Blood Levels of Long-Chain Polyunsaturated Fatty Acids, Aspirin, and the Risk of Colorectal Cancer

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Abstract

Background: ***N*-3 fatty acids may decrease risk of colorectal cancer by inhibiting the cyclooxygenase-2 enzyme and production of proinflammatory eicosanoids derived from arachidonic acid (20:4*n*-6). Aspirin also inhibits the cyclooxygenase-2 enzyme and may share with *n*-3 fatty acids a potential mechanism to decrease the risk of colorectal cancer.**
Methods: **We conducted a nested case-control analysis using blood samples collected from the Physicians' Health Study participants in 1982 to 1984. *N*-3 and *n*-6 fatty acid levels were measured using gas-liquid chromatography for 178 men who developed colorectal cancer through December 31, 1995 and 282 age- and smoking-matched controls. We used conditional logistic regression to examine associations. All statistical tests were two-sided.**
Results: **Total long-chain *n*-3 fatty acids were nonsignificantly inversely associated with colorectal cancer risk [relative risk (RR) for highest versus lowest quartile, 0.60; 95% confidence interval (95% CI), 0.32 to 1.11; $P_{trend} = 0.10$], after adjustment for possible confounders. We observed potential interaction between randomized aspirin assignment and long-chain *n*-3 fatty acid levels ($P_{interaction} = 0.04$). Among men not on aspirin, RRs (95% CI) for increasing quartiles of long-chain *n*-3 fatty acids were 1.00 (reference), 0.60 (0.28-1.28), 0.51 (0.22-1.17), and 0.34 (0.15-0.82), $P_{trend} = 0.006$. For participants taking aspirin, there was no additional benefit of increasing *n*-3 fatty acid levels. The RR (95% CI) for the highest versus lowest quartile of *n*-6 fatty acids was 0.64 (0.35-1.17).**
Conclusions: **Blood levels of long-chain *n*-3 fatty acids were associated with decreased risk of colorectal cancer among men not using aspirin. *N*-6 fatty acids were nonsignificantly inversely associated with colorectal cancer risk.** (Cancer Epidemiol Biomarkers Prev 2007;16(2):314–21)

Introduction

The two classes of polyunsaturated fatty acids (PUFAs), *n*-3 [including α-linolenic acid, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA)] and *n*-6 [including linoleic acid (LA) and arachidonic acid (AA)], play essential roles in human nutrition and health. Sources of α-linolenic acid include flaxseed oil, soybean oil, canola oil, and walnuts, whereas the longer chain *n*-3 fatty acids, EPA and DHA, are mainly found in fish. Most vegetable oils are good sources of LA. Both ecologic and animal studies support an inverse association between the intake of *n*-3 fatty acids and the risk of colorectal cancer (1-7). However, the results of case-control and cohort studies have been inconsistent. Three studies (8-10) support an inverse association between dietary *n*-3 fatty acid intake and colon or colorectal cancer, whereas six others suggest a null association (11-16). In a recent nested case-control study, serum *n*-3 fatty acids were significantly inversely associated with colorectal cancer in males but not females (17).

N-3 fatty acids could potentially affect colorectal cancer development by several mechanisms, including the inhibition of both the cyclooxygenase-2 (COX-2) enzyme and production of eicosanoids derived from AA (18-23). *N*-3 and *n*-6 PUFAs compete for the desaturase and elongase enzymes, which convert α-linolenic acid to the longer chain EPA and DHA and also convert LA to AA (18, 24). In addition, there is competition between *n*-3 and *n*-6 fatty acids for the COX enzymes, which convert EPA to prostaglandin H_3 and AA to prostaglandin H_2 (25-27). Prostaglandin H_3 and prostaglandin H_2 are further converted to other prostaglandins and thromboxanes by additional enzymes. Eicosanoids derived from *n*-6 fatty acids are generally proinflammatory, whereas those derived from *n*-3 fatty acids are antiinflammatory (18, 28). Animal studies also support the hypothesis that high dietary intake of *n*-6 fatty acids increases the incidence of colon tumors in mice and rats treated with the carcinogen azoxymethane (5-7). This has led some researchers to suggest that *n*-6 fatty acids may increase the risk of colorectal cancer, possibly through the effects of these proinflammatory eicosanoids (22, 23). However, five studies (9, 12-15) that examined the association between dietary total *n*-6 or LA (the major dietary *n*-6 fatty acid) and colorectal cancer reported null associations, whereas one reported a nonsignificant positive association (16).

Because *n*-3 fatty acids can inhibit the COX-2 enzyme (3, 29), they may share with aspirin a potential mechanism to decrease the risk of colorectal cancer. Aspirin suppresses both the COX-1 and COX-2 enzymes, and regular aspirin use decreases the risk of both adenoma and colorectal cancer, possibly through its inhibition of COX-2, although other mechanisms have been proposed (30). A shared mechanism would imply that exposure to both would not materially enhance any protective effect over exposure to only one.

We examined the association between blood levels of both *n*-3 and *n*-6 PUFAs and colorectal cancer risk using a prospective, nested case-control design among participants in the Physicians' Health Study (PHS). This study began as a randomized controlled trial of aspirin, and this allowed for the evaluation of potential modification of these associations by aspirin intake.

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Materials and Methods

Study Population. This prospective case-control study was nested within the PHS, a randomized, double-blind, placebo-controlled factorial trial of aspirin (325 mg every other day) and β-carotene (50 mg on alternate days) supplementation for the primary prevention of cancer and cardiovascular disease. The participants were 22,071 male physicians aged 40 to 84 years at baseline in 1982, and with no history of myocardial infarction, stroke, transient ischemic attack, or cancer at the time of enrollment (31). The Institutional Review Board at Brigham and Women's Hospital (Boston, MA) approved the research protocol, and all subjects provided written informed consent. The aspirin component of the trial ended in January 1988 due to the finding of a substantial benefit of aspirin on the incidence of myocardial infarction (31). At that time, the participants had been followed for an average of 5 years. After the end of the aspirin component of the trial, participants chose whether they would receive aspirin or placebo along with their randomly assigned β-carotene treatment. Information on aspirin use was monitored on annual follow-up questionnaires, with 71% of men choosing to take aspirin on ≥3 days per week as of the 7-year follow-up questionnaire.

Before randomization, kits for blood sampling were sent to participants with instructions to have their blood drawn into EDTA-containing vacuum tubes and to centrifuge a portion of the samples to provide both plasma and whole blood. These samples were then placed on ice, sent to the study investigators by prepaid overnight courier, divided into aliquots, and stored at −80°C. Specimens were obtained from 14,916 physicians. Participants also provided information on height, weight, physical activity, multivitamin use, alcohol intake, and history of smoking and diabetes on the baseline questionnaire. The frequency of consumption of red meat, fish, cheese, and ice cream was obtained on the 12-month follow-up questionnaire. Questions inquiring about milk intake were included on the 18-month questionnaire.

Identification of Cases and Controls. The diagnosis of colorectal cancer was reported by participants on annual follow-up questionnaires. Medical records, including the pathology report, were then obtained and reviewed by the end-point committee for the PHS to confirm the diagnosis. Histologic details as well as the site and stage of the disease were documented from the medical record. Information on deaths was obtained from family members, postal authorities, and periodic searches of the National Death Index. Follow-up is over 99% complete for mortality and morbidity.

There were 178 confirmed cases of colorectal cancer through December 31, 1995, among those who provided baseline blood samples. We attempted to select two controls for each case using risk-set sampling. Controls were selected among participants who had provided a blood sample at baseline and had not reported a diagnosis of cancer (except non-melanoma skin cancer) at the time the case reported their diagnosis. Controls were matched on age (±1 year for younger participants, ±5 years for older participants) and smoking status (never, past, current). We were able to identify two controls for 104 of 178 cases, and therefore, a total of 282 participants were selected as controls.

Measurement of Blood Fatty Acids. Fatty acids in whole blood were extracted into an isopropanol-hexane solution containing 2.6-di-*tert*-butyl-*p*-cresol as an antioxidant and were transmethylated with methanol and sulfuric acid. After esterification, fatty acid methyl esters were redissolved in *iso*-octane and assessed by gas-liquid chromatography (32). Peak retention times were identified by injecting known standards, and purity ranges are all above 99% (NuCheck Prep, Elysium, MN), using ChemStation A.08.03 software (Agilent Technologies, Santa Clara, CA) for analysis. Randomly inserted quality-control samples (about 6% of the total real samples analyzed) were used to measure coefficients of variation (CVs) for all fatty acid peaks. Our quality-control samples were whole blood specimens from individuals who gave a blood sample before randomization and were subsequently not randomized into the PHS. These samples were collected at the same time and stored for the same number of years as the real samples.

Cases and their controls were analyzed in the same batch, and laboratory personnel were blinded to case, control, and quality-control status. From this analysis, we obtained information on levels of total *n*-3, *n*-6, saturated, monounsaturated, and trans-unsaturated fatty acids, as well as individual fatty acids [including α-linolenic acid, EPA, docosapentaenoic acid (DPA), DHA, LA, and AA]. The mean intra-assay CVs were 2.3% for α-linolenic acid, 5.4% for EPA, 4.5% for DPA, 2.7% for DHA, 2.3% for LA, and 1.8% for AA. We do not report interassay CVs because all samples were analyzed in the same run.

Statistical Analysis. We first compared the baseline risk factors for colorectal cancer between cases and controls using means or proportions. The correlation between baseline long-chain *n*-3 fatty acid levels and other fatty acids, as well as between long-chain *n*-3 fatty acids and fish intake, were calculated using the Spearman rank-correlation coefficient. Blood fatty acid levels were generally not normally distributed and were log transformed when used as continuous variables. Total and individual *n*-3 and *n*-6 fatty acid levels (as percent of total fatty acids) for cases and controls were compared using the Wilcoxon rank-sum test.

Blood levels of *n*-3 and *n*-6 fatty acids were divided into quartiles based on the distribution of these fatty acids in the controls. Estimates of the RR of colorectal cancer for quartiles of blood *n*-3 and *n*-6 fatty acids were obtained using conditional logistic regression to account for the matched case-control study design. Multivariate models adjusted for potential confounding factors, including body mass index (kg/m^2), randomized aspirin treatment assignment, diabetes, physical activity, multivitamin use, alcohol intake, red meat intake, dairy intake (including milk, cheese, and ice cream), and blood levels of other fatty acids. Tests for trend were done by assigning the median value to each quartile and modeling this as a continuous variable in separate regression models.

To assess potential effect modification by aspirin assignment, we used conditional logistic regression to estimate RRs of colorectal cancer for cross-tabulations of blood fatty acid quartile and aspirin assignment. To formally test for interaction, we included a term that was the product of blood fatty acid level (as a continuous variable) and aspirin assignment in a conditional logistic regression model and used a likelihood ratio test. We also examined the potential trend of the association between *n*-3 fatty acids and risk of colorectal cancer after stratifying by aspirin assignment. Stratification by aspirin breaks the matching of case-control pairs, and therefore, unconditional logistic regression adjusted for the matching factors as well as the covariates mentioned above was used to conduct this analysis and to test for trend within each stratum. In addition, we assessed potential effect modification by aspirin according to follow-up time. This was done using separate conditional logistic regression models, first including cases and their matched controls occurring before the end of the aspirin component of the trial and then including only cases and their matched controls occurring after the end of the aspirin component. To examine the joint effects of *n*-3 and *n*-6 fatty acid levels, we estimated relative risks for cross-tabulations of tertiles of these fatty acids. To test for interaction, we again used a likelihood ratio test to compare a model containing a term that was the product of *n*-3 and *n*-6 fatty acids to a model that

contained the main effects only. All statistical tests were two-sided, and SAS version 8.2 (SAS Institute, Cary, NC) was used for all statistical analyses.

Results

Table 1 shows the baseline characteristics and geometric mean blood *n*-3 and *n*-6 fatty acid levels among the 178 cases and 282 controls. There were no substantial differences in the distribution of baseline variables for these two groups. In addition, geometric mean blood levels of *n*-3 and *n*-6 fatty acids did not differ significantly between those who developed colorectal cancer and their matched controls. The Spearman correlation between blood levels of total long-chain *n*-3 fatty acids and fish intake was 0.24. For dark fish, the correlation was 0.35.

The RR of colorectal cancer by blood levels of total long-chain *n*-3 fatty acids is shown in Table 2. In the basic model accounting for only the matching factors, increasing level of total long-chain *n*-3 fatty acids was inversely related to the risk of colorectal cancer, although this relationship was not statistically significant (P_{trend} = 0.08). Results from the multivariate model adjusting for other potential confounding factors were not materially altered [RR for quartile 4 versus quartile 1, 0.60; 95% confidence interval (95% CI) = 0.32-1.11], and further adjustment for quartiles of dairy intake produced similar results (data not shown). Blood levels of long-chain *n*-3 fatty acids were moderately inversely correlated with total monounsaturated ($r = -0.40$) and total trans-unsaturated ($r = -0.35$) fatty acids. Adjustment for both monounsaturated and trans-unsaturated fatty acids did not substantially change the association between long-chain *n*-3 fatty acids and the risk of colorectal cancer (Table 2, multivariate model 2). The correlation between total long chain *n*-3 fatty acids and total *n*-6 PUFAs was weakly positive (r = 0.09). After additional adjustment of multivariate model 1 for the quartile of total *n*-6 PUFAs, the RRs (95% CI) of colorectal cancer for increasing quartiles of long-chain *n*-3 fatty acids were 0.88 (0.49-1.56), 0.77 (0.43-1.38), and 0.60 (0.32-1.12; P_{trend} = 0.09).

Table 1. Baseline characteristics and blood fatty acid levels of cases and controls

Characteristic	Cases (n = 178)	Controls (n = 282)
Age at randomization (y)	59.2 ± 8.8*	57.2 ± 8.0*
Smoking status (%)		
Never	36.5	39.4
Past	55.1	52.1
Current	8.4	8.5
Multivitamin use (%)		
Never	61.2	62.8
Past	15.2	16.3
Current	23.6	20.9
Diabetes (%)	3.9	1.8
Aspirin assignment (%)	48.3	49.7
Vigorous exercise (%)†		
<Once per week	25.3	25.2
1-4 times per week	56.7	58.9
≥5 times per week	18.0	16.0
Alcohol intake (%)		
≤Once per week	27.0	35.1
2-6 times per week	40.5	35.8
≥1 drink per day	32.6	29.1
Body mass index‡	25.1 ± 2.8*	24.7 ± 2.6*
Short-chain *n*-3 PUFAs§		
α-Linolenic	0.36 (0.35-0.38)§	0.36 (0.35-0.38)
Total long-chain *n*-3 PUFAs§	5.04 (4.90-5.19)	5.17 (5.05-5.29)
EPA	1.84 (1.79-1.90)	1.87 (1.83-1.92)
DHA	2.20 (2.11-2.28)	2.27 (2.20-2.34)
DPA	0.94 (0.92-0.97)	0.96 (0.94-0.98)
Total *n*-6 PUFAs§	37.44 (37.03-37.85)	37.82 (37.49-38.15)
LA	24.16 (23.80-24.53)	24.36 (24.05-24.67)
AA	9.77 (9.57-9.99)	9.93 (9.77-10.10)

*Means ± SD.
†Vigorous exercise defined as ''exercise vigorous enough to work up a sweat.''
‡Body mass index is equal to the weight in kilograms divided by the square of the height in meters.
§Geometric mean (95% CI) expressed as a percentage of total fatty acids.

The major individual long-chain *n*-3 fatty acids, EPA and DHA, were also inversely associated with colorectal cancer risk (Table 2). In addition, the RRs (95% CI) for increasing quartiles of DPA were 1.25 (0.72-2.16), 0.93 (0.52-1.66), and 0.69 (0.35-1.35; P_{trend} = 0.16). α-Linolenic acid was not associated with colorectal cancer risk [RRs (95% CI) for increasing quartiles, 1.25 (0.71-2.19), 1.04 (0.59-1.83), and 1.08 (0.61-1.91)]. This finding was not substantially altered by adjustment for long-chain *n*-3 fatty acids or total *n*-6 PUFAs.

We next assessed whether the association between total long-chain *n*-3 fatty acids and risk of colorectal cancer differed by aspirin assignment. Participants who were not assigned to aspirin and whose blood levels of long-chain *n*-3 fatty acids were in the lowest quartile served as the common reference group (Fig. 1). Compared with the common reference group, the RRs (95% CI) for increasing quartiles of long-chain *n*-3 fatty acids for those assigned to aspirin were 0.55 (0.26-1.15), 0.57 (0.24-1.35), 0.55 (0.25-1.21), and 0.54 (0.22-1.31). Long-chain *n*-3 fatty acids did not provide additional benefits for these participants. However, among those not assigned to aspirin, the RRs (95% CI) for increasing quartiles of long-chain *n*-3 fatty acids were 0.60 (0.28-1.28), 0.51 (0.22-1.17), and 0.34 (0.15-0.82). This interaction was statistically significant (P = 0.04). When we restricted the analysis to participants not assigned to aspirin, the RRs (95% CI) for increasing quartiles of long-chain *n*-3 fatty acids were 0.57 (0.26-1.23), 0.45 (0.20-1.04), and 0.30 (0.13-0.71; P_{trend} = 0.006). The corresponding RRs (95% CI) for participants assigned to aspirin were 1.14 (0.50-2.60), 1.02 (0.46-2.23), and 1.09 (0.48-2.50; P_{trend} = 0.90). We did not observe a statistically significant interaction between α-linolenic acid and aspirin assignment (data not shown).

We further assessed the above interaction for cases (and matched controls) diagnosed before and after January 1988, the time at which the aspirin component of the trial ended. There were 59 cases and 83 controls before and 119 cases and 199 controls after January 1988. Given that aspirin is hypothesized to act in the early promotion stages in the prevention of colorectal cancer, we would expect to see the interaction only in the analysis of cases and matched controls that occurred after the end of the aspirin component of the trial (because it is long-term aspirin use that is associated with decreased risk of colorectal cancer; ref. 33). For the analysis of cases and matched controls that occurred before the end of the aspirin component of the trial, we would expect little or no effect of aspirin (or *n*-3 fatty acids if they play a role during the same stage of carcinogenesis as aspirin). The numbers of cases and controls for the aspirin trial were too small to sustain a meaningful analysis. However, the post-aspirin results were similar to the overall results.

Our results show a nonsignificant inverse association between increasing levels of total *n*-6 fatty acids and colorectal cancer, which is similar in magnitude to the results for *n*-3 fatty acids. (Table 3). This nonsignificant inverse association persisted after adjustment for quartile of long-chain *n*-3 fatty acids. Both LA and AA were not significantly associated with the risk of colorectal cancer. For increasing quartiles, the RRs (95% CI) were 0.77 (0.45-1.33), 0.91 (0.54-1.54), and 0.86 (0.49-1.54) for LA and 1.12 (0.66-1.91), 1.01 (0.58-1.77), and 0.89 (0.50-1.57) for AA. There was no suggestion of an interaction between LA or AA and aspirin assignment (data not shown). The association between total *n*-6 fatty acids and colorectal cancer also did not differ by aspirin assignment ($P_{interaction}$ = 0.57).

Finally, there was no statistically significant interaction between *n*-3 and *n*-6 fatty acids (Table 4). However, results

from the cross-tabulations of *n*-3 and *n*-6 fatty acids suggested that *n*-3 fatty acids were most strongly inversely associated with colorectal cancer risk at low levels of *n*-6 fatty acids [RR (95% CI), 0.50 (0.20-1.24)]. In addition, *n*-6 fatty acids were most strongly inversely associated with colorectal cancer risk when *n*-3 fatty acid levels were low [RR (95% CI) = 0.47 (0.21-1.10)]. These findings do not support the hypothesized adverse effect of a high *n*-6 and low *n*-3 fatty acid intake.

Discussion

In this prospective, nested case-control study among U.S. physicians, we observed nonsignificant inverse associations of blood levels of long-chain *n*-3 and *n*-6 fatty acids with colorectal cancer risk. Thus, our results do not support the hypothesis that *n*-6 fatty acids may increase risk of colorectal cancer. In addition, we observed a possible interaction between aspirin assignment in the PHS trial and blood levels of *n*-3 fatty acids in association with colorectal cancer risk. Blood levels of long-chain *n*-3 fatty acids were significantly associated with decreased risk of colorectal cancer only among men not taking aspirin.

Although our finding of a possible interaction between blood levels of *n*-3 fatty acids and aspirin assignment provides support for the hypothesis that aspirin and *n*-3 fatty acids share a mechanism to decrease the risk of colorectal cancer, these results should be interpreted cautiously. Previous analyses in the PHS found no association between randomized aspirin assignment and colorectal cancer risk after 5 years (34) and 12 (35) years of follow-up. These null findings could be due to the low dose of aspirin used in this trial or to the relatively short period of randomly assigned supplementation. In a recent analysis from the Nurses' Health Study, Chan et al. (33) reported that aspirin use was significantly associated with the risk of colorectal cancer only after ≥10 years of regular use, and that the reduction in risk was greatest for women using more than 14 aspirin tablets per week. It is also possible that low-dose aspirin decreases the risk of colorectal cancer only among those with low *n*-3 fatty acid intake. In the only other study to examine this interaction, Oh et al. (36) found no modification of the effect of dietary long-chain *n*-3 fatty acids on the risk of distal colorectal adenoma by aspirin use.

Our findings are fairly consistent with the only other known prospective, nested case-control study to examine the association between biomarkers of *n*-3 fatty acid intake and

Table 2. Relative risk of colorectal cancer by quartile of baseline blood level of long-chain *n*-3 PUFAs

	Quartile of long-chain *n*-3 PUFAs				*P* value for trend
	1	2	3	4	
Number of cases	52	48	43	35	
Number of controls	70	71	71	70	
Fatty acid level (%)*					
Median	3.95	4.81	5.59	6.67	
Range	2.43-4.43	4.43-5.19	5.21-6.05	6.06-11.41	
Basic model (accounting for age and smoking status)					
Relative risk	1.0	0.83	0.77	0.60	0.08
95% confidence interval		0.49-1.42	0.44-1.34	0.33-1.07	
Multivariate model 1†					
Relative risk	1.0	0.81	0.74	0.60	0.10
95% confidence interval		0.46-1.42	0.41-1.32	0.32-1.11	
Multivariate model 2‡					
Relative risk	1.0	0.78	0.68	0.57	0.13
95% confidence interval		0.42-1.45	0.35-1.33	0.27-1.19	
	Quartile of EPA				*P* value for trend
	1	2	3	4	
Number of cases	41	56	50	31	
Number of controls	70	71	71	70	
Fatty acid level (%)*					
Median	1.42	1.75	2.01	2.52	
Range	0.89-1.55	1.56-1.88	1.89-2.21	2.21-4.07	
Multivariate model 1†					
Relative risk	1.0	1.22	1.09	0.60	0.14
95% confidence interval		0.69-2.14	0.59-1.98	0.29-1.23	
	Quartile of DHA				*P* value for trend
	1	2	3	4	
Number of cases	54	33	49	42	
Number of controls	70	71	71	70	
Fatty acid level (%)*					
Median	1.57	2.08	2.53	3.24	
Range	0.69-1.87	1.87-2.27	2.27-2.79	2.83-6.22	
Multivariate model 1†					
Relative risk	1.0	0.52	0.86	0.69	0.54
95% confidence interval		0.28-0.96	0.51-1.46	0.39-1.23	

NOTE: Long-chain n-3 PUFAs include EPA, DHA, DPA, 20:3*n*-3c, and 22:3*n*-3c.

*Levels are expressed as a percentage of total fatty acids.

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

‡Multivariate model 2 included the variables in multivariate model 1 plus the quartile of monounsaturated fatty acids and trans-unsaturated fatty acids.

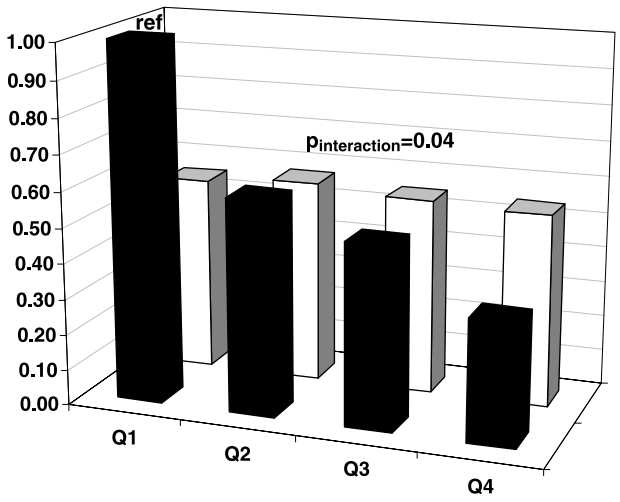

Figure 1. Relative risk of colorectal cancer by quartile of total long-chain *n*-3 fatty acids and aspirin assignment. *Abscissa*, quartile of long-chain *n*-3 fatty acids; *ordinate*, RR. ■, RRs for increasing quartiles of long-chain *n*-3 fatty acids among participants not assigned to aspirin [RRs (95% CI), 1.00; 0.60 (0.28-1.28); 0.51 (0.22-1.17); and 0.34 (0.15-0.82)]. □, RRs for increasing quartiles of long-chain *n*-3 fatty acids among participants assigned to aspirin [RRs (95% CI), 0.55 (0.26-1.15); 0.57 (0.24-1.35); 0.55 (0.25-1.21); and 0.54 (0.22-1.31)].

colorectal cancer risk. Kojima et al. (17) reported significant inverse associations for total *n*-3 fatty acids, α-linolenic acid, DPA, and DHA, in relation to colorectal cancer risk and a nonsignificant inverse association for EPA in men. We observed a null association for α-linolenic acid, and our overall results for total long-chain *n*-3 fatty acids, EPA, and DHA were not statistically significant. One potential explanation for the differences in results is a likely higher intake of *n*-3 fatty acids in the Japanese population, due to higher fish consumption, compared with our group of U.S. physicians (37).

Prospective data on the association between dietary *n*-3 and *n*-6 fatty acids and the risk of colorectal cancer are sparse. Kobayashi et al. (11) reported no association between dietary EPA and incidence of colorectal cancer incidence, and both Terry et al. (12) and Pietinen et al. (13) observed no association between dietary *n*-3 or *n*-6 fatty acids and the risk of colorectal cancer. No association for dietary *n*-3 fatty acids and a nonsignificant positive association for *n*-6 fatty acids were reported by Lin et al. (16). Bostick et al. (8) did observe an inverse association between dietary *n*-3 fatty acids and colorectal cancer incidence that was not statistically significant. Among the case-control studies, Nkondjock et al. (9) reported that dietary total *n*-3 fatty acids were inversely associated with colorectal cancer risk, the ratio of *n*-6 to *n*-3 fatty acids was positively associated with risk, and total *n*-6 fatty acids showed no association. A significant inverse association between total *n*-3 fatty acids and both colon and rectal cancer was reported by Tavani et al. (10), whereas Slattery et al. (14) observed no associations for *n*-3 or *n*-6 fatty acids in relation to the risk of colon cancer.

In addition, several studies have assessed the relationship between fish intake (the main dietary source of long-chain *n*-3 fatty acids) and colorectal cancer risk. Among the prospective cohort studies, seven reported inverse associations (8, 38-43), although only two (38, 40) of these were statistically significant. Another seven reported null associations (11, 13, 16, 44-47), and one reported a nonsignificant positive association (48). In a cohort study conducted in a Finnish population (49), a positive association was reported for smoked and salted fish, whereas intake of other fish was not associated with risk of colorectal cancer.

Larsson has reviewed several potential explanations for the inconsistent findings (18), including nondifferential misclassification of dietary *n*-3 fatty acid intake, which would tend to bias results toward the null, and low between-person variability in intake of *n*-3 fatty acids, which reduces the statistical power to find an association. Many studies have also failed to take into account the intake of *n*-6 fatty acids. This could potentially be an important factor given the metabolic interplay between *n*-3 and *n*-6 fatty acids. In addition, several studies have reported on the association between total fish intake and colorectal cancer risk when fatty fish intake may provide a better measure of long-chain *n*-3 fatty acid intake (18). In our study, we attempted to address some of these issues by adjusting for blood levels of *n*-6 fatty acids and examining the interaction between blood levels of *n*-3 fatty acids and aspirin, another factor that may alter the association between *n*-3 fatty acids and colorectal cancer risk. In addition, our *n*-3 fatty acid biomarker, although certainly not a perfect measure, may provide a more objective assessment of dietary

Table 3. Relative risk of colorectal cancer by quartile of baseline blood level of total *n*-6 PUFAs

	Quartile of total *n*-6 PUFAs				*P* value for trend
	1	2	3	4	
Number of cases	57	41	44	36	
Number of controls	70	71	71	70	
Fatty acid level (%)*					
Median	34.50	37.06	39.10	41.52	
Range	21.21-36.10	36.13-38.16	38.17-40.06	40.12-46.77	
Basic model (accounting for age and smoking status)					
Relative risk	1.0	0.74	0.78	0.64	0.14
95% confidence interval		0.44-1.25	0.46-1.33	0.37-1.12	
Multivariate model 1†					
Relative risk	1.0	0.81	0.79	0.64	0.16
95% confidence interval		0.45-1.44	0.45-1.39	0.35-1.17	
Multivariate model 2‡					
Relative risk	1.0	0.81	0.83	0.63	0.17
95% confidence interval		0.45-1.45	0.47-1.47	0.34-1.17	

*Levels are expressed as a percentage of total fatty acids.

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

‡Multivariate model 2 included the variables in multivariate model 1 plus the quartile of long-chain *n*-3 PUFAs.

Table 4. Multivariate relative risk of colorectal cancer by tertile of baseline blood level of *n*-3 and *n*-6 PUFAs

Level of long-chain *n*-3 PUFAs	Level of total *n*-6 PUFAs					
	Tertile 1		Tertile 2		Tertile 3	
	Number of cases/number of controls	RR (95% confidence interval)	Number of cases/number of controls	RR (95% confidence interval)	Number of cases/number of controls	RR (95% confidence interval)
Tertile 1	31/34	1.00 (reference)	18/27	0.77 (0.33-1.81)	15/33	0.47 (0.21-1.10)
Tertile 2	21/31	0.57 (0.25-1.30)	18/31	0.65 (0.28-1.51)	21/32	0.72 (0.32-1.62)
Tertile 3	14/29	0.50 (0.20-1.24)	21/36	0.58 (0.25-1.35)	19/29	0.62 (0.26-1.48)
			$P_{interaction} = 0.95$			

NOTE: Multivariate relative risk was adjusted for body mass index (<23, 23-24.99, 25-26.99, ≥27), multivitamin use (never use, past use, current use), history of diabetes, random assignment to aspirin or placebo, vigorous exercise (<once per week, 1-4 times per week, ≥5-6 times per week), alcohol intake (≤once per week, 2-6 times per week, ≥once per day), and quartile of red meat intake.

intake than would questionnaire-based information. We estimate that our whole blood PUFA measurements reflect intake over a period of time intermediate between fatty acid measurements from RBC membranes and from plasma. Although we are not aware of any data on the reliability of whole blood fatty acid measurements over time, Ma et al. (50) reported a short-term reliability coefficient (based on three blood samples collected at 2-week intervals) of 0.71 for PUFAs based on cholesterol ester measurements. This decreased to 0.32 when plasma phospholipid measurements were used. The long-term reliability coefficients (based on two samples collected ~3 years apart) for the various PUFAs ranged from 0.41 to 0.83 for the cholesterol ester measurements and from 0.35 to 0.81 for the phospholipid measurements.

The most widely investigated mechanisms by which *n*-3 fatty acids could potentially decrease the risk of colorectal cancer are by inhibiting the COX-2 enzyme and the production of eicosanoids derived from AA, which may lead to decreased cell proliferation and increased apoptosis (18, 19, 21, 22). Increased COX-2 expression has been observed in colorectal cancer and in some colorectal adenomas (51, 52). Clinical trials of fish oil supplementation in patients with sporadic colorectal adenomas have reported reduced proliferation in the rectal mucosa of these patients (53, 54). However, Cheng et al. (55) reported that higher *n*-3 fatty acid intake promotes apoptosis of normal colonic mucosa and has no effect on proliferation. Several other mechanisms by which *n*-3 fatty acids may decrease the risk of colorectal cancer have been proposed. These include the inhibition of ornithine decarboxylase, decreased bile acid excretion, altered protein kinase C activity, decreased NFκB activity, activation of peroxisome proliferator-activated receptor α and γ (PPARα and γ), and decreased nitric oxide production (18, 21-23).

Although eicosanoids derived from *n*-6 fatty acids are generally proinflammatory (18, 28) and therefore suggested to promote carcinogenesis (22, 23), some have been shown to have anticarcinogenic effects. For example, 13-*S*-hydroxyoctadecadienoic acid (13-S-HODE), which is the product of 15-lipoxygenase metabolism of LA, has been shown to be decreased in human colon cancers and to decrease cell proliferation and increase apoptosis in transformed colonic epithelial cells (56). In addition, Bull et al. (57) showed that 13-S-HODE activated the nuclear receptor PPARγ, which is important for differentiation of intestinal epithelial cells, in human colon cancer cell lines. 13-S-HODE has also been shown to inhibit PPARδ activation, leading to increased apoptosis in colon cancer cells (58).

There are limitations of this study that should be noted. First, we used a single baseline measurement for blood levels of *n*-3 fatty acids. This baseline measurement may not be fully representative of long-term *n*-3 fatty acid intake, and we were not able to account for changes in intake over the period of follow-up. In addition, the blood samples were stored at −80 C for ~20 years, and this could have resulted in some degradation of long-chain PUFAs. However, the New York University Women's Health Study (59) showed minimal effect of oxidation in serum samples stored for 7 to 12 years. Although there could have been greater oxidation in our whole blood samples due to the longer storage time or perhaps a greater presence of enzymes compared with plasma samples, our case and control samples were handled identically. As a result, we would expect the rate of oxidation to be the same in these two groups, and any oxidation that occurred would tend to attenuate the observed relative risks toward the null. In addition, the Spearman correlation between long-chain *n*-3 fatty acids and fish intake in our study was 0.24, and for dark fish, this correlation increased to 0.35. These correlations suggest that there was no serious degradation of our samples over time.

Another possible limitation of our study is the fact that not all PHS participants gave blood samples. This could have led to selection bias if those participants who donated blood samples were in some way different (overall healthier lifestyle, for example) than those who did not, and if this difference was associated with both exposure and outcome. However, we have previously compared the distributions of baseline risk factors for colorectal cancer in the blood subgroup with that of the full cohort and found them to be similar. In this prospective analysis, all comparisons are made within the group of physicians who provided blood samples.

We also do not have information on total energy intake in this cohort. Differences in caloric intake are mainly accounted for by varying levels of physical activity. Because physical activity is inversely associated with colorectal cancer risk, we would expect that caloric intake would also be inversely associated with colorectal cancer risk. If blood levels of *n*-3 fatty acids were positively correlated with total energy intake, lack of adjustment for energy intake could result in an observed relative risk that is lower than the true relative risk (i.e., negative confounding). Although we do not have information on total energy intake, plasma levels of *n*-3 fatty acids were not correlated with total energy intake in the Nurses' Health Study (October 30, 2006).[3] Therefore, the association between blood levels of *n*-3 fatty acids and colorectal cancer risk should not be confounded by total energy intake. In addition, we were not able to adjust for dietary fiber intake. This could result in unmeasured confounding by dietary fiber intake. However, the results of some recent prospective cohort studies have called into question whether dietary fiber decreases the risk of colorectal cancer (60, 61).

[3] Q. Sun, personal communication.

There is also the possibility that some of our cases could have had colorectal cancer at the time that the blood samples were collected. However, after excluding cases diagnosed during the first 3 years of follow-up (leaving 145 cases and 236 controls), the RRs (95% CI) from the multivariate model for increasing quartiles of total long-chain *n*-3 fatty acids were 0.71 (0.38-1.31), 0.62 (0.32-1.19), and 0.50 (0.25-0.99; $P_{trend} = 0.05$). Lastly, because this is an observational study, there is the possibility that our results may be affected by residual confounding or other unknown confounders. High blood levels of *n*-3 fatty acids may be a marker of other healthy lifestyle behaviors. To minimize this possibility, we controlled for several potentially confounding variables.

In summary, our findings suggest that long-chain *n*-3 fatty acids may decrease the risk of colorectal cancer, and that this association may be modified by aspirin use. In addition, our results do not support the hypothesis that *n*-6 fatty acids increase the risk of this disease, but rather suggest an inverse association. Further investigation of the possible interaction between long-chain *n*-3 fatty acids and aspirin is warranted.

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