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Long-chain omega-3 polyunsaturated fatty acid intake and risk of colorectal cancer

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Abstract

Research suggests that long-chain omega-3 polyunsaturated fatty acids (LC-PUFAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have anti-neoplastic properties, yet evidence for association between LC-PUFAs and colorectal cancer (CRC) remains inconsistent. Using the VITamins And Lifestyle (VITAL) cohort, we evaluated how EPA/DHA intake, and its primary sources, fish oil supplement use and dark fish consumption, relate to CRC risk. A total of 68,109 Washington residents aged 50-76 completed a questionnaire between 2000-2002 and were followed for CRC through 2008 (n=488). Persons using fish oil supplements on 4+days/week for 3+years experienced 49% lower CRC risk than non-users (HR:0.51; 95% CI:0.26-1.00; p-trend=0.06). The association between fish oil use and decreased CRC risk was primarily observed for men (p-interaction=0.02; p-trend men=0.02; p-trend women=0.88) and for colon cancer (p-difference=0.05; p-trend colon=0.03; p-trend rectum=0.87). While dark fish and total EPA+DHA intake were not associated with CRC risk overall, these associations varied by genetic risk (p-interaction=0.009 and 0.02, respectively), with inverse associations observed among low-moderate genetic risk groups and positive associations observed among high risk groups. Results suggest that associations between LC-PUFA intake and CRC may vary by gender, subsite, and genetic risk, providing additional insight into the potential role of LC-PUFAs in cancer prevention.

Introduction

Colorectal cancer (CRC) is the third most common cancer among men and women in the United States (1), and it is therefore important that we identify potential preventive agents. Inflammation has been implicated in the etiology of several cancers, including CRC, and has gained recent attention as a target for preventive efforts (2, 3).

Recent RCT evidence has demonstrated that long-chain omega-3 polyunsaturated fatty acids (PUFAs) eicosapentanenoic acid (EPA) and docosahexaenoic acid (DHA) reduce inflammation in humans (4, 5). These long-chain omega-3 PUFAs are found in dark fish and

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fish oil supplements, and may have additional anti-neoplastic properties, including anti-proliferative, pro-apoptotic, and anti-angiogenic effects (6-9).

Despite support for several anticancer mechanisms, observational data on the association between total long-chain omega-3 intake and CRC risk is inconsistent. While two meta-analyses have concluded that fish intake is associated with decreased risk of CRC (10, 11), two systematic reviews of omega-3 PUFAs on cancer risk qualitatively concluded that there is inadequate (12) or limited (13) evidence to suggest an association between long-chain omega-3 PUFA intake and CRC risk. Despite the number of studies conducted on dietary long-chain omega-3 PUFA intake and CRC, few studies have assessed the association between fish oil supplement use or total (diet+supplemental) long-chain omega-3 intake and CRC risk. This is important given the popularity of fish oil supplements (14) and the fact that these supplements contain high doses of EPA and DHA, allowing for a broader range of exposure potentially not observable when considering dietary exposure alone.

In this paper, we follow up on a previous finding from the VITamins And Lifestyle (VITAL) study which found that any use of fish oil supplements in the 10 years before baseline was associated with a reduced risk of CRC (15). With additional follow-up, we examine whether there is a exposure-response association between CRC and fish oil supplement use, and further evaluate whether CRC is associated with dark fish intake, dietary and total (diet+supplement) intake of EPA, DHA, EPA+DHA, and the omega-3 to omega-6 ratio. We also assess whether associations vary by anatomic subsite and by gender, body mass index (BMI), aspirin use, dietary fiber intake, history of polyps, and genetic risk.

Materials and Methods

Study Population

The study population includes participants of the VITAL Study, a prospective cohort of persons aged 50-76 years residing in the 13-county Western Washington catchment area of the Surveillance, Epidemiology, and End Results (SEER) cancer registry (16). Between October 2000 and November 2002, 364,418 potential participants identified by purchased commercial mailing list were mailed a 24-page questionnaire and reminder postcard. 77,719 persons returned the questionnaire and were deemed eligible for inclusion in the VITAL cohort. Study procedures were approved by the Fred Hutchinson Cancer Research Center Institutional Review Board.

We excluded persons with a history of CRC as of baseline (n=971), as well as those for whom this information was missing (n=213). Persons with history or unknown history of the following conditions were also excluded: ulcerative colitis or Crohn's disease (n=1030), intestinal polyposis (n=273), and malabsorptive syndromes (n=42). Additional exclusion criteria included diagnosis with *in situ* CRC over follow-up (n=12), cancer noted on death certificate only (n=1), and diagnosis with CRC of certain rare morphologies, such as malignant carcinoid tumors and lymphomas(n=33). Persons missing information on diet (n=6,890) and fish oil supplement use (n=236) were further excluded, leaving 68,109 persons for analyses.

Exposure

Supplemental fish oil use was ascertained by a series of questions about use of fish oil/omega-3 supplements in the 10-year period prior to baseline. Participants reporting use of fish oil/omega-3 supplements at least once a week for a year or more over the 10 years prior to baseline were asked to report the years of use and average number of days/week of use. From this information, we classified use into 3 categories (high use [4+ days/week for 3+ years], low use [<4 days/week or <3 years] or no use), with the high dose category including persons with both high frequency and duration of use.

Dark fish consumption and fatty acid intake were ascertained by a food-frequency questionnaire (FFQ) which captures frequency of intake and serving size of 120 foods and beverages (17). Participants were asked about their consumption of "dark fish such as salmon and fresh tuna" over the last year, from which we classified participants into quartiles of based on serving–size adjusted frequency. The University of Minnesota's Nutrition Coding Center Database was used to convert FFQ data into nutrient intake, including EPA and DHA intake. Participants were excluded from nutrient calculations if they did not complete all pages of the FFQ or if they reported abnormally high or low energy intake (men reporting <800 kcal/day or >5000 kcal/day; women reporting <600 kcal/day or >4000 kcal/day). For long-chain omega-3 intake (EPA, DHA, and EPA+DHA), we present results in terms of i) dietary intake determined by the FFQ, and ii) total dietary +supplement intake. In calculating g/day of long-chain omega-3 PUFA intake from supplements, we calculated the average days/week in which fish oil supplements were used over the 10-year period prior to baseline. We then incorporated information on average EPA (0.64 g) and DHA (0.35 g) contained within popular fish oil supplements to calculate estimated average dose of supplemental EPA, DHA, and EPA+DHA intake over the 10-year period prior to baseline (18). Average daily intake from dietary and supplement sources was summed to estimate total intake (g/day), with results presented in quartiles. We also present quartiles of the omega-3 to omega-6 ratio, with omega-3 intake defined by total EPA+DHA intake and omega-6 intake including dietary arachidonic acid and linoleic acid.

Potential confounders

Age and sex are included in all models, while multivariate analyses include factors associated with risk of CRC. These covariates were selected *a priori* and include: race/ethnicity (white, Hispanic, black, American Indian/Alaska Native, Asian or Pacific Islander, or other), educational status (high school graduate/GED or less, some college or technical school, or college graduate or above), BMI (kg/m^2; classified as normal weight[<25], overweight[25-<30], obese[30-<35], and severely obese[35+]), physical activity (no moderate/vigorous activity, sex-specific tertiles of activity), smoking history (never, quit 10+ years before baseline, quit <10 years before baseline, current), energy intake (quartiles), total calcium intake (quartiles of dietary+supplemental intake), alcohol consumption (none-<1 drink/month, 1 drink/month-<4drinks/week, >4 drinks week/-<2 drinks/day, 2+ drinks/day), multivitamin use (never, past, current), dietary fiber intake (quartiles), fruit/vegetable intake (quartiles), red/processed meat intake (quartiles), omega-6 intake (quartiles), hormone replacement therapy (never, former, current), as well as aspirin use and non-aspirin non-steroidal anti-inflammatory drug (NSAID) use (none, low, high use; high use defined by use

4+ days/week for 4+ years). Analyses also included adjustment for family history of CRC among 1st degree relatives (yes/no), history of sigmoidoscopy/colonoscopy in the 10 years prior to baseline (yes/no), and history of polyp excision (yes/no).

BMI was calculated from self-reported height and weight (kg/m^2) at baseline. For persons missing baseline BMI, but who reported height and weight at age 45 (n=1114), we estimated baseline BMI. This was achieved by first calculating the average BMI change/year within sex-age-race group among those with complete data, after which we applied the group-specific average BMI change/year to the number of years elapsing since age 45.

Physical activity (average MET-hours/week of moderate/vigorous activity) was ascertained by questions about activities in the 10-year period prior to baseline. Participants who reported engaging in a given activity regularly (at least 1 time/week for at least one year) in this 10-year period were asked to report on hours/day, days/week, and years of activity, plus intensity for walking. From this information, MET-hours/week of moderate/vigorous activity was calculated.

We also adjusted for conditions which may have increased fish oil supplement use or dark fish consumption, including: history of cardiovascular disease (coronary bypass surgery, angioplasty, angina, myocardial infarction, or stroke), hypercholesterolemia (use of cholesterol-lowering drugs), and memory loss (memory better/same as age 25, memory somewhat worse than age 25, or memory much worse than age 25).

Outcome

Cases include 488 incident, invasive cancers of the colon and rectum diagnosed between baseline and December 31, 2008. Cases were identified by linkage to the western Washington SEER registry, which uses information from area hospitals, state death certificates, and offices of oncologists, radiologists, and pathologists to identify cases. VITAL is linked to SEER annually in a largely automated process, with datasets matched on data items common to both datasets, such as: name, Social Security number, and date of birth. If too few data items are in common to ensure a match, the datasets are reviewed manually, with participants telephoned directly if needed.

Effect modifiers/Anatomic Subsite

We tested for effect modification and for difference across anatomic subsite (colon vs. rectum) for the following exposures: fish oil supplement use, dark fish consumption, and total long-chain omega-3 intake (EPA+DHA). Factors considered as potential effect modifiers include history of polyp excision (as this may represent a high-risk subgroup for chemoprevention) and factors associated with inflammation, including: gender, BMI (normal weight, overweight, obese), aspirin use (yes/no), and smoking status (never/quit >10 years prior to baseline vs current/quit within 10 years of baseline). Given that long-chain omega-3 PUFAs have anti-inflammatory effects, we hypothesized that the association between long-chain omega-3 PUFA intake and CRC would be strongest among persons with highest inflammation. Given recent evidence to suggest that dietary fiber may interact with long-chain omega-3 intake on CRC risk (19), we also tested for effect modification by dietary fiber intake (above/below median).

We also tested for effect modification by genetic risk score within a nested case-control study. Contributing cases and controls were selected in 2008 and DNA for genotyping was obtained from buccal swabs collected from cohort participants at the time of baseline questionnaire. Samples were processed at the Broad Institute using Illumina's HumanCytoSNP-12v2 beadchip, with samples excluded in the genotyping process if they had low sample volume, low sample concentration, gender mismatch, or call rate <97%. Of those successfully genotyped, duplicates, non-whites and principal components analysis outliers have been further excluded, leaving 250 cases and 260 controls for analyses. An overall genetic risk score was created by enumerating the number of risk alleles present at 16 single nucleotide polymorphisms (SNPs) located within known/recently-identified CRC susceptibility loci including: rs6691170(1q41) rs6687758(1q41), rs10936599(3q26.2), rs16892766(8q23.3), rs6983267(8q24.21), rs10795668(10p14), rs3802842(11q23.1), rs11169552(12q13.13), rs7136702(12q13.13), rs4444235(14q22.2), rs4779584(15q13.3), rs9929218(16q22.1), rs4939827(18q21.1), rs10411210(19q13.1), rs961253(20p12.3), and rs4925386(20q13.33) (20,21). The number of risk alleles present at each SNP was determined by either direct genotype or imputation as part of the Genetics and Epidemiology of Colorectal Cancer Consortium (GECCO). Two of these loci (1q41 and 12q13.13) include more than 1 SNP associated with CRC risk. We decided to include both SNPs at each of these loci in our risk score, as we did not find compelling evidence of correlation between these SNPs. In addition to the overall score, we also created an exploratory transforming growth factor-beta (TGF-β) risk score. This exploratory score included a subset of 6 SNPs (rs4444235, rs4779584, rs4939827, rs10411210, rs961253, rs4925386) included in the overall genetic risk score which have also been associated with the TGF-β pathway, a pathway important to various processes involved in carcinogenesis, including inflammation (20,23,23).

Statistical Analysis

Cox regression was used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) between exposures of interest and CRC risk, with age used as the time metric of analysis. Exposures and covariates were modeled using indicator variables; trend was assessed by use of a single variable with values 1,2,3, etc corresponding to the category of exposure. We tested for interaction using a single interaction term, with both exposure and effect modifier modeled using “trend” variables. Participants entered analysis at the time the baseline questionnaire was received and cases were followed until date of CRC diagnosis, while non-cases were censored at whichever occurred earliest: date of death (6.7%), date of emigration out of the SEER catchment area (5.5%), date of requested removal from study (0.03%), or December 31st, 2008 (87.8%). Deaths occurring within the state of Washington were identified by linkage to the state death file, while emigrations out of area were identified by linkage to the National Change of Address System and by active follow-up involving telephone calls and mailings. Study participants have been followed for an average of 6.7 years.

In determining the subsite-specific HRs associated with colon cancer and rectal cancer, cases of the opposite subsite were censored at the date of diagnosis. To determine the statistical significance of subsite-specific differences, logistic regression was used to model

the association between exposure and outcome (colon cancer vs rectal cancer; non-cases excluded), with the p-trend corresponding to the p-difference across subsite. For analyses involving genetic risk score, we developed a reduced multivariate model by first deciding on a base model of covariates, after which we included additional covariates which changed the beta for interaction by >10%. Our final model for these analyses included age, sex, first 3 principal components to adjust for population substructure, energy intake, and alcohol consumption. All analyses were conducted using Stata (version 12, College Station, TX).

Results

Analyses include 68,109 persons, among whom 488 cases of CRC occurred. In minimally-adjusted models presented in Table 1, increasing age and BMI were associated with increased risk of CRC. Risk of CRC declined with increasing educational status, physical activity, calcium intake, fiber intake, and fruit/vegetable intake. Recent smokers and current smokers were observed to be at increased risk of CRC, as were persons consuming >2 alcoholic drinks/week. High consumption of red/processed meat intake was also associated with increased CRC risk, while aspirin use, HRT, and history of sigmoidoscopy/colonoscopy were associated with decreased risk.

As compared to non-users, persons using fish oil supplements for 4+ days/week for 3+ years had reduced risk of CRC (HR: 0.51, 95% CI: 0.21, 1.00), though the test for trend was not significant (p-trend: 0.06) (Table 2). Dark fish consumption was not associated with CRC risk, nor was dietary or total (diet+supplemental) EPA, DHA or EPA+DHA intake. The omega-3 to omega-6 ratio was not associated with CRC risk.

We conducted a sensitivity analysis in which we evaluated whether the association between the omega-3 to omega-6 ratio and CRC varied if the omega-6 term was defined by arachidonic acid alone or linoleic acid alone, as opposed to total omega-6 PUFA intake. This change made little difference (data not shown).

We also conducted sensitivity analyses for associations between CRC and fish oil supplement use, dark fish consumption, and total omega-3 intake in which we excluded the first two years of follow-up. Exclusion of early follow-up strengthened results: the HR for high use of fish oil supplements strengthened to 0.38 (95% CI: 0.16, 0.93, p-trend:0.05), the HR for highest vs. lowest quartile of dark fish consumption was 0.66 (95% CI: 0.45, 0.99, p-trend:0.15), and the HR for the highest vs. lowest quartile of total EPA+DHA was 0.83 (95% CI: 0.58,1.20; p-trend:0.26).

We observed significant interaction (p-interaction: 0.02) between fish oil supplement use and gender, with increasing dose associated with reduced CRC risk among men (p-trend: 0.02), but not women. As shown in Table 3, male high supplement users experienced 78% lower risk of CRC than non-users (HR: 0.22; 95% CI: 0.06-0.90). Furthermore, we observed significant interaction between dark fish consumption and overall genetic risk (p=0.009) and between total EPA+DHA intake and overall genetic risk (p=0.02) (Table 4). Increasing dark fish consumption and increasing total EPA+DHA intake were inversely associated with CRC risk among persons in the lowest two tertiles of genetic risk, while positive

associations were observed among those in the highest tertile of genetic risk. When the genetic risk score was limited to 6 SNPs associated with the TGF-β pathway, no interaction was observed (results not shown). Given limited sample size of this nested case-control study, we were unable to assess interaction between genetic risk and dose of fish oil supplement use. No other interactions (BMI, aspirin use, dietary fiber intake, smoking status, history of polyp excision) were statistically significant (results not shown).

In subsite-specific analyses, increasing fish oil supplement use was associated with reduced risk of colon cancer, but not rectal cancer (p for difference: 0.05): high supplement users experienced a 63% lower risk of colon cancer than non-users (HR: 0.37; 95% CI: 0.15-0.91; p for trend 0.03) (Table 5). Neither dark fish intake nor total EPA+DHA intake was associated with cancer at either site (Table 5), and no difference in association was observed between proximal and distal colon cancers (results not shown).

Discussion

In this prospective study, persons using fish oil supplements for 4+ days/week for 3+ years had about half the risk of CRC of non-users, with the observed association driven by colon cancer rather than rectal cancer and by findings for men more than women. While dark fish consumption and long-chain omega-3 intake were not associated with CRC risk overall, these associations were significantly modified by genetic risk (p-interaction:0.009 and 0.02, respectively), with inverse associations observed in the low/moderate genetic risk groups and positive associations observed in the high genetic risk group.

Several epidemiologic studies have addressed the association between CRC and fish intake or dietary long-chain omega-3 PUFA intake. Two meta-analyses have reported significant inverse association between fish intake and CRC risk: a 2007 meta-analysis reported a relative risk (RR) of 0.88 (95% CI: 0.78, 1.00) comparing persons of the highest and lowest fish consumption categories (11), while a 2012 meta-analysis reported an odds ratio (OR) of 0.86 (95% CI: 0.79-0.95) (10). While our findings were not statistically significant, these point estimates are comparable in magnitude to the HR observed in our study for dark fish intake (HR: 0.77; 95% CI: 0.55, 1.07). Furthermore, it should be noted that in the 2012 meta-analysis referenced above, the association was stronger in case-control studies (OR: 0.83; 95% CI: 0.72-0.95) than in cohort studies (HR: 0.93, 95% CI: 0.86, 1.01) (10). A previous systematic review of prospective studies in 2006 concluded that there was no evidence to suggest an association between omega-3 PUFA intake and CRC risk (12), though overall point estimates were not presented. An updated systematic review of recently published studies (including both cohort and case-control studies) similarly concluded that there is only limited suggestion of an association between long-chain omega-3 PUFA intake and CRC risk (13), again not presenting an overall effect estimate. In part, these observed inconsistencies may reflect differences in exposure contrast across studies, as there is some suggestion that studies conducted within high exposure populations reveal stronger associations than studies with less exposure contrast (24). Results from the 2006 meta-analysis by Geelen *et al* support this notion: a pooled RR of 0.95 was observed comparing highest to lowest fish consumption groups among ‘low exposure contrast’ studies in which the highest and lowest quartiles were separated by <7 fish eating occasions/month (95% CI:

0.81, 1.11), while a RR of 0.78 was observed among 'high exposure contrast' studies in which the highest and lowest quartiles were separated by 7+ fish eating occasions/month (95% CI: 0.66, 0.92) (11).

While our analyses of dietary exposures may have been influenced by insufficient exposure contrast, analysis of fish oil supplement use may allow for greater contrast, as these supplements contain high EPA and DHA levels. It is estimated that persons using fish oil supplements on 4+days/week consume more than 0.56 grams of supplementary EPA+DHA per day, a much higher threshold of exposure than the cut-point for the upper quartile of dietary EPA+DHA intake in our study (>0.29 grams EPA+DHA/day). We observed a stronger association between fish oil supplement use and CRC than was observed for dietary variables: persons who reported fish oil supplement use on 4+ days/week for 3+years experienced 49% lower CRC risk than non-users (HR: 0.51; 95% CI: 0.26, 1.00) (p-trend: 0.06). To make our results comparable across exposures in terms of EPA+DHA intake, we conducted a sensitivity analysis in which we further divided the fourth quartile of dietary EPA+DHA into two groups (>0.29-≤0.56 grams EPA+DHA/day and >0.56 grams EPA +DHA/day), with those in the highest exposure group (>0.56 grams/day EPA+DHA) consuming comparable amounts of EPA+DHA as high fish oil supplement users. We observed that the association between dietary EPA+DHA intake and CRC was strongest among persons consuming >0.56 grams of dietary EPA+DHA/day (HR: 0.77; 95% CI: 0.46-1.28), though it should be noted that this HR is not as strong as the HR corresponding to high fish oil supplement use (HR: 0.51; 95% CI: 0.26, 1.00). The association between fish oil supplement use and CRC risk remains relatively unexplored in the literature, with the association only previously assessed in the VITAL cohort (15). In an analysis following persons for CRC through 2006, Satia *et al* reported that any use of fish oil supplements in the 10-year period prior to baseline was associated with 35% reduced risk of CRC (HR: 0.65; 95% CI:0.42-0.99) (15). Here, we have 2 additional years of follow-up, providing more statistical power to assess a dose-response relationship and subgroup and subsite specific differences, though inclusion of additional follow-up likely increased measurement error, as cohort members may have changed supplement use over time. A few studies have reported on the association between total (diet+supplement) long-chain omega-3 intake and CRC either by questionnaire of diet and supplement use or by use of blood biomarkers. Results from the questionnaire-based study suggest a significant inverse association between total (dietary+supplement) EPA intake and CRC (25), though results from two small nested case-control studies using blood biomarkers are less strong, with one reporting a non-significant inverse association (26) and another reporting a non-significant inverse association among men only (27).

In our study, the association between fish oil supplement use and CRC risk varied by gender (p-interaction:0.02) and by anatomic subsite (p-difference:0.05), with significant association observed among men (p-trend:0.02), but not among women. Results from prior epidemiologic studies have been inconsistent, with two reporting an inverse association among men only (27,28), one an inverse association for women only (29), and another reporting no difference by gender (30). We also observed the association between fish oil supplement use and cancer risk to vary over anatomic subsite, with increasing fish oil use

supplement use associated with reduced risk of colon cancer (p-trend: 0.03), but not associated with risk of rectal cancer. Given that aspirin is more strongly associated with colon cancer than rectal cancer(31), observation of stronger association with colon cancer might be expected as both aspirin and fish oil supplements are thought to reduce inflammation through the cyclooxygenase pathway (32). Our finding is supported by a previous cohort study by Sasazuki *et al* in which marine omega-3 PUFA intake was significantly associated with reduced risk of proximal colon cancer among men, but not rectal cancer, though significance of subsite-specific differences was not presented and no difference was apparent among women (29). However, the literature is not consistent on this issue, and these results stand in contrast to two meta-analyses of fish consumption and CRC risk, with one reporting a stronger effect estimate for cancers of the rectum than for the colon (10), and another reporting comparable associations across subsite (11).

Lastly, we observed a significant interaction between overall genetic risk based on previously identified CRC susceptibility loci and i) dark fish consumption (p-interaction: 0.009), and ii) total EPA+DHA intake (p-interaction:0.02). In both of these analyses, inverse associations were limited to the low/moderate genetic risk groups while positive associations were observed in the high genetic risk group (Table 4). Our observation of a positive association between long-chain omega-3 intake and CRC risk among persons with high genetic load is supported by a recent study conducted among persons with Lynch Syndrome (32), a form of hereditary CRC caused by mutations in DNA mismatch repair genes. In this prospective cohort, study authors observed that fish oil supplement users had a 1.74-fold higher risk of colorectal adenoma than non-users (95% CI: 1.00-3.01) (33). While the association between long-chain omega-3 intake and CRC has not been previously studied in terms of effect modification by overall genetic risk, it is notable that prior studies have also shown the association between omega-3 intake and cancer risk to be modified by genetic variation. For example, a recent case-control study suggested that genetic variation at a tagging SNP in the *PARP* gene may modify the association between marine omega-3 intake and rectal cancer, with inverse association observed between marine omega-3 intake and rectal cancer among persons of the wild-type, and positive association observed among those with variation at this SNP (34). Additional observational research suggests that genetic variation in inflammation-related genes may modify the associations between long-chain omega-3 intake and risk of cancers of the colon and rectum (35) and colon polyp formation (36). Our results, in combination with these prior studies, suggest that the association between long-chain omega-3 intake and CRC risk may vary by underlying genetic risk, a point which may merit further attention when considering the potential role of long-chain omega-3 PUFAs in cancer prevention.

Research indicates that long-chain omega-3 PUFAs have several biologic effects which may be relevant to CRC prevention. Animal models have demonstrated that omega-3 PUFA-rich diets reduce the release of inflammatory biomarker, PGE_2(37), and despite initially inconclusive small trials (38,39), two recent RCTs have shown omega-3 PUFA supplementation reduces inflammation in humans (4, 5). Epidemiologic studies corroborate this growing body of evidence, with a recent study observing that regular fish oil supplement users have significantly lower levels of inflammation measured by high-sensitivity C-

reactive protein (hsCRP) than non-users (40). Omega-3 PUFAs are thought to reduce inflammation by competitively inhibiting pro-inflammatory omega-6 PUFAs via competition for cyclooxygenase enzyme activity and by displacement of omega-6 PUFA stores from cell membranes (41-43). Furthermore, *in vitro* studies demonstrate that omega-3 PUFAs inhibit the activity of nuclear factor kappa B (NFkB), a transcription factor central to the inflammatory cascade and which has been implicated in the etiology of several cancers (43,44).

Beyond posited anti-inflammatory effects, *in vitro* and animal studies suggest that these long-chain omega-3 PUFAs may also have several additional anti-neoplastic effects, including inhibition of tumor growth or increased apoptosis (6, 7,45) and suppression of angiogenesis (9). Furthermore, animal models have demonstrated that omega-3 PUFAs reduce the incidence of azoxymethane-induced colon tumors in rats by increasing cell differentiation and apoptosis (45). A recent small randomized trial in humans suggests that these mechanisms may extend beyond the animal model: after 3 months of treatment, persons supplemented with EPA had decreased mucosal proliferation and increased apoptosis at colonoscopy as compared to those in the control group (8).

A limitation of this study is that we were unable to assess changes in exposure over follow-up. Given that fish oil supplement use has increased over recent years (14), we might expect more non-users to become users over time. If a genuine association exists, one would expect the lack of additional exposure assessment to attenuate the association towards the null. Another limitation is the narrow range of exposure for dietary variables, limiting our ability to create substantial exposure contrast and detect significant association, potentially explaining why the association between fish oil supplement dose and CRC risk is stronger than other observed associations. Furthermore, those with symptoms of CRC at baseline may have changed their diet, and in order to address this concern of reverse causality, we conducted a sensitivity analysis excluding the first two years of follow-up. The associations strengthened upon exclusion of early follow-up, with the association between fish oil supplement use and CRC becoming statistically significant. Given that a substantial change was observed for fish oil supplement use, a behavior unlikely to be affected by early symptoms of CRC, it seems more likely that this difference is related to the etiologically relevant time frame. By including the first two years of follow-up, we may have diluted results by including cases unlikely to have been impacted by exposure at baseline. However, given the *a priori* decision to treat this as a sensitivity analysis, emphasis should be placed on the full cohort.

In addition to the above-listed limitations, our study has several strengths. Notably, we were able to assess the associations between CRC and fish oil supplement use and total (dietary +supplemental) intake of long-chain omega-3 PUFAs. Furthermore, the FFQ used to assess dietary intake was enriched to better ascertain fatty acid intake, while many previous studies have not considered non-fish sources of EPA and DHA and many have studied total fish intake with no distinction made between fish of high and low EPA/DHA content (24). Lastly, the use of a cohort specifically designed to study the association between supplement use and cancer provided detailed information on exposures and covariates of interest, including omega-6 intake and reasons for why persons may increase omega-3 intake. Apart

from results of fish oil supplements, these methodologic advantages did not lead to stronger results than have been observed in similar studies.

In our study, we were able to examine both dietary and supplementary sources of omega-3 PUFAs in a large prospective study. Furthermore, we were able to examine these associations across anatomic subsite and across risk subgroups. These subgroup and subsite specific differences merit further attention, as it is possible that future preventive efforts may focus on specific subgroups. Given the relative popularity of fish oil supplements and the high doses of long-chain omega-3 PUFAs in these supplements, future research is needed to better understand the association between fish oil supplement use and CRC risk.

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References

1. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA Cancer J Clin. 2010 Sep-Oct;60(5): 277–300. [PubMed: 20610543]
2. Cai Q, Gao YT, Chow WH, et al. Prospective study of urinary prostaglandin E2 metabolite and colorectal cancer risk. J Clin Oncol. 2006 Nov; 24(31):5010–6. [PubMed: 17075120]
3. Schottenfeld D, Beebe-Dimmer J. Chronic inflammation: a common and important factor in the pathogenesis of neoplasia. CA Cancer J Clin. 2006 Mar-Apr;56(2):69–83. [PubMed: 16514135]
4. Ebrahimi M, Ghayour-Mobarhan M, Rezaiean S, et al. Omega-3 fatty acid supplements improve the cardiovascular risk profile of subjects with metabolic syndrome, including markers of inflammation and auto-immunity. Acta Cardiol. 2009 Jun; 64(3):321–7. [PubMed: 19593941]
5. Micallef MA, Garg ML. Anti-inflammatory and cardioprotective effects of n-3 polyunsaturated fatty acids and plant sterols in hyperlipidemic individuals. Atherosclerosis. 2009 Jun; 204(2):476–82. [PubMed: 18977480]
6. Latham P, Lund EK, Johnson IT. Dietary n-3 PUFA increases the apoptotic response to 1,2-dimethylhydrazine, reduces mitosis and suppresses the induction of carcinogenesis in the rat colon. Carcinogenesis. 1999 Apr; 20(4):645–50. [PubMed: 10223194]
7. Clarke RG, Lund EK, Latham P, Pinder AC, Johnson IT. Effect of eicosapentaenoic acid on the proliferation and incidence of apoptosis in the colorectal cell line HT29. Lipids. 1999 Dec; 34(12): 1287–95. [PubMed: 10652988]
8. Courtney ED, Matthews S, Finlayson C, et al. Eicosapentaenoic acid (EPA) reduces crypt cell proliferation and increases apoptosis in normal colonic mucosa in subjects with a history of colorectal adenomas. Int J Colorectal Dis. 2007 Jul; 22(7):765–76. [PubMed: 17216221]
9. Calviello G, Di Nicuolo F, Gragnoli S, et al. n-3 PUFAs reduce VEGF expression in human colon cancer cells modulating the COX-2/PGE2 induced ERK-1 and -2 and HIF-1alpha induction pathway. Carcinogenesis. 2004 Dec; 25(12):2303–10. [PubMed: 15358633]
10. Wu S, Feng B, Li K, et al. Fish Consumption and Colorectal Cancer Risk in Humans: A Systematic Review and Meta-analysis. Am J Med. 2012 Jun; 125(6):551–9. e5. [PubMed: 22513196]
11. Geelen A, Schouten JM, Kamphuis C, et al. Fish consumption, n-3 fatty acids, and colorectal cancer: a meta-analysis of prospective cohort studies. Am J Epidemiol. 2007 Nov; 166(10):1116–25. [PubMed: 17823383]
12. MacLean CH, Newberry SJ, Mojica WA, et al. Effects of omega-3 fatty acids on cancer risk: a systematic review. JAMA. 2006 Jan; 295(4):403–15. [PubMed: 16434631]

13. Gerber M. Omega-3 fatty acids and cancers: a systematic update review of epidemiological studies. Br J Nutr. 2012 Jun; 107(2):S228–39. [PubMed: 22591896]
14. Wu CH, Wang CC, Kennedy J. Changes in herb and dietary supplement use in the U.S. adult population: a comparison of the 2002 and 2007 National Health Interview Surveys. Clin Ther. 2011 Nov; 33(11):1749–58. [PubMed: 22030445]
15. Satia JA, Littman A, Slatore CG, Galanko JA, White E. Associations of herbal and specialty supplements with lung and colorectal cancer risk in the VITamins and Lifestyle study. Cancer Epidemiol Biomarkers Prev. 2009 May; 18(5):1419–28. [PubMed: 19423520]
16. White E, Patterson RE, Kristal AR, et al. VITamins And Lifestyle cohort study: study design and characteristics of supplement users. Am J Epidemiol. 2004 Jan; 159(1):83–93. [PubMed: 14693663]
17. Patterson RE, Kristal AR, Tinker LF, Carter RA, Bolton MP, Agurs-Collins T. Measurement characteristics of the Women's Health Initiative food frequency questionnaire. Ann Epidemiol. 1999 Apr; 9(3):178–87. [PubMed: 10192650]
18. Kristal AR, Brasky TM, Lampe JE, Patterson RE, White E. Dietary intake of specific fatty acids and breast cancer risk among postmenopausal women in the VITAL cohort. Under Review.
19. Kato I, Majumdar AP, Land SJ, Barnholtz-Sloan JS, Severson RK. Dietary fatty acids, luminal modifiers, and risk of colorectal cancer. Int J Cancer. 2010 Aug; 127(4):942–51. [PubMed: 19998336]
20. He J, Wilkens LR, Stram DO, et al. Generalizability and epidemiologic characterization of eleven colorectal cancer GWAS hits in multiple populations. Cancer Epidemiol Biomarkers Prev. 2011 Jan; 20(1):70–81. [PubMed: 21071539]
21. Houlston RS, Cheadle J, Dobbins SE, et al. Meta-analysis of three genome-wide association studies identifies susceptibility loci for colorectal cancer at 1q41, 3q26.2, 12q13.13 and 20q13.33. Nat Genet. 2010 Nov; 42(11):973–7. [PubMed: 20972440]
22. Slattery ML, Lundgreen A, Herrick JS, Wolff RK, Caan BJ. Genetic variation in the transforming growth factor-β signaling pathway and survival after diagnosis with colon and rectal cancer. Cancer. 2011 Sep; 117(18):4175–83. [PubMed: 21365634]
23. Lubbe SJ, Whiffin N, Chandler I, Broderick P, Houlston RS. Relationship between 16 susceptibility loci and colorectal cancer phenotype in 3146 patients. Carcinogenesis. 2012 Jan; 33(1):108–12. [PubMed: 22045029]
24. Larsson SC, Kumlin M, Ingelman-Sundberg M, Wolk A. Dietary long-chain n-3 fatty acids for the prevention of cancer: a review of potential mechanisms. Am J Clin Nutr. 2004 Jun; 79(6):935–45. [PubMed: 15159222]
25. Theodoratou E, McNeill G, Cetnarskyj R, et al. Dietary fatty acids and colorectal cancer: a case-control study. Am J Epidemiol. 2007 Jul; 166(2):181–95. [PubMed: 17493949]
26. Hall MN, Campos H, Li H, et al. Blood levels of long-chain polyunsaturated fatty acids, aspirin, and the risk of colorectal cancer. Cancer Epidemiol Biomarkers Prev. 2007 Feb; 16(2):314–21. [PubMed: 17301265]
27. Kojima M, Wakai K, Tokudome S, et al. Serum levels of polyunsaturated fatty acids and risk of colorectal cancer: a prospective study. Am J Epidemiol. 2005 Mar; 161(5):462–71. [PubMed: 15718482]
28. Theodoratou E, McNeill G, Cetnarskyj R, et al. Dietary fatty acids and colorectal cancer: a case-control study. Am J Epidemiol. 2007 Jul; 166(2):181–95. [PubMed: 17493949]
29. Sasazuki S, Inoue M, Iwasaki M, et al. Intake of n-3 and n-6 polyunsaturated fatty acids and development of colorectal cancer by subsite: Japan public health center-based prospective study. Int J Cancer. 2011 Oct 1; 129(7):1718–29. [PubMed: 21120874]
30. Butler LM, Wang R, Koh WP, Stern MC, Yuan JM, Yu MC. Marine n-3 and saturated fatty acids in relation to risk of colorectal cancer in Singapore Chinese: a prospective study. Int J Cancer. 2009 Feb; 124(3):678–86. [PubMed: 18973226]
31. Rothwell PM, Wilson M, Elwin CE, et al. Long-term effect of aspirin on colorectal cancer incidence and mortality: 20-year follow-up of five randomised trials. Lancet. 2010 Nov; 376(9754):1741–50. [PubMed: 20970847]

32. Botma A, Nagengast FM, Braem MG, et al. Body mass index increases risk of colorectal adenomas in men with Lynch syndrome: the GEOLynch cohort study. J Clin Oncol. 2010 Oct; 28(28):4346–53. [PubMed: 20733131]
33. Heine-Broring RC, Winkels RM, Botma A, van Duijnhoven FJB, Jung AY, et al. Dietary supplement use and colorectal adenoma risk in individuals with Lynch Syndrome: The GEOLynch Cohort Study. PloS ONE. 2013; 8:e66819. [PubMed: 23825568]
34. Stern MC, Butler LM, Corral R, et al. Polyunsaturated fatty acids, DNA repair single nucleotide polymorphisms and colorectal cancer in the Singapore Chinese Health Study. J Nutrigenet Nutrigenomics. 2009; 2(6):273–9. [PubMed: 20559012]
35. Habermann N, Ulrich CM, Lundgreen A, et al. PTGS1, PTGS2, ALOX5, ALOX12, ALOX15, and FLAP SNPs: interaction with fatty acids in colon cancer and rectal cancer. Genes Nutr. 2012 Jun.
36. Poole EM, Bigler J, Whitton J, et al. Genetic variability in prostaglandin synthesis, fish intake and risk of colorectal polyps. Carcinogenesis. 2007 Jun; 28(6):1259–63. [PubMed: 17277229]
37. Mollard RC, Gillam ME, Wood TM, Taylor CG, Weiler HA. (n-3) fatty acids reduce the release of prostaglandin E2 from bone but do not affect bone mass in obese (fa/fa) and lean Zucker rats. J Nutr. 2005 Mar; 135(3):499–504. [PubMed: 15735084]
38. Balk EM, Lichtenstein AH, Chung M, Kupelnick B, Chew P, Lau J. Effects of omega-3 fatty acids on serum markers of cardiovascular disease risk: a systematic review. Atherosclerosis. 2006 Nov; 189(1):19–30. [PubMed: 16530201]
39. Fritsche K. Fatty acids as modulators of the immune response. Annu Rev Nutr. 2006; 26:45–73. [PubMed: 16848700]
40. Kantor ED, Lampe JW, Vaughan TL, Peters U, Rehm CD, White E. Association between use of specialty dietary supplements and C-reactive protein concentrations. American Journal of Epidemiology. 2012; 176(11):1002–13. [PubMed: 23139249]
41. Chapkin RS, Kim W, Lupton JR, McMurray DN. Dietary docosahexaenoic and eicosapentaenoic acid: emerging mediators of inflammation. Prostaglandins Leukot Essent Fatty Acids. 2009 Aug-Sep;81(2-3):187–91. [PubMed: 19502020]
42. Wall R, Ross RP, Fitzgerald GF, Stanton C. Fatty acids from fish: the anti-inflammatory potential of long-chain omega-3 fatty acids. Nutr Rev. 2010 May; 68(5):280–9. [PubMed: 20500789]
43. Zhao Y, Joshi-Barve S, Barve S, Chen LH. Eicosapentaenoic acid prevents LPS-induced TNF-alpha expression by preventing NF-kappaB activation. J Am Coll Nutr. 2004 Feb; 23(1):71–8. [PubMed: 14963056]
44. Li Q, Withoff S, Verma IM. Inflammation-associated cancer: NF-kappaB is the lynchpin. Trends Immunol. 2005 Jun; 26(6):318–25. [PubMed: 15922948]
45. Chang WL, Chapkin RS, Lupton JR. Fish oil blocks azoxymethane-induced rat colon tumorigenesis by increasing cell differentiation and apoptosis rather than decreasing cell proliferation. J Nutr. 1998 Mar; 128(3):491–7. [PubMed: 9482754]

Abbreviations

95% CI	95% confidence interval
BMI	body mass index
CRC	colorectal cancer
EPA	eicosapentaenoic acid
DHA	docosahexaenoic acid
FFQ	food-frequency questionnaire
GECCO	Genetics and Epidemiology of Colorectal Cancer Consortium
hsCRP	high-sensitivity C-reactive protein

HR	hazard ratio
LC-PUFAs	long-chain omega-3 polyunsaturated fatty acids
NSAID	non-steroidal anti-inflammatory drug
RR	relative risk
SEER	Surveillance, Epidemiology, and End Results
TGF-β	transforming growth factor-beta
VITAL	VITamins And Lifestyle study

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Table 1

Participant Characteristics and Age and Sex-Adjusted Risk Ratios of Colorectal Cancer Risk, the VITAL Study (*n*=68,109)

	Cohort n=68,109 *n* (%)	Cases n=488 *n (%)*	Age and Sex Adjusted Risk Ratios[a]	95% Confidence Interval
Demographic				
Age at Baseline (years)				
50- <55	16,383 (24.1)	49 (10.0)	1.00	Ref
55- <60	15,805 (23.2)	63 (12.9)	1.36	0.93, 1.97
60- <65	12,516 (18.4)	82 (16.8)	2.24	1.57, 3.19
65- <70	11,020 (16.2)	131 (26.8)	4.08	2.94, 5.68
70+	12,385 (18.2)	163 (33.4)	4.64	3.37, 6.38
Sex				
Female	34,745 (51.0)	230 (47.1)	1.00	Ref
Male	33,364 (49.0)	258 (52.9)	1.17	0.98, 1.40
Lifestyle/Diet				
Body Mass Index (kg/m^2)				
Normal Weight (<25)	22,769 (34.1)	141 (29.6)	1.00	Ref
Overweight (≥25-<30)	27,750 (41.3)	189 (39.6)	1.09	0.88, 1.36
Obese (≥30-<35)	11,053 (16.6)	96 (20.1)	1.51	1.17, 1.97
Severely Obese (≥35)	5,358 (8.03)	51 (10.7)	1.92	1.39, 2.65
Smoking Status				
Never Smoker	32,256 (47.8)	193 (40.0)	1.00	Ref
Former Smoker (Quit 10+ Years Before Baseline)	25,160 (37.3)	196 (40.7)	1.13	0.92, 1.39
Former Smoker (Quit <10 Years Before Baseline)	4,516 (6.70)	42 (8.71)	1.67	1.19, 2.33
Current Smoker	5,543 (8.21)	51 (10.6)	1.73	1.27, 2.36
Dietary Fiber Intake (g/day)				
Quartile 1: <12.4	17,028 (25.0)	129 (26.4)	1.00	Ref
Quartile 2: 12.4-<17.4	17,027 (25.0)	140 (28.7)	1.01	0.79, 1.29
Quartile 3: 17.4-<23.7	17,027 (25.0)	116 (23.8)	0.80	0.62, 1.04
Quartile 4: 23.7+	17,027 (25.0)	103 (21.1)	0.68	0.52, 0.90
Medication				
Aspirin Use[c]				

	Cohort n=68,109 *n* (%)	Cases n=488 *n (%)*	Age and Sex Adjusted Risk Ratios[a]	95% Confidence Interval
Non-user	36,345 (54.8)	284 (59.9)	1.00	Ref
Low	15,636 (23.6)	106 (22.4)	0.77	0.62, 0.96
High	14,331 (21.6)	84 (17.7)	0.55	0.43, 0.70
Other				
History of Polyp Excision				
No	59,717 (87.7)	423 (86.7)	1.00	Ref
Yes	8,392 (12.3)	65 (13.3)	0.88	0.67, 1.14
Genetic Risk Score (number risk alleles)[d]				
Lowest Risk (<14.5)	96 (36.9)	74 (29.6)	1.00	Ref
Mid Risk (14.5- <16.9)	86 (33.1)	84 (33.6)	1.28	0.83. 1.98
High Risk (16.9+)	78 (30.0)	92 (36.8)	1.52	0.99, 2.33

Abbreviations: 95% CI (95% confidence interval)

[a]Hazard ratios presented for all risk factors except genetic risk score, for which odds ratios are presented

[b]Tertiles of physical activity among those engaging in moderate/vigorous leisure time physical activity determined within gender; women (T1: <2.81; T2: 2.81-<9.48; T3: 9.48+); men (T1: <-4.38; T2: 4.38- <13.3; T3: 13.3+)

[c]Use of aspirin (including both low-dose and regular aspirin) and non-aspirin NSAID defined by use over 10 years prior to baseline, with low use defined as use <4 days per week or <4 years, and high use defined as 4+ days per week for 4+ years

[d]Genetic risk score determined by risk score generated from 16 single nucleotide polymorphisms associated with colorectal cancer risk (260 controls. 250 cases); in addition to adjusting for age and sex, genetic risk scores are additionally adjusted for population substructure; the first column corresponds to the number of controls in this nested case control study

Table 2

Hazard Ratios (HR) of Colorectal Cancer Associated with Fish Oil Supplement Use, Dark Fish Consumption, and Long-Chain Omega-3 Polyunsaturated Fatty Acid Intake

	Cohort	Cases	Age and Sex Adjusted		Multivariate Adjusted [a]	
	n=68,109 N (%)	*n*=488 N (%)	HR	95% CI	HR	95% CI
Fish Oil Supplement Use [b]						
Average 10-yr use						
No Use	61,936 (90.1)	456 (93.4)	1.00	Ref	1.00	Ref
Low Use (<4 days/week or <3 yrs)	3,806 (5.59)	21 (4.30)	0.78	0.51, 1.22	0.93	0.59, 1.49
High Use (≥4 days/week and ≥3 yrs)	2,907 (4.27)	11 (2.25)	0.48	0.26, 0.87	0.51	0.26, 1.00
			P-trend: 0.009		*P-trend: 0.06*	
Dietary Fish Consumption [b]						
Dark Fish (Salmon +Tuna) (servings/week)						
Quartile 1: None	24,021 (35.3)	202 (41.4)	1.00	Ref	1.00	Ref
Quartile 2: >0 - <0.26	15,978 (23.5)	107 (21.9)	0.79	0.62, 0.99	0.88	0.68, 1.14
Quartile 3: 0.26-<0.80	16,178 (23.8)	118 (24.2)	0.85	0.68, 1.07	1.07	0.83, 1.38
Quartile 4: 0.80+	11,932 (17.5)	61 (12.5)	0.62	0.46, 0.82	0.77	0.55, 1.07
			P-trend: 0.002		*P-trend: 0.40*	
Eicosapentaenoic Acid (EPA) [b]						
Dietary EPA (g/day)						
Quartile 1: <0.02	17,028 (25.0)	141 (28.9)	1.00	Ref	1.00	Ref
Quartile 2: 0.02-<0.05	17,027 (25.0)	118 (24.2)	0.82	0.64, 1.05	0.89	0.68, 1.17
Quartile 3: 0.05-<0.09	17,027 (25.0)	111 (22.8)	0.77	0.60, 0.99	0.93	0.70, 1.23
Quartile 4: 0.09+	17,027 (25.0)	118 (24.2)	0.79	0.62, 1.02	0.99	0.74, 1.33
			P-trend: 0.05		*P-trend: 1.00*	
Total EPA [d] (g/day)						
Quartile 1: <0.03	17,029 (25.0)	146 (29.9)	1.00	Ref	1.00	Ref
Quartile 2: 0.03-<0.06	17,026 (25.0)	116 (23.8)	0.78	0.61, 1.00	0.86	0.66, 1.13
Quartile 3: 0.06-<0.11	17,027 (25.0)	117 (24.0)	0.78	0.61, 1.00	0.96	0.72, 1.26
Quartile 4: 0.11+	17,027 (25.0)	109 (22.3)	0.71	0.55, 0.91	0.91	0.67, 1.22

	Cohort	Cases	Age and Sex Adjusted		Multivariate Adjusted [a]	
	***n*=68,109 N (%)**	***n*=488 N (%)**	**HR**	**95% CI**	**HR**	**95% CI**
			P-trend: 0.01		*P-trend: 0.67*	
Docosahexaenoic Acid (DHA) [b]						
Dietary DHA (g/day)						
Quartile 1: <0.05	17,028 (25.0)	146 (29.9)	1.00	Ref	1.00	Ref
Quartile 2: 0.05-<0.11	17,027 (25.0)	122 (25.0)	0.82	0.64, 1.04	0.90	0.69, 1.17
Quartile 3: 0.11-<0.19	17,027 (25.0)	112 (23.0)	0.75	0.59, 0.96	0.94	0.71, 1.23
Quartile 4: 0.19+	17,027 (25.0)	108 (22.1)	0.71	0.55, 0.91	0.89	0.66, 1.21
			P-trend: 0.005		*P-trend: 0.56*	
Total DHA [d] (g/day)						
Quartile 1: <0.05	17,028 (25.0)	145 (29.7)	1.00	Ref	1.00	Ref
Quartile 2: 0.05-<0.11	17,027 (25.0)	127 (26.0)	0.86	0.68, 1.09	0.96	0.74, 1.25
Quartile 3: 0.11-<0.21	17,027 (25.0)	116 (23.8)	0.78	0.61, 1.00	0.98	0.74, 1.29
Quartile 4: 0.21+	17,027 (25.0)	100 (20.5)	0.66	0.51, 0.85	0.86	0.63, 1.16
			P-trend: 0.001		*P-trend: 0.38*	
EPA + DHA [b]						
Dietary EPA + DHA (g/day)						
Quartile 1: <0.08	17,123 (25.1)	145 (29.7)	1.00	Ref	1.00	Ref
Quartile 2: 0.08- <0.16	17,103 (25.1)	124 (25.4)	0.84	0.66, 1.06	0.91	0.70, 1.19
Quartile 3: 0.16- <0.29	16,870 (24.8)	110 (22.5)	0.75	0.58, 0.96	0.92	0.69, 1.22
Quartile 4: 0.29+	17,013 (25.0)	109 (22.3)	0.72	0.56, 0.93	0.92	0.68, 1.24
			P-trend: 0.007		*P-trend: 0.61*	
Total EPA +DHA [d] (g/day)						
Quartile 1: <0.08	17,134 (25.2)	142 (29.1)	1.00	Ref	1.00	Ref
Quartile 2: 0.08- <0.17	16,955 (24.9)	126 (25.8)	0.88	0.69, 1.12	1.00	0.77, 1.31
Quartile 3: 0.17- <0.32	17,037 (25.0)	120 (24.6)	0.83	0.65, 1.06	1.07	0.81, 1.41
Quartile 4: 0.32+	16,983 (24.9)	100 (20.5)	0.67	0.52, 0.87	0.88	0.65, 1.20
			P-trend: 0.003		*P-trend: 0.56*	
Total EPA/DHA to Omega-6 Ratio [b,c]						
Quartile 1: <0.007	17,007 (25.0)	141 (28.9)	1.00	Ref	1.00	Ref

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	Cohort	Cases	Age and Sex Adjusted		Multivariate Adjusted [a]	
	***n*=68,109 N (%)**	***n*=488 N (%)**	**HR**	**95% CI**	**HR**	**95% CI**
Quartile 2: 0.007- <0.01	17,021 (25.0)	137 (28.1)	0.96	0.76, 1.21	1.06	0.82, 1.38
Quartile 3: 0.01- <0.03	17,047 (25.0)	113 (23.2)	0.79	0.62, 1.02	1.01	0.77, 1.33
Quartile 4: 0.03+	17,034 (25.0)	97 (19.9)	0.68	0.52, 0.88	0.85	0.63, 1.16
			P-trend: 0.001		*P-trend: 0.33*	

Abbreviations: DHA (docosahexaenoic acid); EPA (eicosapentaenoic acid); HR (hazard ratio); 95% CI (95% confidence interval).

[a] Multivariate analyses include 59,500 study participants, including 419 cases

[b] Multivariate model adjusted for age, sex, race/ethnicity, education, BMI, energy intake, MET-hours per week of moderate/vigorous activity, alcohol intake, smoking history, multivitamin use, calcium intake, dietary fiber intake, fruit and vegetable intake, red/processed meat intake, aspirin use, non-aspirin NSAID use, family history of colorectal cancer, history of sigmoidoscopy/colonoscopy, history of polyps, hormone replacement therapy, cardiovascular disease, memory loss, use of cholesterol-lowering drugs, and omega-6 (linoleic +arachidonic) intake

[c] Ratio of (total EPA + DHA) to (lineolic acid + arachidonic acid)

[d] Includes both dietary intake and supplementary intake (estimated from fish oil supplement use)

Table 3

Hazard Ratios (HR) of Colorectal Cancer Associated with Fish Oil Supplement Use, Dark Fish Consumption, and Total EPA+DHA, by Gender

	Male			Female			P-interaction
	Case/Cohort	**HR**	**95% CI**	**Case/Cohort**	**HR**	**95% CI**	
10-yr Fish Oil Use[a]							
No Use	224/27,654	1.00	Ref	167/26,000	1.00	Ref	0.02
Low Use (<4 days/week or <3 years)	6/1,410	0.66	0.29,1.49	13/1,891	1.16	0.66,2.05	
High Use (≥4 days/week and ≥3 yrs)	2/1,271	0.22	0.06,0.90	7/1,274	0.85	0.39,1.80	
		P-trend: 0.02			*P-trend: 0.88*		
Dark Fish (Salmon +Tuna)(servings/week)[a]							
Quartile 1: None	94/10,409	1.00	Ref	77/10,440	1.00	Ref	0.87
Quartile 2: >0 - <0.26	50/7,300	0.82	0.58,1.16	42/6,687	0.96	0.65,1.41	
Quartile 3: 0.26-<0.80	64/7,205	1.18	0.85,1.64	42/6,956	0.91	0.62,1.34	
Quartile 4: 0.80+	24/5,421	0.69	0.43,1.11	26/5,082	0.86	0.54,1.38	
		P-trend: 0.57			*P-trend: 0.49*		
Total EPA +DHA (g/day)[a,b]							
Quartile 1: <0.08	54/5,719	1.00	Ref	61/9,044	1.00	Ref	0.72
Quartile 2: 0.08- <0.17	50/6,588	0.84	0.57,1.25	58/8,131	1.14	0.79,1.65	
Quartile 3: 0.17- <0.32	67/8,187	1.01	0.70,1.47	43/6,824	1.08	0.72,1.64	
Quartile 4: 0.32+	61/9,841	0.89	0.59,1.32	25/5,166	0.82	0.50,1.34	
		P-trend: 0.78			*P-trend: 0.56*		

Abbreviations: DHA (docosahexaenoic acid); EPA (eicosapentaenoic acid); HR (hazard ratio); 95% CI (95% confidence interval).

[a]Multivariate model adjusted for age, sex, race/ethnicity, education, BMI, energy intake, MET-hours per week of moderate/vigorous activity, alcohol intake, smoking history, multivitamin use, calcium intake, dietary fiber intake, fruit and vegetable intake, red/processed meat intake, aspirin use, non-aspirin NSAID use, family history of colorectal cancer, history of sigmoidoscopy/colonoscopy, history of polyps, hormone replacement therapy, cardiovascular disease, memory loss, use of cholesterol-lowering drugs, and omega-6 (linoleic +arachidonic) intake

[b]Includes both dietary intake and supplementary intake (estimated from fish oil supplement use)

Table 4

Odds Ratios (OR) of Colorectal Cancer Associated with Dark Fish Consumption and Total EPA+DHA Intake, by Genetic Risk[a]

	Overall			Low Genetic Risk			Mid-Genetic Risk			High-Genetic Risk			
	Case/Control	OR	95% CI	Case/Control	OR	95% CI	Case/Control	OR	95% CI	Case/Control	OR	95% CI	P
Dark Fish (servings/week)[b]													
Quartile 1: None	91/76	1.00	Ref	29/27	1.00	Ref	30/21	1.00	Ref	32/28	1.00	Ref	*0.009*
Quartile 2: >0 -<0.26	41/53	0.62	0.37,1.06	14/18	0.63	0.24,1.67	10/18	0.25	0.08,0.75	17/17	0.92	0.35,2.39	
Quartile 3:0.26<0.80	59/54	0.87	0.52,1.44	14/23	0.47	0.19,1.21	24/21	0.55	0.21,1.49	21/10	2.16	0.80,5.80	
Quartile 4: 0.80+	25/43	0.41	0.22,0.76	5/19	0.13	0.04,0.48	8/15	0.14	0.04,0.53	12/9	1.59	0.51,4.97	
		p-trend:0.02			*p-trend:0.002*			*p-trend:0.01*			*p-trend:0.17*		
Total EPA +DHA (g/day)[b,c]													
Quartile 1: <0.08	58/60	1.00	Ref	16/22	1.00	Ref	23/18	1.00	Ref	19/20	1.00	Ref	*0.02*
Quartile 2:0.08<0.17	60/54	1.15	0.68,1.97	20/18	1.40	0.51,3.87	17/20	0.69	0.26,1.81	23/16	1.82	0.66,5.00	
Quartile 3: 0.17<0.32	48/58	0.82	0.47,1.44	16/20	0.84	0.30,2.34	17/20	0.36	0.12,1.05	15/18	1.07	0.37,3.10	
Quartile 4: 0.32+	50/54	0.92	1.51,1.66	10/27	0.23	0.07,0.78	15/17	0.43	0.12,1.41	25/10	5.79	1.79,18.7	
		p-trend: 0.54			*p-trend:0.02*			*p-trend:0.08*			*p-trend:0.01*		

Abbreviations: DHA (docosahexaenoic acid); EPA (eicosapentaenoic acid); OR (odds ratio); 95% CI (95% confidence interval).

[a] Genetic risk defined by number of risk alleles at 16 SNPs at susceptibility loci shown to be associated with colorectal cancer risk (see Methods).

[b] Adjusted for age, sex, principal components (population substructure), energy intake, alcohol intake (see Methods).

[c] Includes both dietary intake and supplementary intake (estimated from fish oil supplement use).

Table 5

Hazard Ratios (HR) of Colorectal Cancer Associated with Fish Oil Supplement Use, Dark Fish Consumption, and Total EPA+DHA Intake, by Subsite

	Colon Cancer(n=311)			Rectal Cancer (n=108)			P for difference
	Case/Cohort	HR	95% CI	Case/Cohort	HR	95% CI	
10-yr Fish Oil Use[a]							
No Use	293/53,654	1.00	Ref	98/53,654	1.00	Ref	0.05
Low Use (<4 days/week or <3 yrs)	13/3,301	0.84	0.48,1.47	6/3,301	1.22	0.53,2.82	
High Use (≥4 days/week and ≥3 yrs)	5/2,545	0.37	0.15,0.91	4/2,545	0.98	0.35,2.69	
		P trend: 0.03			*P trend: 0.87*		
Dark Fish (Salmon +Tuna): (servings/week)[a]							
Quartile 1: None	126/20,849	1.00	Ref	45/20,849	1.00	Ref	0.46
Quartile 2: >0 - <0.26	67/13,987	0.88	0.65,1.19	25/13,987	0.86	0.53,1.42	
Quartile 3: 0.26-<0.80	85/14,161	1.18	0.88,1.57	21/14,161	0.77	0.45,1.33	
Quartile 4: 0.80+	33/10,503	0.71	0.47,1.06	17/10,503	0.90	0.50,1.64	
		P trend: 0.53			*P trend: 0.53*		
Total EPA +DHA (g/day)[a,b]							
Quartile 1: <0.08	90/14,763	1.00	Ref	25/14,763	1.00	Ref	0.39
Quartile 2: 0.08- <0.17	81/14,719	0.98	0.72,1.33	27/14,719	1.10	0.63,1.91	
Quartile 3: 0.17- <0.32	83/15,011	1.06	0.77,1.46	27/15,011	1.11	0.63,1.97	
Quartile 4: 0.32+	57/15,007	0.78	0.54,1.12	29/15,007	1.22	0.67,2.21	
		P trend:0.30			*P trend: 0.53*		

Abbreviations: DHA (docosahexaenoic acid); EPA (eicosapentaenoic acid); HR (hazard ratio); 95% CI (95% confidence interval).

[a]Multivariate model adjusted for age, sex, race/ethnicity, education, BMI, energy intake, MET-hours per week of moderate/vigorous activity, alcohol intake, smoking history, multivitamin use, calcium intake, dietary fiber intake, fruit and vegetable intake, red/processed meat intake, aspirin use, non-aspirin NSAID use, family history of colorectal cancer, history of sigmoidoscopy/colonoscopy, history of polyps, hormone replacement therapy, cardiovascular disease, memory loss, use of cholesterol-lowering drugs, and omega-6 (linoleic +arachidonic) intake.

[b]Includes both dietary intake and supplementary intake (estimated from fish oil supplement use).