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Aspirin and the Risk of Colorectal Cancer in Relation to the Expression of COX-2

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ABSTRACT

BACKGROUND

Regular use of aspirin reduces the risk of a colorectal neoplasm, but the mechanism by which aspirin affects carcinogenesis in the colon is not well understood.

METHODS

We estimated cyclooxygenase-2 (COX-2) expression by immunohistochemical assay of sections from paraffin-embedded colorectal-cancer specimens from two large cohorts of participants who provided data on aspirin use from a questionnaire every 2 years. We applied Cox regression to a competing-risks analysis to compare the effects of aspirin use on the relative risk of colorectal cancer in relation to the expression of COX-2 in the tumor.

RESULTS

During 2,446,431 person-years of follow-up of 82,911 women and 47,363 men, we found 636 incident colorectal cancers that were accessible for determination of COX-2 expression. Of the tumors, 423 (67%) had moderate or strong COX-2 expression. The effect of aspirin use differed significantly in relation to COX-2 expression (P for heterogeneity=0.02). Regular aspirin use conferred a significant reduction in the risk of colorectal cancers that overexpressed COX-2 (multivariate relative risk, 0.64; 95% confidence interval [CI], 0.52 to 0.78), whereas regular aspirin use had no influence on tumors with weak or absent expression of COX-2 (multivariate relative risk, 0.96; 95% CI, 0.73 to 1.26). The age-standardized incidence rate for cancers that overexpressed COX-2 was 37 per 100,000 person-years among regular aspirin users, as compared with 56 per 100,000 person-years among those who did not use aspirin regularly; in contrast, the rate for cancers with weak or absent COX-2 expression was 27 per 100,000 person-years among regular aspirin users, as compared with 28 per 100,000 person-years among nonregular aspirin users.

CONCLUSIONS

Regular use of aspirin appears to reduce the risk of colorectal cancers that overexpress COX-2 but not the risk of colorectal cancers with weak or absent expression of COX-2.

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Observational studies and randomized intervention trials have found that regular use of aspirin reduces the risk of colorectal neoplasms.[1-4] The mechanism by which aspirin influences the risk of colorectal cancer is not well understood. Aspirin inhibits cyclooxygenase, which catalyzes the rate-limiting step in the metabolic conversion of arachidonic acid to prostaglandins and related eicosanoids.[5] One form of cyclooxygenase, termed cyclooxygenase-2 (COX-2), promotes inflammation and cell proliferation, and colorectal cancers often overexpress this enzyme.[6-9] Randomized trials have demonstrated that selective inhibitors of COX-2 reduce the risk of recurrent adenoma in participants at high risk.[10-13] However, aspirin and nonsteroidal antiinflammatory drugs (NSAIDs) decrease proliferation and increase apoptosis of colorectal cancer cell lines that have no detectable cyclooxygenase activity.[14] Aspirin has other effects that are unrelated to cyclooxygenase, including inhibition of nuclear factor-κB,[15] induction of apoptosis by activation of p38 kinase,[16] and catabolism of polyamines.[17] If aspirin exerts its effect on the formation of adenomas and cancers by inhibiting COX-2 or its downstream effectors, then the use of aspirin should preferentially reduce the risk of tumors for which growth depends on COX-2 function.

We investigated whether the influence of aspirin on the risk of colorectal cancer varied with the expression of COX-2 in the tumor. For this purpose, we used tumor specimens from two large prospective studies in which an association was found between the regular use of aspirin and a reduced risk of colorectal cancer.[18-20]

METHODS

STUDY POPULATION

The Nurses' Health Study (NHS) was initiated in 1976 when 121,701 U.S. female registered nurses, 30 through 55 years of age, completed a mailed questionnaire.[19,20] The Health Professionals Follow-up Study (HPFS) was established in 1986 with a parallel cohort of 51,529 U.S. male dentists, optometrists, osteopaths, podiatrists, pharmacists, and veterinarians who were 40 through 75 years of age at entry.[18] The cohorts had a follow-up rate of 92%. We mailed questionnaires every 2 years to obtain updated information and identify new cases of cancer. In 1980, the NHS questionnaire was expanded to include a validated assessment of diet and aspirin use[21]; a similar instrument had been used in the 1986 HPFS questionnaire.[22] The overall response rate for these questionnaires was 90%. The institutional review boards at Brigham and Women's Hospital and the Harvard School of Public Health approved this study; completion of the questionnaire was considered to imply informed consent.

ASSESSMENT OF MEDICATION USE

Assessment of aspirin use in both the NHS and the HPFS has been described in detail previously.[18-20,23] Briefly, in 1980 we asked NHS participants whether they used aspirin, the number of pills taken each week, and the number of years of use. We updated this information every 2 years except for 1986 with specific questions on the number of aspirin tablets taken per week (in categories of number taken). In the 1986 HPFS questionnaire and in questionnaires every 2 years thereafter, we inquired whether the men in the study used aspirin two or more times per week. Beginning in 1992, we also asked these men the average number of tablets taken per week (in categories of number taken). In both cohorts, we specifically inquired about standard-dose (325-mg) aspirin tablets. However, to reflect secular trends in consumption of low-dose (baby) aspirin, the questionnaires after 1992 asked participants to convert four baby aspirin tablets to one adult standard-dose tablet when responding. We did not collect consistent data on use of nonaspirin NSAIDs in either cohort. We did not evaluate the use of selective inhibitors of COX-2 that were introduced in the United States in 1999.

ASCERTAINMENT OF CASES

We requested permission to acquire medical records and pathology reports from participants who reported colorectal cancer on our biennial questionnaire. We identified deaths from the National Death Index and from next of kin.[24] A study physician who was unaware of information on the participants' intake of aspirin reviewed medical and pathological records to extract information on histologic types and anatomic locations of the cancers. A single study pathologist reviewed all of the cases that were retrieved for determination of COX-2 expression.

ASSESSMENT OF COX-2 EXPRESSION

Beginning in 1997 in the HPFS and 2001 in the NHS, we began retrieving, from the pathology de-

partments of treating hospitals, available pathological specimens from participants whom we confirmed had received a diagnosis of colorectal cancer. We obtained specimens from 648 cases (76%) over 16 years of follow-up in the HPFS and 662 cases (58%) over 22 years of follow-up in the NHS. We limited our analysis of the expression of COX-2 to unstained paraffin blocks with amounts of tumor tissue and adjacent mucosa that were sufficient for immunohistochemical analysis (636 cases: 368 from the NHS and 268 from the HPFS). The baseline characteristics of participants with colorectal cancer whose tumors we analyzed were similar to those of participants whose tumors we did not analyze (mean age, 54.4 vs. 54.9 years; nonwhite, 3% vs. 3%; former or current smoker, 60% vs. 58%; mean body-mass index [the weight in kilograms divided by the square of the height in meters], 25.5 vs. 25.3; mean metabolic equivalent [MET; i.e., exercise intensity] score per week, 16.6 vs. 15.3; current multivitamin use, 36% vs. 33%; previous lower gastrointestinal endoscopy, 13% vs. 12%; folate intake, 401 vs. 408 μg per day; calcium intake, 798 vs. 786 mg per day; alcohol intake, 9.9 vs. 10.2 g per day; P>0.25 for all comparisons).

We used a previously described method for COX-2 immunostaining on whole tissue sections or on microassays constituted from specimens.[25,26] We incubated deparaffinized tissue sections with citrate buffer (BioGenex) in a microwave oven for 15 minutes, then cooled them for 40 minutes. Tissue sections were then incubated with 3% hydrogen peroxide for 20 minutes, avidin block for 15 minutes, and biotin block for 15 minutes. A mouse anti–COX-2 antibody (Cayman Chemical) diluted 1:300 in phosphate-buffered saline was applied, and the slides were maintained overnight at 4°C. Next, we applied an antimouse horse IgG antibody (Vector Laboratories) for 20 minutes, followed by an avidin–biotin complex conjugate (Vector Laboratories). The immunochemical reaction was revealed by diaminobenzidine and methyl-green counterstain. For each assay run, we included a cancer tissue with COX-2 overexpression and a negative control (normal colonic tissue). We also examined a specimen with known COX-2 overexpression treated with phosphate-buffered saline, but not the anti-COX-2 antibody, as a control for nonspecific binding of the immunohistochemical marker.

A pathologist who was unaware of any data concerning the participants interpreted COX-2 expression, using a standardized grading system (absent, weak, moderate, or strong). The pathologist classified staining of tumor cells as "absent" if COX-2 expression was at the same level of intensity as that in adjacent normal colonic epithelium; weak, moderate, or strong staining indicated progressively increasing degrees of overexpression. A random sample of 108 cancers was reread by a second pathologist unaware of data on the participants; the concordance between the two pathologists was 0.92 (κ=0.62, P<0.001).[26] If the intensity of immunostaining was moderate or strong, tumors were classified as cancers with COX-2 overexpression (COX-2–positive). If immunostaining intensity was weak or absent, tumors were classified as negative for COX-2 overexpression (COX-2–negative) (Fig. 1).

STATISTICAL ANALYSIS

At baseline, we excluded men and women who did not complete the baseline dietary questionnaire or medication questions, who recorded implausible dietary or aspirin data (e.g., responded "yes" to use but then recorded use of zero aspirin tablets per week), or reported a baseline history of cancer (except nonmelanoma skin cancer). We also excluded participants with inflammatory bowel disease, familial polyposis, or hereditary nonpolyposis colon cancer. After these exclusions, 82,911 women and 47,363 men were eligible for analysis. They accrued follow-up time beginning on the month of return of their baseline questionnaire and ending on the month of diagnosis of colorectal cancer, death from other causes, or June 2002, whichever came first. Participants in whom colorectal cancer was adjudicated as the cause of death by study physicians after review of medical records were included as cases. Data from cases of colorectal cancer for which we were unable to assay COX-2 expression in the tumor were censored from the analysis at the date of diagnosis.

To reduce within-person variation and to better estimate long-term intake, we used the cumulative average intake of aspirin as reported on all available questionnaires up to the start of each 2-year follow-up interval.[27] In the NHS, women who reported taking two or more standard (325-mg) aspirin tablets per week were classified as regular users, whereas those who reported less aspirin use were classified as nonusers. We also estimated the duration of the regular use of aspirin by NHS participants from the number of years of regular use

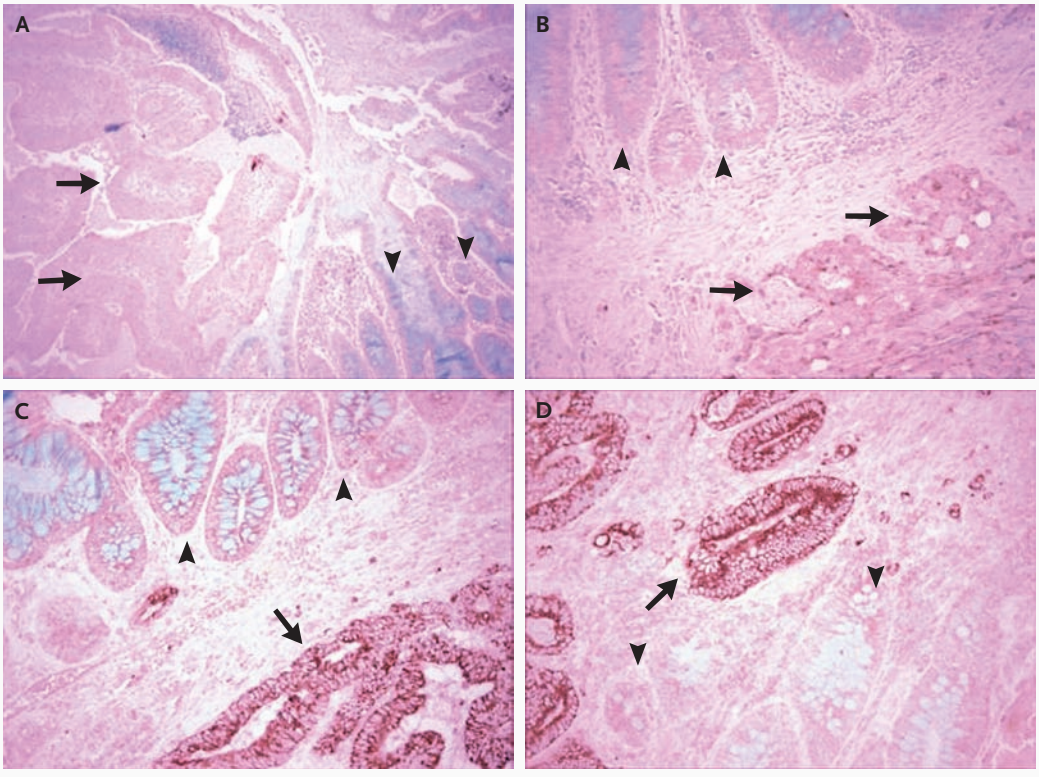

Figure 1. COX-2 Expression in Immunohistochemical Assays of Colorectal-Cancer Tissue and Mucosa.

In Panel A, COX-2 is not overexpressed in carcinoma (arrows) as compared with normal mucosa (arrowheads). Panel B shows weak expression of COX-2 in carcinoma (arrows) as compared with normal mucosa (arrowheads). Panel C shows moderate overexpression of COX-2 in carcinoma (arrow) as compared with normal mucosa (arrowheads). Panel D shows strong overexpression of COX-2 in carcinoma (arrow) as compared with normal mucosa (arrowheads).

reported in 1980, with updating of this variable every 2 years.[19,20,23] In the HPFS, men who reported using aspirin at least two times per week were classified as regular users, whereas those who did not report aspirin use at least two times per week were classified as nonusers.[18] For our main analyses, we pooled data from both cohorts. We conducted tests of heterogeneity using the Q statistic before pooling.[28] In this analysis, we observed no heterogeneity between the cohorts regarding the association of regular aspirin use and risk of colorectal cancer ($P=0.33$ for Cochran's Q test).

We calculated the incidence rates of colorectal cancer for participants in a specific category of aspirin use by dividing the number of incident cases by the number of person-years of follow-up. We calculated relative risks by dividing the incidence rate in one category by the incidence rate in the reference category; we used Cox proportional-hazards modeling to control for multiple variables simultaneously and to calculate 95% confidence intervals. Multivariate relative risks are adjusted for age, sex, smoking before the age of 30 years (0, 1 to 4, 5 to 10, 11 to 15, or more than 15 pack-years); body-mass index (in quintiles); regular vigorous exercise (in quintiles of MET task score per week); colorectal cancer in a parent or sibling (yes or no); previous lower gastrointestinal endoscopy (yes or no); history of polyps (yes or no); current multivitamin use (yes or no); consumption of beef, pork, or lamb as a main dish (0 to 3 times per month, 1 time per week, 2 to 4 times per week, or 5 or more times per week); alcohol consumption (0, 0.1 to 4.9, 5.0 to 14.9, or 15 g or more per day); and energy-adjusted folate and calcium intake (in quintiles). For the analyses restricted to women, the multivariate models additionally adjusted for postmenopausal hormone-replacement therapy (premenopausal women and postmenopausal women who never used, formerly used, or were

currently using hormone-replacement therapy) and did not include sex. For the analyses restricted to men, the multivariate models also did not include sex. We used the most recent information for all covariates.

To compare the association between aspirin use and the risk of colorectal cancer in relation to COX-2 expression in the cancer, we used a competing-risk analysis that uses a duplication-method Cox regression.[29,30] This method permits estimation of separate regression coefficients for aspirin use stratified according to the type of outcome (e.g., cancer with COX-2 overexpression vs. cancer with weak or absent COX-2 expression). We assessed the statistical significance of the difference between the risk estimates according to tumor type by a likelihood-ratio test that compared the model that allowed for separate associations of aspirin according to COX-2 expression with a model that assumed a common association. We used SAS software (version 9.1.3) for all analyses. All P values are two-sided.

RESULTS

Among the 82,911 eligible women and 47,363 eligible men at the time of the baseline questionnaire, participants reporting the regular use of aspirin (taking two or more standard aspirin tablets per week or using aspirin at least two times per week) were older, were slightly less physically active, were more likely to be current or former smokers, were more likely to use multivitamins regularly, were more likely to use postmenopausal hormones (among women), consumed more alcohol, and consumed more folate (P<0.001 for all unadjusted comparisons) (Table 1). On the basis of the updated data on aspirin use we obtained on biennial questionnaires, we observed that patterns of aspirin use changed over time. Averaged over the total number of person-years of follow-up (2,446,431), the difference in mean age between regular aspirin users and nonusers gradually increased (59.0 years for regular aspirin users and 56.5 years for nonusers, P<0.001). For this analysis, we identified 636 incident cases of colorectal cancer among users and nonusers of aspirin that were available for analysis of COX-2 expression (Table 1). Among these 636 tumors, 423 (67%) had moderate or strong COX-2 expression (i.e., were COX-2–positive), whereas 213 (33%) had weak or absent COX-2 expression (i.e., were COX-2–negative).

As in our previous studies,[18-20] we observed in both cohorts a significantly lower risk of colorectal cancer among regular aspirin users than among participants who did not regularly use aspirin, after controlling for other known or suspected colorectal cancer risk factors (Table 2). For the combined cohorts, regular aspirin use was associated with a multivariate relative risk of colorectal cancer of 0.73 (95% confidence interval [CI], 0.62 to 0.86) for cases with tissue available for COX-2 analysis. Inclusion of all documented cases of colorectal cancer that were found during follow-up, irrespective of the availability of tissue for analysis of COX-2 expression, did not materially alter these results (multivariate relative risk, 0.76; 95% CI, 0.70 to 0.84 for 1142 cases in the NHS and 853 cases in the HPFS; P<0.001). Regular aspirin use was associated with a multivariate relative risk of 0.78 (95% CI, 0.69 to 0.87; P<0.001) among participants with colorectal cancer in whom COX-2 expression was not assayed.

We evaluated the influence of aspirin on the risk of colorectal cancer according to overexpression of COX-2 in the tumor (Table 2). The benefit of regular aspirin use appeared to be confined to cancers with COX-2 overexpression (multivariate relative risk, 0.64; 95% CI, 0.52 to 0.78). In contrast, aspirin use did not appear to be associated with the risk of colorectal cancer with weak or absent COX-2 expression (multivariate relative risk, 0.96; 95% CI, 0.73 to 1.26). A test for heterogeneity for the association of regular aspirin use with COX-2–positive or COX-2–negative tumors found a statistically significant association (P for heterogeneity = 0.02).

Table 3 shows the association between the duration of regular use of aspirin and the risk of a COX-2–positive or COX-2–negative colorectal cancer. A statistically significant reduction in the number of cases of COX-2–positive cancer was not evident until aspirin had been used regularly for more than 10 years (multivariate relative risk, 0.59; 95%, CI, 0.42 to 0.82; P for trend, <0.001); there was no statistically significant reduction in the number of COX-2–negative cancers with increasing duration of aspirin use (P for trend = 0.25).

Table 4 shows the association between the dose of aspirin (tablets per week) and the risk of colorectal cancer. A reduction in the multivariate relative risk of colorectal cancer became apparent with an intake of more than five tablets per week, and the relative risk was further reduced as the

Table 1. Baseline Characteristics of the Study Cohort.*

Characteristic	Women		Men		Total	
	Nonregular Users of Aspirin (N=53,827)	Regular Users of Aspirin (N=29,084)	Nonregular Users of Aspirin (N=33,441)	Regular Users of Aspirin (N=13,922)	Nonregular Users of Aspirin (N=87,268)	Regular Users of Aspirin (N=43,006)
Median age (yr)	46	47	53	56	48	49
Race (%)†						
Nonwhite	3	2	6	4	4	2
White	97	98	94	96	96	98
Former or current smoker						
%	56	58	49	58	53	58
No. of pack-years‡	20.4	20.8	24.2	25.6	21.8	22.5
Body-mass index§	24.1	24.6	25.5	25.7	24.6	24.9
Physical activity (METs/wk)¶	14.3	13.5	21.0	20.8	17.3	16.0
Postmenopausal women (%)‖	43	46				
Never used hormones (%)	62	58				
Formerly used hormones (%)	19	20				
Currently using hormones (%)	19	22				
Current multivitamin use (%)	31	38	39	49	34	42
Previous lower gastrointestinal endoscopy (%)	2	2	25	27	11	10
Colorectal cancer in a parent or sibling (%)	8	8	9	8	8	8
Dietary intake**						
Beef, pork, or lamb as a main dish (servings/wk)	2.5	2.6	1.8	1.8	2.2	2.3
Folate (μg/day)	360	371	473	498	404	411
Alcohol (g/day)	6.1	6.8	10.8	12.6	7.9	8.7
Calcium (mg/day)	736	726	890	917	795	787

* Data are from the baseline questionnaire administered in 1980 to women enrolled in the Nurses' Health Study (NHS)[19,20] and in 1986 to men enrolled in the Health Professionals Follow-up Study (HPFS).[18] In the NHS, regular aspirin use was based on previously described categorization as the consumption of at least two 325-mg tablets per week. Nonregular use was defined as the consumption of fewer than two tablets per week. In the HPFS, regular aspirin use was based on previously described categorization as the consumption of aspirin at least two times per week. Nonregular use was defined as the consumption of aspirin fewer than two times per week. All values, other than age, have been directly standardized according to the age distribution of the cohort. Values are means unless otherwise indicated.

† Race was self-assessed by the participants on questionnaires.

‡ Pack-years were calculated for former and current smokers only.

§ The body-mass index is the weight in kilograms divided by the square of the height in meters.

¶ MET denotes metabolic equivalent, calculated according to the frequency of a range of physical activities (e.g., jogging) in 1986 for both women and men.

‖ Hormones are defined as postmenopausal estrogen or estrogen plus progesterone preparations. The percentages of those never using, formerly using, and currently using hormones were calculated for postmenopausal women only.

** Nutrient values (folate and calcium) represent the mean of energy-adjusted intakes.

Table 2. Relative Risk of Colorectal Cancer in Relation to COX-2 Expression and Regular Aspirin Use.*

Variable	Women		Men		Total†	
	Nonregular Users of Aspirin	Regular Users of Aspirin	Nonregular Users of Aspirin	Regular Users of Aspirin	Nonregular Users of Aspirin	Regular Users of Aspirin
All colorectal cancer						
No. of cases/no. of person-yr	225/1,037,122	143/704,361	161/402,337	107/302,612	386/1,439,458	250/1,006,973
Age-adjusted relative risk (95% CI)	1.0	0.80 (0.65–0.99)	1.0	0.72 (0.56–0.92)	1.0	0.76 (0.65–0.89)
Multivariate relative risk (95% CI)‡	1.0	0.80 (0.65–0.99)	1.0	0.67 (0.52–0.86)	1.0	0.73 (0.62–0.86)
COX-2–positive cancer§						
No. of cases/no. of person-yr	154/1,037,181	88/704,404	117/402,372	64/302,652	271/1,439,553	152/1,007,056
Age-adjusted relative risk (95% CI)	1.0	0.72 (0.55–0.94)	1.0	0.59 (0.44–0.81)	1.0	0.66 (0.54–0.81)
Multivariate relative risk (95% CI)‡	1.0	0.72 (0.56–0.94)	1.0	0.56 (0.41–0.76)	1.0	0.64 (0.52–0.78)¶
COX-2–negative cancer‖						
No. of cases/no. of person-yr	71/1,037,259	55/704,435	44/402,442	43/302,668	115/1,439,702	98/1,007,104
Age-adjusted relative risk (95% CI)	1.0	0.98 (0.69–1.39)	1.0	1.04 (0.68–1.59)	1.0	0.99 (0.76–1.30)
Multivariate relative risk (95% CI)‡	1.0	0.98 (0.69–1.40)	1.0	0.97 (0.63–1.49)	1.0	0.96 (0.73–1.26)¶

* The women were participants in the Nurses' Health Study (NHS)[19,20] enrolled in 1980 and followed until 2002. The men were participants in the Health Professionals Follow-up Study (HPFS)[18] enrolled in 1986 and followed until 2002. In the NHS, regular aspirin use was based on previously described categorization as the consumption of at least two 325-mg tablets per week. Nonregular use was defined as the consumption of fewer than two tablets per week. In the HPFS, regular aspirin use was based on previously described categorization as the consumption of aspirin at least two times per week. Nonregular use was defined as the consumption of aspirin fewer than two times per week. Relative risks are for regular users as compared with nonregular users.

† Pooled data are from NHS and HPFS (P=0.33 with the use of Cochran's Q test for heterogeneity).

‡ Multivariate relative risks are adjusted for age, sex, smoking before 30 years of age (0 pack-years, 1 to 4 pack-years, 5 to 10 pack-years, 11 to 15 pack-years, or >15 pack-years); body-mass index (in quintiles); regular vigorous exercise (in quintiles of metabolic equivalent [MET] task score per week); colorectal cancer in a parent or sibling (yes or no); previous endoscopy (yes or no); history of polyps (yes or no); current multivitamin use (yes or no); consumption of beef, pork, or lamb as a main dish (0 to 3 times per month, once a week, 2 to 4 times per week, or ≥5 times per week); alcohol consumption (0 g, 0.1 to 4.9 g, 5.0 to 14.9 g, or ≥15.0 g per day); and energy-adjusted folate and calcium intake (in quintiles). For the analyses restricted to women, the multivariate models were also adjusted for postmenopausal hormone-replacement therapy (premenopausal women and postmenopausal women who never used, formerly used, or were currently using hormone-replacement therapy) and did not include sex. For the analyses restricted to men, the multivariate models did not include sex.

§ Cancers with immunohistochemical COX-2 staining of moderate to strong intensity are classified as COX-2–positive cancers.

¶ The P value for heterogeneity of the association of regular aspirin use with COX-2–positive cancer and of regular aspirin use with COX-2– negative cancer is 0.02 (χ^2=5.7, 1 degree of freedom).

‖ Cancers with no immunohistochemical COX-2 staining or with staining of weak intensity are classified as COX-2–negative cancers.

Table 3. Relative Risk of Colorectal Cancer in Relation to COX-2 Expression and Duration of Regular Aspirin Use.*

Variable	Years of Regular Aspirin Use					P Value†
	0	1–5	6–10	11–20	>20	
All colorectal cancer						
No. of cases/no. of person-yr	244/1,012,439	139/481,563	135/404,348	78/286,056	40/262,025	
Age-adjusted relative risk (95% CI)	1.0	0.98 (0.80–1.21)	0.92 (0.74–1.14)	0.69 (0.53–0.90)	0.60 (0.43–0.84)	<0.001
Multivariate relative risk (95% CI)‡	1.0	0.92 (0.74–1.13)	0.87 (0.70–1.08)	0.66 (0.51–0.86)	0.58 (0.42–0.82)	<0.001
COX-2–positive cancer§						
No. of cases/no. of person-yr	169/1,012,502	94/481,604	86/404,385	48/286,080	26/262,039	
Age-adjusted relative risk (95% CI)	1.0	0.96 (0.74–1.24)	0.85 (0.65–1.11)	0.61 (0.44–0.85)	0.56 (0.37–0.85)	0.001
Multivariate relative risk (95% CI)‡	1.0	0.90 (0.69–1.16)	0.80 (0.61–1.04)	0.59 (0.42–0.82)	0.55 (0.36–0.84)	<0.001
COX-2–negative cancer¶						
No. of cases/no. of person-yr	75/1,012,593	45/481,647	49/404,422	30/286,094	14/262,050	
Age-adjusted relative risk (95% CI)	1.0	1.03 (0.71–1.50)	1.08 (0.75–1.56)	0.86 (0.56–1.33)	0.68 (0.38–1.20)	0.26
Multivariate relative risk (95% CI)‡	1.0	0.96 (0.66–1.40)	1.01 (0.70–1.46)	0.83 (0.54–1.29)	0.66 (0.37–1.18)	0.24

* In the Nurses' Health Study (NHS), regular aspirin use was based on previously described categorization as the consumption of at least two 325-mg tablets per week. Nonregular use was defined as the consumption of fewer than two tablets per week. In the Health Professionals Follow-up Study (HPFS), regular aspirin use was based on previously described categorization as the consumption of aspirin at least two times per week. Nonregular use was defined as the consumption of aspirin fewer than two times per week. Relative risks are for regular users as compared with nonregular users.

† P values are for linear trend.

‡ Multivariate relative risks are adjusted for age, sex, smoking before 30 years of age (0 pack-years, 1 to 4 pack-years, 5 to 10 pack-years, 11 to 15 pack-years, or >15 pack-years); body-mass index (in quintiles); regular vigorous exercise (in quintiles of metabolic equivalent [MET] task score per week); colorectal cancer in a parent or sibling (yes or no); previous endoscopy (yes or no); history of polyps (yes or no); current multivitamin use (yes or no); consumption of beef, pork, or lamb as a main dish (0 to 3 times per month, once a week, 2 to 4 times per week, or ≥5 times per week); alcohol consumption (0 g, 0.1 to 4.9 g, 5.0 to 14.9 g, or ≥15.0 g per day); and energy-adjusted folate and calcium intake (in quintiles). For the analyses restricted to women, the multivariate models were also adjusted for postmenopausal hormone-replacement therapy (premenopausal women and postmenopausal women who never used, formerly used, or were currently using hormone-replacement therapy) and did not include sex. For the analyses restricted to men, the multivariate models did not include sex.

§ Cancers with immunohistochemical COX-2 staining of moderate to strong intensity are classified as COX-2–positive cancers.

¶ Cancers with no immunohistochemical COX-2 staining or with staining of weak intensity are classified as COX-2–negative cancers.

Table 4. Relative Risk of Colorectal Cancer in Relation to COX-2 Expression and Aspirin Dose.*

Cancer	No. of Aspirin Tablets per Week					P Value†
	0	0.5–1.5	2–5	6–14	>14	
All colorectal cancer						
No. of cases/no. of person-yr	126/523,876	204/712,066	118/453,234	91/339,473	13/103,731	
Age-adjusted relative risk (95% CI)	1.0	0.94 (0.75–1.18)	0.77 (0.60–1.00)	0.75 (0.57–0.98)	0.47 (0.26–0.83)	0.002
Multivariate relative risk (95% CI)‡	1.0	0.93 (0.74–1.17)	0.75 (0.58–0.97)	0.71 (0.54–0.94)	0.46 (0.26–0.81)	0.001
COX-2–positive cancer§						
No. of cases/no. of person-yr	82/523,912	145/712,116	73/453,273	54/339,502	7/103,737	
Age-adjusted relative risk (95% CI)	1.0	1.03 (0.78–1.36)	0.73 (0.53–1.01)	0.68 (0.48–0.96)	0.39 (0.18–0.85)	<0.001
Multivariate relative risk (95% CI)‡	1.0	1.02 (0.77–1.34)	0.71 (0.52–0.98)	0.65 (0.46–0.92)	0.38 (0.18–0.83)	<0.001
COX-2–negative cancer¶						
No. of cases/no. of person-yr	44/523,950	59/712,197	45/453,297	37/339,521	6/103,736	
Age-adjusted relative risk (95% CI)	1.0	0.78 (0.52–1.16)	0.85 (0.56–1.29)	0.87 (0.56–1.35)	0.62 (0.26–1.44)	0.58
Multivariate relative risk (95% CI)‡	1.0	0.77 (0.52–1.14)	0.82 (0.54–1.25)	0.83 (0.53–1.29)	0.60 (0.26–1.41)	0.52

* Aspirin dose is classified according to the number of standard 325-mg tablets taken per week. In the Health Professionals Follow-up Study (HPFS), data on the number of tablets of aspirin taken per week were not collected until 1992. Thus, this analysis includes 368 incident cases from the Nurses' Health Study (NHS) from 1980 through 2002 and 184 incident cases from the HPFS from 1992 through 2002. Relative risks are calculated for each dose category as compared with no aspirin use.

† P values are for linear trend.

‡ Multivariate relative risks are adjusted for age, sex, smoking before 30 years of age (0 pack-years, 1 to 4 pack-years, 5 to 10 pack-years, 11 to 15 pack-years, or >15 pack-years); body-mass index (in quintiles); regular vigorous exercise (in quintiles of metabolic equivalent [MET] task score per week); colorectal cancer in a parent or sibling (yes or no); previous endoscopy (yes or no); history of polyps (yes or no); current multivitamin use (yes or no); consumption of beef, pork, or lamb as a main dish (0 to 3 times per month, once a week, 2 to 4 times per week, or ≥5 times per week); alcohol consumption (0 g, 0.1 to 4.9 g, 5.0 to 14.9 g, or ≥15.0 g per day); and energy-adjusted folate and calcium intake (in quintiles). For the analyses restricted to women, the multivariate models additionally adjusted for postmenopausal hormone-replacement therapy (premenopausal women and postmenopausal women who never used, formerly used, or were currently using hormone-replacement therapy) and did not include sex. For the analyses restricted to men, the multivariate models did not include sex.

§ Cancers with immunohistochemical COX-2 staining of moderate to strong intensity are classified as COX-2–positive cancers.

¶ Cancers with no immunohistochemical COX-2 staining or with staining of weak intensity are classified as COX-2–negative cancers.

number of tablets per week increased (P for trend = 0.001). This association was also noted among participants with COX-2–positive tumors (P for trend, <0.001) but not among those with COX-2–negative tumors (P for trend = 0.52). After adjustment for the number of years of use of aspirin, the association between dose and COX-2–negative tumors remained nonsignificant (P for trend = 0.91).

On the basis of the overall incidence of colorectal cancer in these cohorts and the prevalence of COX-2–positive and COX-2–negative tumors among participants in whom we assessed COX-2 expression, we estimated the age-standardized incidence rate for COX-2–positive and COX-2–negative colorectal cancers in relation to aspirin use in the entire cohort. The age-standardized incidence rate of COX-2–positive tumors was 37 per 100,000 person-years for regular aspirin users as compared with 56 per 100,000 person-years for nonusers. In contrast, the age-standardized incidence rate of COX-2–negative tumors was 27 per 100,000 person-years for regular aspirin users as compared with 28 per 100,000 person-years for nonusers.

DISCUSSION

We found that participants in two large prospective cohorts who reported long-term, regular aspirin use (at least two standard tablets per week or use of aspirin at least two times per week) had a significant reduction in the relative risk of colorec-

tal cancer, as compared with participants who reported less use of aspirin. This reduction in risk was due almost entirely to a reduction in the number of COX-2–positive colorectal cancers among regular aspirin users. In contrast, the difference in the incidence of COX-2–negative colorectal cancers between regular users and nonusers of aspirin was not statistically significant. A reduction of the risk of COX-2–positive tumors was also found with increasing aspirin dose and increasing duration of use. No significant dose or duration effect was observed for COX-2–negative tumors.

These results are consistent with a cross-sectional case–control study in which the association of NSAIDs with a reduced risk of adenomatous polyps was strongest in cases with high expression of COX-2 messenger RNA.[31] Our finding that the association with a reduced risk of colorectal cancer is most apparent at intakes of more than five aspirin tablets per day is consistent with the results of studies in which higher doses of aspirin were required to inhibit COX-2 than to inhibit COX-1.[1,5] Moreover, the association with a reduction in cancer risk was found only after several years of aspirin use, a finding that suggests an effect of aspirin on the early stages of colorectal adenoma or cancer.[1-4]

COX-2 is progressively overexpressed during the stepwise sequence from adenoma to carcinoma,[7] and randomized, placebo-controlled trials have shown that selective COX-2 inhibitors prevent recurrence of adenoma among patients with a history of adenoma or familial polyposis.[10-13] Our data suggest that the anticancer benefit of aspirin is mediated, at least in part, by inhibition of COX-2. Experimental studies have shown that aspirin and NSAIDs, especially at high doses, have a range of other potentially antineoplastic actions[15-17,32-37]; these results indicate that further work is needed to elucidate the effects of these agents and COX-2 (or its downstream effectors) on the development of colorectal cancer.[38,39] COX-2, possibly through production of inflammatory prostaglandins or eicosanoids, may regulate angiogenesis, apoptosis, or tumor-cell invasiveness.[6]

Our study has several strengths. First, because we collected detailed, updated information on aspirin use during 22 years of follow-up, we were able to evaluate the effects of long-term use across a broad range of intakes. Second, we obtained aspirin data prospectively, before the diagnosis of colorectal cancer. Any errors in recall would have tended to attenuate rather than exaggerate true associations. Third, because the participants were all health professionals, the accuracy of self-reported aspirin use is likely to be high. Fourth, we collected detailed data on potential confounders and had a high follow-up rate (92% of participants returned questionnaires or were identified as deceased). Finally, our results were remarkably consistent between the two independent cohorts.

There are several limitations to our study. The study was observational, and aspirin use was self-selected; thus, among aspirin users and nonusers, there were small, albeit statistically significant, differences in risk factors for colorectal cancer, including smoking history, physical activity, and use of multivitamins. However, adjustment for these characteristics, as well as for a wide range of other potential risk factors, had minimal influence on our findings, suggesting little potential for residual or uncontrolled confounding. We did not have sufficient data on use of nonaspirin NSAIDs in both cohorts to reliably examine their use in relation to COX-2–expressing tumors. However, it is improbable that use of nonaspirin NSAIDs influenced our findings with aspirin; our previous study did not demonstrate any modification by NSAID use of the reduced risk of colorectal cancer observed with aspirin.[20] We were unable to obtain tumor tissue from all cases of confirmed colorectal cancer detected in the two cohorts, but the risk factors in these cases did not appreciably differ from those in cases with tumor tissue available.

Another limitation of our study is the lack of a widely accepted, standardized classification scheme for COX-2 expression in colorectal cancer. Nevertheless, previous studies have demonstrated that the results of Western and Northern blot analyses correlate well with immunohistochemical grading of COX-2.[40] With our classification of COX-2 expression, we found, in our overall population, a proportion of tumors that overexpressed COX-2 similar to those found by other investigators.[8,9,41] In validation studies of the central, blinded review of tumor specimens, we observed substantial interobserver agreement (92%). Any significant misclassification of COX-2 overexpression would be expected to bias our results toward finding no significant differences in the effect of aspirin on tumors in relation to COX-2 expression.

Our results support the importance of continued investigation into COX-2 and related pathways for the development of new treatments and the potential use of COX-2 as a molecular marker for

tailoring chemoprevention in participants with a history of colorectal cancer.

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