

A 22-year Prospective Study of Fish, *n*-3 Fatty Acid Intake, and Colorectal Cancer Risk in Men

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Abstract

Background: Fish is the main dietary source of long-chain *n*-3 fatty acids, which have been suggested to play a protective role in colorectal cancer development in laboratory and animal studies. Human studies have not shown consistent results. We examined the association between intakes of fish and *n*-3 fatty acids from fish and colorectal cancer risk in men enrolled in the Physicians' Health Study.

Methods: The Physicians' Health Study began as a randomized trial to examine the effect of aspirin and β -carotene supplementation on cancer and cardiovascular disease. Fish intake was assessed at the 12-month follow-up with an abbreviated food-frequency questionnaire. Cox proportional hazards models were used to estimate multivariate relative risks for colorectal cancer for the categories of fish intake and quartiles of *n*-3 fatty acid intake.

Results: During 22 years of follow-up, 500 men had a confirmed diagnosis of colorectal cancer. Fish intake was inversely associated with colorectal cancer risk [multivariate relative risk (95% confidence interval) for highest versus lowest category, 0.60 (0.40-0.91); $P_{\text{trend}} = 0.01$]. The inverse association was observed for both colon and rectal cancers. Our findings for *n*-3 fatty acids were similar to those for fish; the multivariate relative risk (95% confidence interval) of total colorectal cancer for the highest versus lowest quartile of *n*-3 fatty acids was 0.74 (0.57-0.95; $P_{\text{trend}} = 0.01$).

Conclusions: Our results from this long-term prospective study suggest that intakes of fish and long-chain *n*-3 fatty acids from fish may decrease the risk for colorectal cancer. (Cancer Epidemiol Biomarkers Prev 2008;17(5):1136-43)

Introduction

Fish is the main dietary source of long-chain *n*-3 fatty acids, which have been suggested to play a protective role in colorectal cancer development (1, 2). Although animal studies provide support for this hypothesis (3, 4), consistent results have not emerged from studies conducted with human populations. Of the 15 prospective cohort studies that have examined the association between fish intake and colorectal cancer risk, 7 reported inverse associations [5-11; 2 of which were statistically significant (refs. 6, 8)], 7 reported null associations (12-18), and 2 reported positive associations (19, 20). A recent meta-analysis (21) reported an overall summary relative risk [95% confidence interval (95% CI)] for colorectal cancer incidence of 0.88 (0.78-1.00) for the highest versus lowest category of fish intake based on 14 of these prospective cohort studies. The results of case-control studies have also been mixed (22-32).

n-3 Fatty acids may protect against colorectal cancer by inhibiting the cyclooxygenase-2 (COX-2) enzyme and

the production of arachidonic acid (*n*-6) derived eicosanoids (1, 2). In general, eicosanoids produced from arachidonic acid are proinflammatory whereas those produced from *n*-3 fatty acids are anti-inflammatory (1, 33). Because *n*-3 and *n*-6 fatty acids compete for both the enzymes that convert shorter- to longer-chain fatty acids (1, 34) and the COX-2 enzyme (35-37), which converts the longer-chain fatty acids to precursors for eicosanoid synthesis, higher *n*-3 fatty acid intake may result in decreased production of proinflammatory eicosanoids that could play a role in the development of colorectal cancer.

Aspirin also inhibits the COX-2 enzyme, and regular use has been shown to decrease the risk for both colorectal adenoma and cancer (38). Although it is not known whether aspirin exerts its protective effect through COX-2 inhibition or other mechanisms, it is possible that *n*-3 fatty acids and aspirin may share a mechanism to decrease colorectal cancer risk.

We previously reported an inverse association between whole blood biomarker levels of long-chain *n*-3 fatty acids and colorectal cancer risk from a case-control study nested within the Physicians' Health Study (PHS; ref. 39). In that analysis, we also observed a statistically significant interaction between aspirin and *n*-3 fatty acids levels, which indicated that the benefit of increasing *n*-3 fatty acid levels was only apparent among participants who were not taking aspirin. Here, we report on the association between fish intake and colorectal cancer risk using data from the full PHS cohort followed for 22 years.

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Materials and Methods

Study Population. We carried out this prospective cohort study using data from the PHS, a randomized, double-blind, placebo-controlled, factorial trial designed to examine the effect of aspirin (325 mg every other day) and β -carotene (50 mg on alternate days) supplementation on the incidence of cancer and cardiovascular disease. The 22,071 participants had no history of myocardial infarction, stroke, transient ischemic attack, or cancer at the start of the study in 1982 (40). A baseline questionnaire inquired about previous medical diagnoses and lifestyle factors, including height, weight, physical activity level, multivitamin use, alcohol intake, history of smoking, and other lifestyle habits. The research protocol was approved by the Institutional Review Board at Brigham and Women's Hospital, and all subjects provided written informed consent. Because of the emergence of a statistically extreme ($P < 0.00001$) 44% reduction in the risk for first myocardial infarction, the aspirin component of the trial was terminated in January 1988 (40).

Identification of Cases. Participants reported new diagnoses, including colorectal cancer, on annual follow-up questionnaires. The end-point committee for the PHS obtained and reviewed the medical records, including the pathology report, to confirm the diagnosis of colorectal cancer. In addition, the histologic details and the site and stage of the disease were recorded from the medical record. Information on deaths was gathered from family members, periodic searches of the National Death Index, and postal authorities. Death certificates and medical records were acquired to determine cause of death. Follow-up is more than 99% complete for mortality and morbidity.

Measurement of Fish Intake. An assessment of fish intake was obtained at the 12-month follow-up with an abbreviated semiquantitative food-frequency questionnaire (41). The questionnaire asked about the average intake of four types of fish or shellfish: (a) canned tuna fish, (b) dark meat fish (mackerel, salmon, sardines, bluefish, and swordfish; 113.4-170.1 g 4-6), (c) other fish (113.4-170.1 g 4-6), and (d) shrimp, lobster, or scallops as a main dish. Seven frequency response categories were used on the questionnaire: rarely or never, 1 to 3 times per month, 1 time per week, 2 to 4 times per week, 5 to 6 times per week, daily, and ≥ 2 times per day. This information was used to calculate both the average daily intake of fish and n -3 fatty acids from fish. For fish, we summed the frequency responses to each of the four fish or shellfish items. Intake of n -3 fatty acids was calculated by multiplying the frequency of intake of each fish or shellfish item with the grams of n -3 fatty acids per serving of that item (0.17 g for other fish, 0.46 g for shellfish, 0.69 g for tuna fish, and 1.37 g for dark fish) and summing all four items. n -3 Fatty acid values per serving of each type of fish were obtained from the U.S. Department of Agriculture food composition tables (42).

Although a study on the reproducibility and validity of these questionnaire items has not been carried out in the PHS, this has been done in a similar population of 127 male health professionals involving the same fish items (43). Correlations between two food-frequency questionnaires completed approximately 1 year apart were 0.48

for other fish, 0.54 for canned tuna fish, 0.63 for dark meat fish, and 0.67 for shrimp, lobster, and scallops (43). As a measure of validity, correlations between intakes from the second food-frequency questionnaire and 14 days of diet records were 0.23 for shrimp, lobster, and scallops; 0.39 for other fish; 0.42 for dark meat fish; and 0.56 for canned tuna fish (43). The correlation between eicosapentaenoic acid intake estimated from the second food-frequency questionnaire and the percentage measured from adipose tissue from 118 of the 127 participants was 0.49 ($P < 0.001$; ref. 44). In addition, we further assessed the validity of the self-reported intakes of fish or n -3 fatty acids from fish by correlating these with the whole blood biomarkers of long-chain n -3 fatty acid levels (45) in 456 participants from our previously reported nested case-control study (39). The Spearman correlation between these two variables was 0.24 (Fig. 1). Stronger correlations were observed between total fish intake and blood levels of docosahexaenoic acid ($r = 0.34$), as well as between the intake of dark meat fish and docosahexaenoic acid ($r = 0.35$). These data suggest a reasonable validity of the self-reported fish intake in our study.

Statistical Analysis. We excluded 232 participants who died before or did not return the 12-month questionnaire, participants who developed cancer before returning the 12-month questionnaire, and those who did not respond to 2 or more questions about fish or shellfish consumption. We also excluded those who had missing information for 1 item and responded with "rarely/never" or "1 to 3 times per month" for the other 3 to minimize misclassification in the lower categories of fish intake. These exclusions left 21,406 participants for the analysis.

Participants were followed from the date of return of the 12-month follow-up questionnaire until the date of death, date of diagnosis of colorectal cancer, or date of last returned questionnaire until March 1, 2006, whichever came first. Dietary fish intake was divided into four categories (<1 time per week, 1-2 times per week, 2-5 times per week, and ≥ 5 times per week), and dietary n -3 fatty acid intake was categorized into quartiles.

We first examined the distribution of baseline risk factors among categories of fish intake using means or proportions. We used the Cox proportional hazards model (46) with age (in months) as the time metric to

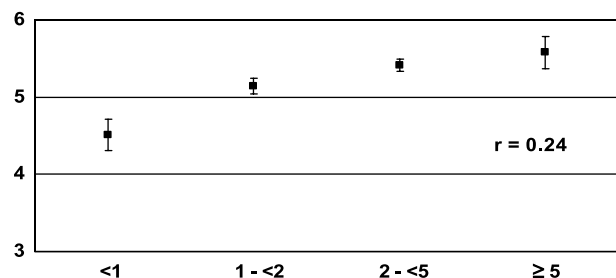


Figure 1. Blood levels of long-chain n -3 fatty acids by category of fish intake. *Abscissa*, fish intake (servings per week); *ordinate*, mean blood level of total long-chain n -3 fatty acids (percentage of total fatty acids).

Table 1. Distribution of baseline risk factors for colorectal cancer by category of fish intake at 12 months

Variable	Average frequency of fish intake (times per week)			
	<1	1 to <2	2 to <5	≥5
Number of men	2,060	6,656	10,350	2,343
Age at randomization	54.0 ± 10.2*	53.6 ± 9.6	53.6 ± 9.4	53.4 ± 9.2
Smoking status (%)				
Never	54.0	49.1	48.6	51.7
Past	33.6	38.4	41.1	40.2
Current	12.4	12.5	10.3	8.1
Multivitamin use (%)				
Never	64.3	65.1	64.7	61.0
Past	16.3	16.6	15.4	15.7
Current	19.4	18.3	19.9	23.4
Diabetes (%)	3.1	2.3	2.2	2.4
Aspirin assignment (%)	51.2	49.2	50.4	49.5
Vigorous exercise (%)				
<1 time per wk	31.8	29.7	25.8	24.1
1-4 times per wk	52.7	54.8	58.0	56.9
≥5 times per wk	15.5	15.5	16.1	19.1
Alcohol intake (%)				
≤1 time per wk	53.4	42.1	36.3	36.8
2-6 times per wk	26.9	34.0	37.7	38.7
≥1 drink per day	19.7	23.9	26.1	24.5
BMI [‡]	24.6 ± 2.7	24.8 ± 2.8	24.8 ± 2.8	24.7 ± 2.8

*Plus-minus values are means ± SD.

† Vigorous exercise defined as "exercise vigorous enough to work up a sweat."

‡ BMI is equal to the weight in kilograms divided by the square of the height in meters.

estimate the relative risk for colorectal cancer for categories of fish intake using the <1 time per week category as the reference and for quartiles of *n*-3 fatty acid intake using the lowest quartile as the reference group. We then conducted multivariate analyses to assess the independent association of fish intake with the risk for colorectal cancer by adjusting for random aspirin assignment, smoking, body mass index (BMI), history of diabetes, physical activity, alcohol intake, multivitamin use, and red meat intake. Tests for trend were done by assigning the median value to each category of consumption and modeling this as a continuous variable in separate Cox models. To test the assumption of proportional hazards, we fit a model that included an interaction term between age and fish intake (as a continuous variable), as well as a model that included an interaction term between age and *n*-3 fatty acid intake from fish (also as a continuous variable). We then conducted likelihood ratio tests and found no evidence of violation of the proportional hazards assumption.

To assess potential effect modification by aspirin assignment or BMI, we used Cox models to estimate relative risks for colorectal cancer for strata of aspirin assignment or BMI. We hypothesized that there could be effect modification by BMI because overweight and obesity are strong risk factors for colorectal cancer and overweight men may have a different dietary pattern than normal weight men. To formally test for interaction, we included a term that was the product of the natural log of *n*-3 fatty acid intake from fish (as a continuous variable) and randomized aspirin assignment or BMI (continuous) in a Cox regression model, and used a likelihood ratio test. Because we were not able to adjust for colorectal cancer screening in our multivariate models, we examined the association between *n*-3 fatty acid intake from fish and colorectal cancer risk before and after 1995, the time at which we estimated that screening by colonoscopy

became widely available, to assess the degree of potential confounding by colonoscopy utilization. In the examination of the association before 1995, participants contributed follow-up time from the date of return of the 12-month follow-up questionnaire to the date of diagnosis of colorectal cancer, date of death, date of last returned questionnaire, or December 31, 1994, whichever came first. For the analysis after 1995, we excluded participants who died, developed colorectal cancer, or were lost to follow-up before January 1, 1995. Participants then contributed follow-up time from January 1, 1995, to date of diagnosis, date of death, date of last returned questionnaire, or March 1, 2006.

Results

During the 22 years of follow-up, we recorded 500 confirmed cases of colorectal cancer. Of these cases, 388 were colon and 112 were rectal cancers. Overall, 9.6% of the men consumed fish <1 time per week; 31.1%, 1 to <2 times per week; 48.3%, 2 to <5 times per week; and 10.9%, >5 times per week. Men with higher fish intake were more likely to use multivitamins and to drink alcohol and exercise more frequently (Table 1). They were also less likely to be current smokers and to have a history of diabetes.

After adjustment for age, fish intake was inversely associated with the risk for colorectal cancer (Table 2; $P_{\text{trend}} = 0.05$). The multivariate relative risk (95% CI) for those consuming fish ≥5 times per week compared with <1 time per week was 0.63 (0.42-0.95; $P_{\text{trend}} = 0.02$), adjusting for random aspirin assignment, smoking, BMI, history of diabetes, physical activity, alcohol intake, multivitamin use, and red meat intake (Table 2). Additional adjustment for quartiles of dairy intake or β-carotene assignment produced similar results (data not shown).

Table 2. Relative risk for colorectal cancer by fish intake

	Fish intake (times per week)				P_{trend}
	<1	1 to <2	2 to <5	≥ 5	
Cases	54	162	243	41	
Person-years	35,661	116,957	182,668	40,886	
Age-adjusted RR (95% CI)	1.00	0.93 (0.68-1.26)	0.88 (0.66-1.18)	0.69 (0.46-1.03)	0.05
Multivariate adjusted RR* (95% CI)	1.00	0.88 (0.65-1.20)	0.82 (0.61-1.10)	0.63 (0.42-0.95)	0.02

Abbreviation: RR, relative risk.

*Multivariate model adjusted for age, smoking (never smoked, past smoking, and current smoking), BMI (<23, 23-24.99, 25-26.99, and ≥ 27), multivitamin use (never use, past use, and current use), history of diabetes, random assignment to aspirin or placebo, vigorous exercise (<1 time per week, 1 to 4 times per week, and ≥ 5 to 6 times per week), alcohol intake (≤ 1 time per week, 2-6 times per week, and ≥ 1 time per day), and quartile of red meat intake.

To assess whether change of dietary intake due to preclinical cancer could influence the association, we repeated the analysis by excluding cases that occurred during the first 5 years of follow-up. The multivariate relative risks (95% CI) for increasing categories of fish intake after excluding these cases were 1.00 (0.70-1.43), 0.84 (0.60-1.20), and 0.66 (0.41-1.07; $P_{\text{trend}} = 0.03$).

We also examined the association between each type of fish or shellfish and the risk for colorectal cancer. The multivariate relative risk (95% CI) comparing men who consumed fish or shellfish >1 time per week compared with never was 0.98 (0.55-1.75) for dark fish, 0.76 (0.52-1.11) for other fish, 0.67 (0.31-1.42) for shrimp, and 0.95 (0.68-1.32) for tuna fish.

The relative risks for colorectal cancer across quartiles of *n*-3 fatty acid intake from fish are shown in Table 3. Overall, the results for *n*-3 fatty acids were very similar to those for fish intake. In the model adjusted for age only, *n*-3 fatty acids were inversely associated with the risk for colorectal cancer ($P_{\text{trend}} = 0.05$). In the multivariate model, the relative risk (95% CI) for the highest versus lowest quartile was 0.76 (0.59-0.98; $P_{\text{trend}} = 0.02$).

Because aspirin and *n*-3 fatty acids can both inhibit the COX-2 enzyme and may share a mechanism to decrease the risk for colorectal cancer, we assessed the possibility of an interaction between these two variables. We did not observe a statistically significant modification of the effect of *n*-3 fatty acid intake from fish by aspirin assignment ($P_{\text{interaction}} = 0.83$; Table 4). Because the aspirin component of the trial lasted only 5 years, we further examined the possibility that any interaction between *n*-3 fatty acid intake and randomized aspirin assignment in relation to colorectal cancer could vary over time. There was no evidence of a statistically significant interaction when we limited the analysis to the first 10 years of follow-up ($P_{\text{interaction}} = 0.51$) or when we started follow-up at year 10 ($P_{\text{interaction}} = 0.76$). Although the inverse association was stronger among overweight men, the test for interaction between *n*-3 fatty acid intake and BMI was not statistically significant ($P_{\text{interaction}} = 0.14$; Table 4). For the analysis of the association between *n*-3 fatty acid intake from fish and colorectal cancer risk by period, results showed that the inverse association was stronger for cases occurring in 1995 or later (Table 4), although the test for interaction was not statistically significant ($P_{\text{interaction}} = 0.18$).

To further assess whether the inverse association between fish intake and colorectal cancer could be due to decreased red meat intake rather than an effect of fish itself, we examined the association between the ratio of red meat to fish intake and colorectal cancer risk. The

relative risks (95% CI) for increasing quartiles of the ratio of red meat to fish intake were 1.01 (0.78-1.31), 1.15 (0.89-1.48), and 1.08 (0.84-1.40), suggesting that the inverse association between fish intake and colorectal cancer is not solely due to decreasing red meat intake with increasing fish intake.

We also examined the association between fish intake and colon and rectal cancers separately. The relative risks (95% CI) across increasing categories of fish intake were 0.91 (0.64-1.31), 0.90 (0.64-1.27), and 0.62 (0.38-1.00; $P_{\text{trend}} = 0.04$) when we included only the 388 cases of colon cancer and 0.79 (0.43-1.46), 0.58 (0.32-1.06), and 0.65 (0.30-1.41; $P_{\text{trend}} = 0.29$) for the 112 rectal cancer cases.

Discussion

We observed an inverse association between fish intake and colorectal cancer risk in this prospective study of U.S. physicians followed for 22 years. *n*-3 Fatty acid intake estimated from fish was also inversely associated with risk, and the trends were statistically significant for both of these associations. The inverse associations persisted after controlling for known risk factors of colorectal cancer and after further excluding cases diagnosed during the first 5 years after the dietary report, suggesting an independent association of fish intake with low risk for colorectal cancer that is unlikely to be affected by change of diet due to pre-cancer symptoms. The inverse associations were also similar for both colon and rectal cancer.

Shrimp and other fish were most strongly inversely associated with colorectal cancer risk. Given that dark meat fish and tuna fish contain higher amounts of *n*-3 fatty acids than does shrimp or other fish, this did not seem to support the hypothesis that *n*-3 fatty acids are the component of fish responsible for the inverse association with colorectal cancer risk. However, the confidence intervals for the relative risks for dark meat fish and tuna fish are wide, and an inverse association cannot be excluded. Only 513 men were in the highest category of dark meat fish intake, which would limit the power to detect an association. It is also possible that the participants in this study did not discriminate well between dark meat fish and other fish when they completed the abbreviated food-frequency questionnaire, and the validity of the food-frequency questionnaire for assessing total fish intake may therefore have been better than for individual types of fish.

We did not find a statistically significant interaction between randomized aspirin assignment and *n*-3 fatty acid intake in this analysis, as we did in our previous

nested case-control study using blood levels of long-chain *n*-3 fatty acids. One possible explanation for this difference may be the length of follow-up. Our previous nested case-control study included cases that developed over the first 13 years of PHS follow-up, whereas the cases in this analysis occurred over 22 years of follow-up. Aspirin use was monitored on annual follow-up questionnaires, and 71% of the men reported regular aspirin use (≥ 3 days per week) as of the 7-year follow-up questionnaire (47). However, when we ran an analysis with follow-up time through the end of 1995 here, there was again no statistically significant evidence of an interaction between *n*-3 fatty acid intake from fish and random aspirin assignment ($P_{\text{interaction}} = 0.44$).

Another possible explanation for this difference is that blood levels of *n*-3 fatty acids may provide a more biologically relevant measure of intake than questionnaire-based data. There may be metabolic steps that occur after intake (that is, competition with *n*-6 fatty acids for enzymes and incorporation into cell membranes) that may make the blood measure a better assessment of what is occurring biologically in relation to pathways that *n*-3 fatty acids may share with aspirin. In addition, a study by Baylin et al. (45) suggests that the whole blood measure of long-chain *n*-3 fatty acids may provide a better assessment of long term intake (particularly for docosahexaenoic acid) than does the food-frequency questionnaire. In this study, the correlations between *n*-3 fatty acid intake as measured by food-frequency questionnaire and in adipose samples (which reflect long-term intake) were -0.08 for eicosapentaenoic acid, 0.26 for docosahexaenoic acid, and 0.39 for total *n*-3 fatty acids. The correlations between the whole blood measurement and the adipose tissue measure were 0.20 for eicosapentaenoic acid, 0.49 for docosahexaenoic acid, and 0.32 for total *n*-3 fatty acids.

Whereas more than 30 published studies have examined the association between *n*-3 fatty acid and/or fish intake and colorectal cancer risk, the results are inconsistent. For *n*-3 fatty acids, 5 studies (5, 32, 48-50) suggest an inverse relationship whereas 7 (12-14, 51-54) reported no association. The results for studies looking at fish intake are similarly mixed with 11 studies (5-11, 22-24, 30) reporting inverse, 11 reporting null (12-18, 25-28), and 4 reporting positive associations (19, 20, 29, 31). Two (20, 31) of these four positive associations were for smoked or salted fish, and one was in females only (29). A systematic review (55) of nine prospective cohort studies that examined the association between *n*-3 fatty acid intake and cancer risk reported that there was "little to suggest that omega-3 fatty acids reduce the risk of any single type of cancer," including colorectal cancer. In

contrast, the recent meta-analysis (21) supports an inverse association between fish intake and colorectal cancer incidence. There are several potential explanations for the inconsistent results across studies, and plausible biological mechanisms support an inverse relation between *n*-3 fatty acids and colorectal cancer risk.

If *n*-3 fatty acids do indeed play a role in colorectal cancer development, null findings from epidemiologic studies could result from several factors, as reviewed by Larsson et al. (1), including an intake of *n*-3 fatty acids that is too low to have any effect; low within-study variation in intake, which reduces statistical power; and nondifferential misclassification of *n*-3 fatty acid intake, which can bias results toward the null. Larsson et al. (1) also suggest that studies should take into account other factors that can influence *n*-3 fatty acid metabolism or function such as intake of *n*-6 fatty acids and use of anti-inflammatory drugs (1). In addition to the possible explanations suggested by Larsson et al., there are others including differences in the length of follow-up and number of cases, inclusion of potential confounders in multivariate models, and the types of fish consumed.

Despite several possibilities, a clear explanation for the inconsistent results across studies is not readily apparent. Among the prospective studies on fish intake, those that reported null results include studies with both relatively small (13, 17, 18) and large (12, 15, 16) numbers of cases. In addition, whereas most of the prospective studies on fish intake that reported inverse associations had follow-up times of <10 years (5-8, 10, 11), our average length of follow-up was 17.6 years. In the meta-analysis by Geelen et al. (21), the summary relative risk for studies in which the difference between the highest and lowest intake categories was ≥ 7 times per month [0.78 (0.66-0.92)] was stronger than the overall summary relative risk. The results of our study, in which the median intakes were 2.2 times per month for the lowest category and 26.1 times per month for the highest category, are consistent with the findings from the meta-analysis.

Most of the potential confounders of the association between fish and colorectal cancer (such as BMI, physical activity, and multivitamin use) are most likely negative confounders, and not adjusting for these covariates would lead to relative risks that are lower (in absolute magnitude) than the "true" relative risk. Therefore, inverse associations could be explained by a lack of adjustment for these covariates. Of the two previous prospective studies on fish intake and colorectal cancer reporting statistically significant inverse associations, Norat et al. (6) adjusted for most potentially confounding variables whereas Kato et al. (8) did not. However, Norat et al. (6) was not able to adjust for colonoscopy

Table 3. Relative risk for colorectal cancer by dietary *n*-3 fatty acid intake from fish

	Dietary <i>n</i> -3 fatty acid intake				P_{trend}
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	
Cases	137	139	118	106	
Person-years	91,686	99,676	93,282	91,528	
Age-adjusted RR (95% CI)	1.00	0.95 (0.75-1.21)	0.84 (0.66-1.08)	0.79 (0.61-1.02)	0.05
Multivariate Adjusted RR* (95% CI)	1.00	0.93 (0.73-1.18)	0.81 (0.63-1.04)	0.76 (0.59-0.98)	0.02

*Adjusted for the same covariates as the multivariate model in Table 2.

Table 4. Relative risk for colorectal cancer for strata of aspirin assignment, BMI, and period by quartile of dietary *n*-3 fatty acid intake from fish

	Dietary intake of <i>n</i> -3 fatty acids from fish								<i>P</i> _{trend}
	Quartile 1		Quartile 2		Quartile 3		Quartile 4		
	Cases/ person-years	RR (95% CI)	Cases/ person-years	RR (95% CI)	Cases/ person-years	RR (95% CI)	Cases/ person-years	RR (95% CI)	
Aspirin									
No	73/46,358	1.00 (Ref)	78/49,824	1.00 (0.73-1.38)	53/46,488	0.72 (0.50-1.02)	55/45,295	0.79 (0.55-1.12)*	0.08
Yes	64/45,329	1.00 (Ref)	61/49,852	0.85 (0.60-1.21)	65/46,794	0.92 (0.65-1.30)	51/46,233	0.72 (0.50-1.05)*	0.12
BMI									
<25	69/53,728	1.00 (Ref)	71/57,094	0.99 (0.71-1.38)	55/54,549	0.77 (0.54-1.10)	59/53,925	0.85 (0.60-1.21) [†]	0.24
≥25	68/37,958	1.00 (Ref)	68/42,583	0.89 (0.64-1.25)	63/38,733	0.87 (0.62-1.23)	47/37,603	0.68 (0.47-0.98) [†]	0.04
Period									
Before 1995	79/53,729	1.00 (Ref)	72/58,066	0.87 (0.64-1.20)	67/54,369	0.82 (0.59-1.13)	72/53,656	0.89 (0.65-1.23) [‡]	0.54
1995 or later	58/43,126	1.00 (Ref)	67/47,208	1.03 (0.72-1.47)	51/44,139	0.82 (0.56-1.20)	34/43,263	0.58 (0.38-0.89) [‡]	0.005

Abbreviation: Ref, reference.

*Adjusted for the same covariates as the multivariate model in Table 2 except random assignment to aspirin or placebo ($P_{\text{interaction}} = 0.83$).

[†] Adjusted for the same covariates as the multivariate model in Table 2 except BMI ($P_{\text{interaction}} = 0.14$).

[‡] Adjusted for the same covariates as the multivariate model in Table 2 ($P_{\text{interaction}} = 0.18$).

utilization, another potential negative confounder. To our knowledge, the only study to examine potential confounding by colonoscopy utilization was that by Giovannucci et al. (17). This study reported no association between fish intake and colorectal cancer risk in an age- and sex-adjusted model and additionally adjusted for colonoscopy utilization but did not report the relative risk from this model.

There is also the possibility that some of the differences between studies could be explained by the type of fish consumed. Three of the prospective studies on fish intake and colorectal cancer risk that reported no association were conducted in Scandinavian countries (13, 15) or in Japan (12), where smoked and salted fish are regularly consumed, and these studies did not distinguish between types of fish in their analyses. In fact, Knekt et al. (20) reported a significant positive association between smoked and salted fish, as well as between calculated *N*-nitrosodimethylamine intake and colorectal cancer risk, and suggested that this finding may have been due to the nitrosamine content of these types of fish (56).

The finding of increased COX-2 expression in colorectal cancers and some colorectal adenomas (57, 58) provides support for the hypothesis that *n*-3 fatty acids could decrease colorectal cancer risk through the ability to inhibit the COX-2 enzyme and the production of eicosanoids derived from arachidonic acid, which could contribute to decreased cell proliferation and increased apoptosis (1). Fish oil supplementation has been reported to decrease proliferation in the rectal mucosa of patients with sporadic colorectal adenomas (59, 60) or to increase apoptosis in normal mucosa (61).

There are several limitations of this study that should be noted. First, fish intake was assessed only once on the 12-month follow-up questionnaire, and this measure may not be representative of fish intake over time. However, we would expect the resulting misclassification to be nondifferential and to bias relative risk estimates toward the null. In addition, PHS participants did not complete a full dietary assessment, and the inverse association observed with fish intake could be

due to some other unmeasured nutrient (such as vitamin D) that is correlated with fish intake.

There are also potential nondietary confounders that we were not able to take into account in our analyses. As mentioned previously, colonoscopy utilization is likely positively correlated with fish intake. We did examine the association between *n*-3 fatty acid intake from fish and colorectal cancer risk before and after 1995 to gauge the extent of potential confounding by colonoscopy utilization. Our results showed a stronger inverse association for cases occurring after 1995. However, there are other potential explanations for this difference, and confounding by colonoscopy utilization may not be the most likely explanation. Because the PHS is a relatively homogeneous group, there may be less confounding by colonoscopy utilization than there would be in the general population. In the Health Professionals Follow-up Study, a similar group of men, 22.7% of the men in the lowest quintile and 27.5% of the men in the highest quintile of fish intake had a history of endoscopy at baseline in 1986. Despite the fact that there is some difference across quintiles, history of endoscopy is generally not a strong confounder of diet-disease associations in this cohort. In addition, for colonoscopy utilization to explain this difference, this variable would need to be negatively associated with colorectal cancer risk. Whereas colonoscopy screening does lower colorectal cancer incidence in the long term through the removal of adenomas, this relationship may not be quite as clear in the early years of screening because it may also lead to the increased detection of slow-growing cancers. Another potential explanation for the difference in results before and after 1995 is that *n*-3 fatty acids from fish could exert a protective effect early in colorectal carcinogenesis. However, findings from studies of *n*-3 fatty acids and colorectal adenoma risk are also inconsistent. Busstra et al. (62) reported a nonsignificant inverse association between *n*-3 fatty acids measured in adipose tissue and the risk for colorectal adenoma. Using a measure of dietary *n*-3 fatty acid intake from a food-frequency questionnaire, Oh et al. (54) observed no association for total colorectal adenomas and a

nonsignificant inverse association for large adenomas. In addition, Giovannucci et al. (63) reported a nonsignificant inverse association between dietary fish intake and colorectal adenomas.

We also do not have information on total energy intake in this cohort. Adjustment for energy intake accounts for extraneous variation in nutrient intake that is due to its correlation with total energy intake (rather than true differences in diet composition) and also adjusts for confounding by energy intake. However, because long-chain *n*-3 fatty acids are found in a small number of foods, their correlation with total caloric intake is lower than other nutrients that are found in many foods. In the Health Professionals Follow-up Study, a similar cohort of men, the Spearman correlation between eicosapentaenoic acid and docosahexaenoic acid (the main long-chain *n*-3 fatty acids found in fish) and total energy intake is not strong ($r = 0.18$). Although we could not adjust for total energy intake, we did adjust for BMI (an important determinant of total energy intake) and physical activity (the major determinant of between-person variation in total energy intake).

Our findings may also be limited by the validity of the food-frequency questionnaire for assessing fish intake. The correlation with the dietary records was low for shrimp or shellfish ($r = 0.23$). The correlations were higher, although still modest, for the other fish items. Albert et al. (64) reported a strong inverse association between fish intake and risk for sudden death in this cohort; this result is consistent with the results from the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico randomized controlled trial (65) of *n*-3 fatty acids. This consistency lends support to the idea that these four food-frequency questionnaire items can discriminate fish intake between individuals. The correlation between fish intake and blood levels of long-chain *n*-3 fatty acids in this study is low. This could be partially explained by the fact that the food-frequency questionnaire and blood measures of intake reflected slightly different periods. The blood samples were donated at baseline and reflect intake for some period of time before then (somewhere between a few weeks and a few months). The abbreviated food-frequency questionnaire was completed at the 12-month follow-up, and participants reported average intake over the previous year.

Fish intake may also be a marker of decreased meat intake, which is associated with increased colorectal cancer risk (66). We addressed this issue by adjusting for red meat intake in our multivariate model and by examining the association between the ratio of red meat to fish intake and colorectal cancer risk. Although we did adjust for red meat intake, there is only a weak correlation between red meat and fish intake ($r = -0.08$) and no association between red meat intake and colorectal cancer risk [relative risk for highest versus lowest quintile of red meat intake was 0.91 (0.71-1.16)] in this cohort.

Given that colorectal cancer takes many years to develop, it is possible that some of our study participants could have had undiagnosed colorectal cancer at the time that fish intake was assessed. This could result in bias if the preclinical disease altered the self-report of fish intake. To address this possible issue, we conducted a sensitivity analysis excluding cases that developed

during the first 5 years of follow-up, and our results suggest little, if any, bias in our relative risks resulting from preclinical disease.

Lastly, the participants are U.S. physicians, most of whom are Caucasian. Although the results of this study may not be directly generalizable to other populations, it is unlikely that the biological mechanism underlying the association between *n*-3 fatty acids and colorectal cancer would differ between populations.

In conclusion, the results from this large prospective cohort of physicians followed for 22 years suggest that intake of fish and long-chain *n*-3 fatty acids from fish may decrease the risk for colorectal cancer. These findings are consistent with our previous study involving blood biomarker levels of long-chain *n*-3 fatty acids in a nested case-control study within the same cohort (39).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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