (0.73–1.12)	0.93 (0.75–1.15)	0.80 (0.64–1.00)	0.90 (0.80–1.02)	0.09	0.44
Men													
Median, g/day	1.10	1.97	2.67	3.31	3.93	4.58	5.28	6.15	7.43	10.2			
No. of cases	110	110	149	117	111	121	147	141	138	132			
Age-adjusted HR (95% CI)	1 (ref)	0.91 (0.70–1.19)	1.16 (0.90–1.49)	0.89 (0.68–1.15)	0.77 (0.59–1.00)	0.81 (0.62–1.05)	0.93 (0.72–1.19)	0.85 (0.66–1.10)	0.77 (0.59–0.99)	0.72 (0.56–0.94)	0.83 (0.74–0.93)	0.001	
Multivariable HR (95% CI)	1 (ref)	0.97 (0.74–1.27)	1.25 (0.97–1.61)	1.00 (0.76–1.30)	0.87 (0.66–1.15)	0.96 (0.73–1.26)	1.13 (0.87–1.47)	1.06 (0.81–1.39)	0.98 (0.74–1.29)	0.97 (0.73–1.28)	0.97 (0.85–1.10)	0.61	
Vegetable fiber													
Women													
Median, g/day	2.79	3.77	4.37	4.87	5.33	5.80	6.34	6.98	7.90	9.87			
No. of cases	172	166	191	192	177	188	196	217	203	200			
Age-adjusted HR (95% CI)	1 (ref)	0.93 (0.75–1.15)	1.06 (0.86–1.30)	1.05 (0.85–1.29)	0.97 (0.79–1.20)	1.00 (0.81–1.23)	1.02 (0.83–1.25)	1.12 (0.91–1.37)	1.02 (0.83–1.25)	0.99 (0.81–1.21)	1.02 (0.91–1.14)	0.73	0.32
Multivariable HR (95% CI)	1 (ref)	0.96 (0.77–1.19)	1.11 (0.90–1.37)	1.11 (0.90–1.37)	1.04 (0.84–1.28)	1.09 (0.88–1.34)	1.11 (0.90–1.37)	1.24 (1.01–1.52)	1.15 (0.93–1.41)	1.14 (0.92–1.42)	1.12 (0.99–1.27)	0.06	0.21
Men													
Median, g/day	3.30	4.40	5.13	5.80	6.40	7.05	7.80	8.77	10.2	13.1			
No. of cases	126	133	123	113	135	138	125	119	134	130			
Age-adjusted HR (95% CI)	1 (ref)	1.02 (0.80–1.31)	0.96 (0.75–1.24)	0.87 (0.67–1.12)	1.05 (0.82–1.34)	1.03 (0.81–1.31)	0.93 (0.73–1.20)	0.84 (0.65–1.08)	0.96 (0.75–1.22)	0.91 (0.71–1.16)	0.95 (0.85–1.05)	0.27	
Multivariable HR (95% CI)	1 (ref)	1.06 (0.83–1.36)	1.01 (0.79–1.30)	0.92 (0.71–1.20)	1.11 (0.87–1.42)	1.11 (0.87–1.42)	1.01 (0.79–1.30)	0.92 (0.71–1.19)	1.07 (0.83–1.38)	1.05 (0.81–1.37)	1.01 (0.91–1.13)	0.80	

(Continues)

Table 2. Association between intake of total fiber, fiber from different food sources and whole grains[2] and colorectal cancer risk in women (NHS, 1980–2012) and men (HPFS, 1986–2012) (Continued)

	Decile 1	Decile 2	Decile 3	Decile 4	Decile 5	Decile 6	Decile 7	Decile 8	Decile 9	Decile 10	HR (95% CI) per 5 g/day [1]	P_{trend}	$P_{heterogeneity}$ by sex
Whole grains													
Women													
Median, g/day	3.18	6.54	9.00	11.3	13.7	16.2	19.0	22.6	28.2	39.1			
No. of cases	151	143	142	159	143	131	139	131	148	160			
Age-adjusted HR (95% CI)	1 (ref)	0.93 (0.74–1.17)	0.90 (0.72–1.13)	1.01 (0.80–1.26)	0.89 (0.70–1.11)	0.79 (0.62–0.99)	0.79 (0.63–1.00)	0.74 (0.58–0.93)	0.78 (0.62–0.98)	0.79 (0.63–0.99)	0.86 (0.78–0.95)	0.003	0.41
Multivariable HR (95% CI)	1 (ref)	0.99 (0.78–1.24)	0.99 (0.79–1.25)	1.14 (0.91–1.42)	1.02 (0.81–1.29)	0.93 (0.73–1.18)	0.98 (0.77–1.24)	0.93 (0.73–1.19)	1.01 (0.80–1.29)	1.08 (0.84–1.38)	1.02 (0.91–1.14)	0.74	0.08
Men													
Median, g/day	4.00	9.70	13.8	17.6	21.4	25.5	29.8	35.3	43.1	58.3			
No. of cases	144	150	127	119	151	128	116	121	126	94			
Age-adjusted HR (95% CI)	1 (ref)	1.08 (0.85–1.36)	0.90 (0.71–1.14)	0.84 (0.66–1.08)	1.01 (0.80–1.28)	0.85 (0.67–1.09)	0.74 (0.58–0.95)	0.76 (0.60–0.97)	0.77 (0.61–0.98)	0.56 (0.43–0.73)	0.81 (0.76–0.88)	<0.001	
Multivariable HR (95% CI)	1 (ref)	1.12 (0.88–1.41)	0.94 (0.73–1.19)	0.92 (0.72–1.18)	1.13 (0.89–1.43)	0.97 (0.76–1.25)	0.86 (0.67–1.12)	0.91 (0.71–1.18)	0.96 (0.74–1.24)	0.73 (0.55–0.96)	0.90 (0.83–0.98)	0.01	

Cox proportional hazards model was used with adjustment for age, family history of colorectal cancer (yes or no), history of lower gastrointestinal endoscopy (yes or no), pack-years of smoking before age of 30 (for women: no, <5 years, ≥5 years; for men: no, <10 years, ≥10 years), smoking status (never, past, current smokers with 1–14 pack-years, current smokers with 15–24 pack-years, current smokers with ≥25 pack-years), body mass index (kg/m^2, continuous), physical activity (for women: <5, 5–11.4, 11.5–21.9, ≥22 METS/week; for men: <10, 10–22.5, 22.5–41.5, ≥41.5 METS/week), alcohol intake (for women: 0.0–0.14, 0.15–1.9, 2.0–7.4, ≥7.5 g/day; for men: 0.0–0.9, 1.0–5.9, 6.0–14.9, ≥15 g/day), regular aspirin use (yes or no), regular multivitamin use (yes or no), total folate intake (median in quartile), calcium intake (median in quartile), vitamin D intake (median in quartile), glycemic load (median in quartile) and processed red meat intake (median in quartile), hormone use (only for women: premenopausal, postmenopausal hormone use no/past/current).

[1]For whole grains, the HR is per 20 g/day increment.

[2]For whole grain analysis, the follow-up started in 1984 in women.

Abbreviations: NHS, the Nurses' Health Study; HPFS, the Health Professionals Follow-up Study; HR, hazard ratio; CI, confidence interval.

the European Prospective Investigation into Cancer and Nutrition (EPIC) study observed a statistically significant 40% lower risk of CRC when comparing participants who consumed the highest amount to the lowest amount, confirmed by several follow-up analyses in the cohort.[2,40,41] Although the exact reasons for the discrepant findings between the US studies and EPIC cohort remain unclear, several explanations can be speculated. First, the amount of total fiber consumption was higher in the EPIC cohort than in our cohorts, with the baseline mean intake of 19.8 and 14.0 g/day in women and 23.7 and 20.0 g/day in men, respectively. It is possible that the anti-CRC effect of fiber may not be detected until certain threshold of intake is achieved. This is supported by our data that cereal fiber and whole grains were associated with lower risk of CRC in men but not in women since the amount of intake was much higher in men than in women. Moreover, these associations did not achieve statistical significance until the intake reached the top decile for cereal fiber and whole grains (Table 2). Second, different types of fiber consumed in the US and European countries may have contributed to the discrepant findings. While cereals are the main source of fiber intake in the EPIC,[2] they only account for one-quarter of total fiber intake in our cohorts. Moreover, there is substantial variation in the specific types of whole grain products consumed in different countries. Within the EPIC cohort, Scandinavians tend to consume rye bread, rather than wheat bread, as a staple food.[42,43] Accordingly, a stronger inverse association between fiber intake and CRC risk has been reported in the Scandinavian than other European countries.[40] Mechanistically, compared to wheat, rye has been suggested to have stronger metabolic benefits, such as lowering body weight and fat mass,[44,45] decreasing plasma leptin[45] and improving insulin sensitivity.[45] Therefore, further studies are needed to examine the dose–response relationship and influence of different food sources on the fiber–CRC relationship, preferably by pooling studies from different regions that provide a wider range of intake for a variety of fiber sources.

Despite the overall null association, we found that higher intake of cereal fiber and whole grains were associated with lower risk of CRC in men. This agrees with findings of other studies that dietary fiber was more strongly associated with lower risk of colorectal neoplasia in men than in women.[9,13,46] As discussed above, a potential explanation for the sex difference may be related to the higher absolute intake of fiber in men than in women. In addition, several metabolic factors, including type 2 diabetes and insulin resistance, have been more strongly associated with higher risk of CRC in men than in women,[47,48] in part because of the potential anti-CRC effect of estrogen. Also, these metabolic conditions such as insulin resistance appear more prevalent in men than in women,[47,49] possibly due to greater amounts of visceral and hepatic adipose tissue. Since one of the major mechanisms through which fiber may protect against CRC is improvement in insulin sensitivity and metabolic regulation,[50,51] it is possible that

Table 3. Multivariable-adjusted hazard ratio of colorectal cancer by subsite according to intake of total fiber intake and fiber from different food sources and whole grains in women (NHS, 1980–2012) and men (HPFS, 1986–2012)

	Total fiber (per 5 g/day)	Cereal fiber (per 5 g/day)	Fruit fiber (per 5 g/day)	Vegetable fiber (per 5 g/day)	Whole grain (per 20 g/day)[1]
Women					
Proximal colon cancer (n = 887)					
MV-adjusted HR (95% CI)	1.02 (0.93–1.11)	1.20 (0.97–1.49)	0.89 (0.75–1.05)	1.05 (0.88–1.24)	1.09 (0.94–1.27)
p_{trend}	0.68	0.09	0.16	0.62	0.26
Distal colon cancer (n = 551)					
MV-adjusted HR (95% CI)	1.04 (0.94–1.16)	1.01 (0.77–1.32)	0.94 (0.76–1.15)	1.12 (0.90–1.39)	1.04 (0.85–1.26)
p_{trend}	0.46	0.96	0.55	0.32	0.72
Rectal cancer (n = 420)					
MV-adjusted HR (95% CI)	0.97 (0.86–1.09)	0.84 (0.62–1.15)	0.90 (0.71–1.13)	1.23 (0.96–1.57)	0.84 (0.66–1.06)
p_{trend}	0.60	0.29	0.36	0.10	0.13
$p_{heterogeneity}$	0.64	0.14	0.91	0.56	0.14
Men					
Proximal colon cancer (n = 434)					
MV-adjusted HR (95% CI)	1.01 (0.92–1.11)	0.91 (0.75–1.11)	1.03 (0.84–1.25)	1.09 (0.92–1.30)	0.92 (0.81–1.05)
p_{trend}	0.84	0.36	0.78	0.33	0.23
Distal colon cancer (n = 370)					
MV-adjusted HR (95% CI)	0.96 (0.86–1.06)	0.86 (0.70–1.07)	0.88 (0.71–1.09)	0.95 (0.78–1.15)	0.87 (0.76–1.01)
p_{trend}	0.38	0.17	0.25	0.59	0.06
Rectal cancer (n = 263)					
MV-adjusted HR (95% CI)	0.98 (0.87–1.09)	0.77 (0.60–0.99)	1.00 (0.77–1.28)	1.00 (0.80–1.25)	0.88 (0.74–1.04)
p_{trend}	0.66	0.04	0.98	0.97	0.14
$p_{heterogeneity}$	0.68	0.54	0.52	0.55	0.82

Cox proportional hazards model was used with adjustment for age, family history of colorectal cancer (yes or no), history of lower gastrointestinal endoscopy (yes or no), pack-years of smoking before age of 30 (for women: no, ‹5 years, ≥5 years; for men: no, ‹10 years, ≥10 years), smoking status (never, past, current smokers with 1–14 pack-years, current smokers with 15–24 pack-years, current smokers with ≥25 pack-years), body mass index (kg/m^2, continuous), physical activity (for women: ‹5, 5–11.4, 11.5–21.9, ≥22 METS/week; for men: ‹10, 10–22.5, 22.5–41.5, ≥41.5 METS/week), alcohol intake (for women: 0.0–0.14, 0.15–1.9, 2.0–7.4, ≥7.5 g/day; for men: 0.0–0.9, 1.0–5.9, 6.0–14.9, ≥15 g/day), regular aspirin use (yes or no), regular multivitamin use (yes or no), total folate intake (median in quartile), calcium intake (median in quartile), vitamin D intake (median in quartile), glycemic load (median in quartile) and processed red meat intake (median in quartile), hormone use (only for women: premenopausal, postmenopausal hormone use no/past/current).

[1]For whole grain analysis, the follow-up started in 1984 in women and the case numbers of proximal colon cancer, distal colon cancer and rectal cancer are 720, 401 and 314, respectively.

Abbreviations: CRC, colorectal cancer; NHS, the Nurses' Health Study; HPFS, the Health Professionals Follow-up Study; HR, hazard ratio; CI, confidence interval.

the beneficial effect of cereal fiber and whole grains on CRC, if there is any, may be stronger in men than in women.

Interestingly, we found that cereal fiber showed a suggestive association with a higher risk of proximal colon cancer in women. While this may be a chance finding due to the multiple testing conducted in the study, it is consistent with several other studies showing that the inverse association of dietary fiber intake with risk of CRC was stronger for rectal cancer and distal colon cancer than proximal colon cancer.[3,52,53] Also, in line with our results, Kunzmann *et al.* found a positive association of dietary fiber with risk of proximal colon cancer.[3] Substantial evidence supports the heterogeneity of CRC arising from different subsites. While some of the heterogeneity may reflect the differences in molecular alterations (e.g., proximal colon cancers are more likely to show MSI, CIMP and BRAF mutation than distal cancers)[54], our observation for a consistently null association between fiber and CRC according to these molecular markers suggests that the subsite findings are unlikely to be explained by these molecular differences across subsites. On the other hand, increasing evidence supports the role of the interplay between fiber and the gut microbiota in CRC development.[55] We recently reported that higher intake of dietary fiber was more strongly associated with lower risk for *Fusobacterium nucleatum*-positive CRC but not *Fusobacterium nucleatum*-negative CRC.[56] Moreover, fiber undergoes gut bacterial fermentation, mostly in the proximal colon, to produce short-chain fatty acids, which have been suggested to protect against CRC by epigenetic regulation and immune modulation. While this seems contradictory to our observed positive association of fiber with proximal colon cancer, given the complexity of the gut microbiota in relation to environmental exposures and the high consumption of processed foods enriched with purified fibers nowadays, it is possible that other components in fiber-rich processed foods may counteract or even reverse

Table 4. Multivariable-adjusted hazard ratio of colorectal cancer by MSI, *BRAF*, CIMP and *KRAS* status according to intake of total fiber intake and fiber from different food sources and whole grains in women (NHS, 1980–2012) and men (HPFS, 1986–2012)

	Total fiber (per 5 g/day)	Cereal fiber (per 5 g/day)	Fruit fiber (per 5 g/day)	Vegetable fiber (per 5 g/day)	Whole grain (per 20 g/day)[1]
Women					
MSS/MSI-low cancer (n = 602)					
MV-adjusted HR (95% CI)	1.05 (0.94–1.18)	1.00 (0.76–1.31)	0.92 (0.75–1.14)	1.23 (0.99–1.53)	0.93 (0.76–1.13)
p_{trend}	0.38	0.98	0.47	0.06	0.45
MSI-high cancer (n = 153)					
MV-adjusted HR (95% CI)	1.03 (0.85–1.26)	1.13 (0.69–1.86)	0.78 (0.53–1.16)	1.21 (0.80–1.81)	1.07 (0.76–1.50)
p_{trend}	0.74	0.62	0.22	0.37	0.71
$p_{heterogeneity}$	0.87	0.65	0.45	0.92	0.47
BRAF-wild-type cancer (n = 604)					
MV-adjusted HR (95% CI)	1.04 (0.92–1.16)	1.00 (0.76–1.31)	0.89 (0.72–1.11)	1.20 (0.96–1.48)	0.95 (0.78–1.15)
p_{trend}	0.54	0.99	0.30	0.10	0.60
BRAF-mutant cancer (n = 160)					
MV-adjusted HR (95% CI)	1.06 (0.87–1.28)	1.37 (0.84–2.21)	0.80 (0.54–1.17)	1.29 (0.87–1.91)	1.10 (0.79–1.54)
p_{trend}	0.59	0.21	0.25	0.21	0.57
$p_{heterogeneity}$	0.86	0.25	0.60	0.74	0.43
CIMP-low/negative cancer (n = 592)					
MV-adjusted HR (95% CI)	1.04 (0.93–1.17)	1.09 (0.83–1.43)	0.92 (0.74–1.14)	1.18 (0.95–1.47)	0.96 (0.79–1.17)
p_{trend}	0.47	0.55	0.47	0.13	0.71
CIMP-high cancer (n = 166)					
MV-adjusted HR (95% CI)	1.04 (0.86–1.26)	1.11 (0.69–1.78)	0.82 (0.56–1.19)	1.24 (0.84–1.82)	1.00 (0.72–1.40)
p_{trend}	0.71	0.67	0.29	0.28	0.98
$p_{heterogeneity}$	0.96	0.95	0.55	0.84	0.83
KRAS-wild-type cancer (n = 521)					
MV-adjusted HR (95% CI)	1.05 (0.93–1.18)	0.94 (0.70–1.25)	0.90 (0.72–1.13)	1.23 (0.98–1.55)	0.88 (0.71–1.08)
p_{trend}	0.47	0.66	0.35	0.07	0.21
KRAS-mutant cancer (n = 246)					
MV-adjusted HR (95% CI)	1.06 (0.90–1.25)	1.41 (0.95–2.09)	0.85 (0.61–1.16)	1.22 (0.88–1.68)	1.25 (0.95–1.63)
p_{trend}	0.49	0.09	0.30	0.23	0.12
$p_{heterogeneity}$	0.89	0.09	0.75	0.95	0.03
Men					
MSS/MSI-low cancer (n = 497)					
MV-adjusted HR (95% CI)	0.95 (0.87–1.05)	0.99 (0.81–1.20)	0.84 (0.68–1.03)	1.00 (0.84–1.19)	0.96 (0.84–1.10)
p_{trend}	0.32	0.89	0.09	0.99	0.57
MSI-high cancer (n = 64)					
MV-adjusted HR (95% CI)	1.04 (0.83–1.29)	1.13 (0.71–1.79)	1.09 (0.67–1.76)	0.86 (0.54–1.37)	1.21 (0.88–1.65)
p_{trend}	0.74	0.61	0.75	0.52	0.24
$p_{heterogeneity}$	0.46	0.59	0.33	0.55	0.18
BRAF-wild-type cancer (n = 525)					
MV-adjusted HR (95% CI)	0.97 (0.88–1.06)	0.98 (0.81–1.19)	0.86 (0.71–1.05)	0.99 (0.84–1.17)	0.99 (0.87–1.12)
p_{trend}	0.46	0.86	0.14	0.94	0.85
BRAF-mutant cancer (n = 44)					
MV-adjusted HR (95% CI)	1.04 (0.80–1.35)	1.55 (0.90–2.67)	0.91 (0.50–1.65)	0.75 (0.41–1.35)	1.10 (0.75–1.63)
p_{trend}	0.77	0.12	0.76	0.34	0.62
$p_{heterogeneity}$	0.59	0.12	0.86	0.35	0.59
CIMP-low/negative cancer (n = 459)					
MV-adjusted HR (95% CI)	0.97 (0.88–1.08)	0.93 (0.76–1.14)	0.91 (0.74–1.12)	0.98 (0.82–1.17)	0.98 (0.86–1.13)
p_{trend}	0.61	0.51	0.38	0.79	0.81

(Continues)

Table 4. Multivariable-adjusted hazard ratio of colorectal cancer by MSI, *BRAF*, CIMP and *KRAS* status according to intake of total fiber intake and fiber from different food sources and whole grains in women (NHS, 1980–2012) and men (HPFS, 1986–2012) (Continued)

	Total fiber (per 5 g/day)	Cereal fiber (per 5 g/day)	Fruit fiber (per 5 g/day)	Vegetable fiber (per 5 g/day)	Whole grain (per 20 g/day)[1]
CIMP-high cancer (n = 64)					
MV-adjusted HR (95% CI)	1.03 (0.83–1.29)	1.23 (0.77–1.96)	0.90 (0.55–1.48)	1.13 (0.72–1.77)	1.14 (0.83–1.57)
p_{trend}	0.76	0.39	0.68	0.60	0.42
$p_{heterogeneity}$	0.61	0.28	0.97	0.56	0.39
KRAS-wild-type cancer (n = 345)					
MV-adjusted HR (95% CI)	0.96 (0.86–1.07)	0.96 (0.76–1.20)	0.88 (0.69–1.11)	1.01 (0.83–1.24)	0.96 (0.82–1.12)
p_{trend}	0.42	0.71	0.27	0.91	0.59
KRAS-mutant cancer (n = 225)					
MV-adjusted HR (95% CI)	1.01 (0.89–1.15)	1.10 (0.84–1.43)	0.91 (0.68–1.20)	0.96 (0.75–1.22)	1.06 (0.88–1.26)
p_{trend}	0.89	0.50	0.49	0. 71	0.56
$p_{heterogeneity}$	0.48	0.41	0.85	0.71	0.39

Cox proportional hazards model was used with adjustment for age, family history of colorectal cancer (yes or no), history of lower gastrointestinal endoscopy (yes or no), pack-years of smoking before age of 30 (for women: no, ‹5 years, ≥5 years; for men: no, ‹10 years, ≥10 years), smoking status (never, past, current smokers with 1–14 pack-years, current smokers with 15–24 pack-years, current smokers with ≥25 pack-years), body mass index (kg/m^2, continuous), physical activity (for women: ‹5, 5–11.4, 11.5–21.9, ≥22 METS/week; for men: ‹10, 10–22.5, 22.5–41.5, ≥41.5 METS/week), alcohol intake (for women: 0.0–0.14, 0.15–1.9, 2.0–7.4, ≥7.5 g/day; for men: 0.0–0.9, 1.0–5.9, 6.0–14.9, ≥15 g/day), regular aspirin use (yes or no), regular multivitamin use (yes or no), total folate intake (median in quartile), calcium intake (median in quartile), vitamin D intake (median in quartile), glycemic load (median in quartile) and processed red meat intake (median in quartile), hormone use (only for women: premenopausal, postmenopausal hormone use no/past/current).

[1]For whole grain analysis, the follow-up started in 1984 in women and the case numbers of colorectal cancer with MSS/MSI-low, MSI-high, *BRAF*-wild-type, *BRAF*-Mutant, CIMP-low/negative, CIMP-high, *KRAS*-wild-type and *KRAS*-Mutant are 483, 124, 491, 129, 484, 135, 419 and 204, respectively.

Abbreviations: NHS, the Nurses' Health Study; HPFS, the Health Professionals Follow-up Study; HR, hazard ratio; CI, confidence interval.

the beneficial effect of fiber on the gut microbiota, thereby promoting the development of proximal colon cancer.[57] However, given the limited data, further studies are needed to examine the interplay between the gut bacteria, fiber and different food sources of fiber in CRC.

Limited data reported that fiber intake was not differentially associated with CRC risk according to *KRAS* or *BRAF* mutation status.[20–22] Consistent with these data, we did not find any statistically significant heterogeneity in the relationship between fiber intake and CRC risk according to common molecular markers, including MSI, CIMP, *KRAS* and *BRAF*. These findings contrast with a recent laboratory report that butyrate, a bacterial fermentation product of fiber, promoted the development of CRC by driving aberrant proliferation of MSH2$^{-/-}$ colon epithelial cells.[58] On the other hand, increasing evidence supports the role of the interplay between fiber and the gut microbiota in CRC development. We recently reported that higher intake of dietary fiber was more strongly associated with lower risk for *Fusobacterium nucleatum*-positive CRC but not *Fusobacterium nucleatum*-negative CRC.[56] Given the limited data, future studies are needed to examine how other tumor markers, including the gut bacteria, may modify the effect of fiber intake on CRC.

Our study has several strengths, including the large sample size, long-term follow-up, detailed and repeated data collection for both fiber intake and various confounders, as well as molecular characterization of tumor subtypes. Several limitations of our study need to be noted as well. First, dietary factors assessed by FFQ including fiber and other covariates are subject to measurement error, which may have attenuated the association between fiber and CRC risk. Second, we did not collect data on food preparation methods, such as mashing and cooking, which could alter the physiological properties of fiber. Third, some subgroup results should be interpreted cautiously given the multiple testing. Finally, our study participants are predominantly Caucasian health professionals, therefore the findings may not be generalizable to other populations. However, the homogeneity of our study population helps minimize the likelihood of uncontrolled confounding.

In conclusion, our updated analysis from the two large US cohorts indicated no association between total dietary fiber intake and risk of CRC, with no heterogeneity observed by tumor subsite and common molecular markers. However, a potential benefit of cereal fiber and whole grains in men cannot be excluded and needs to be confirmed in further studies.

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Author contributions

Drs. Chan and Song have full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Chan AT and Song M. Acquisition of data: He X, Wu K, Chan AT, Song M and Ogino S. Analysis and interpretation of data: He X, Giovannucci EL, Chan AT and Song M. Drafting of the article: He X and Song M. Critical revision of the article for important intellectual content: Wu K, Ogino S, Giovannucci EL, Chan AT, Song M, Cao Y, Zhang X and Nishihara R. Statistical analysis: He X, Song M. Obtained funding: Ogino S, Giovannucci EL, Chan AT and Song M. Administrative, technical, or material support: Wu K, Giovannucci EL, Chan AT, Song M and Ogino S. Study supervision: Chan AT and Song M.

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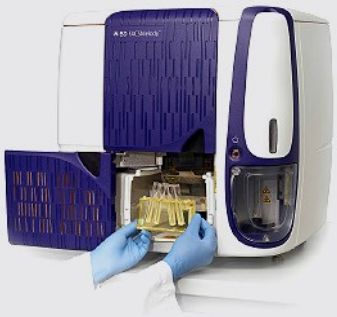

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