Consumption of Fish and Long-chain n-3 Polyunsaturated Fatty Acids is Associated With Reduced Risk of Colorectal Cancer in a Large European Cohort

Short title: Fish, n-3 LC-PUFA and colorectal cancer

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Abbreviations used: BMI, Body mass index; CI, confidence interval; DHA, Docosahexaenoic acid; DPA, Docosapentaenoic acid; ENDB, EPIC Nutrient Database; EPA, Eicosapentaenoic acid; EPIC, European Prospective Investigation into Cancer and Nutrition; FAME, Fatty acid methyl ester; HR, Hazard ratio; IARC, International Agency for Research on Cancer; LC-PUFA, long-chain polyunsaturated fatty acid; MSI, microsatellite instability; OR, Odds ratio; USDA, United States Department of Agriculture; WCRF, World Cancer Research Fund

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Data sharing statement: For information on how to submit an application for gaining access to EPIC data and/or biospecimens, please follow the instructions at http://epic.iarc.fr/access/index.php

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Abstract

Background & Aims: There is an unclear association between intake of fish and long-chain n-3 polyunsaturated fatty acids (n-3 LC-PUFAs) and colorectal cancer (CRC). We examined the association between fish consumption, dietary and circulating levels of n-3 LC-PUFAs, and ratio of n-6:n-3 LC-PUFA with CRC using data from the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort.

Methods: Dietary intake of fish (total, fatty/oily, lean/white) and n-3 LC-PUFA were estimated by food frequency questionnaires given to 521,324 participants in the EPIC study; among these, 6291 individuals developed CRC (median follow up, 14.9 years). Levels of phospholipid LC-PUFA were measured by gas chromatography in plasma samples from a sub-group of 461 CRC cases and 461 matched individuals without CRC (controls). Multivariable Cox proportional hazards and conditional logistic regression models were used to calculate hazard ratios (HRs) and odds ratios (ORs), respectively, with 95% CIs.

Results: Total intake of fish (HR for quintile 5 vs 1, 0.88; 95% CI, 0.80–0.96; P_{trend}=.005), fatty fish (HR for quintile 5 vs 1, 0.90; 95% CI, 0.82–0.98; P_{trend}=.009), and lean fish (HR for quintile 5 vs 1, 0.91; 95% CI, 0.83–1.00; P_{trend}=.016) were inversely associated with CRC incidence. Intake of total n-3 LC-PUFA (HR for quintile 5 vs 1, 0.86; 95% CI, 0.78–0.95; P_{trend}=.010) was also associated with reduced risk of CRC, whereas dietary ratio of n-6:n-3 LC-PUFA was associated with increased risk of CRC (HR for quintile 5 vs 1, 1.31; 95% CI, 1.18–1.45; P_{trend}<.001). Plasma levels of phospholipid n-3 LC-PUFA was not associated with overall CRC risk, but an inverse trend was observed for proximal compared with distal colon cancer ($P_{heterogeneity}$=.026).

Conclusions: In an analysis of dietary patterns of participants in the EPIC study, we found regular consumption of fish, at recommended levels, to be associated with a lower risk of CRC, possibly through exposure to n-3 LC-PUFA. Levels of n-3 LC-PUFA in plasma were not associated with CRC risk, but there may be differences in risk at different regions of the colon.

KEY WORDS: epidemiologic, seafood, omega 3, tumorigenesis

What you need to know

Background: Dietary intake of fish might reduce risk of colorectal cancer, possibly through exposure to marine n-3 fatty acids. Epidemiology studies have not provided a consensus view on the link between fatty acids from seafood and colorectal cancer.

Findings: In an analysis of data from more than 500,000 participants in the European Prospective Investigation into Cancer and Nutrition cohort, we associated intake of fish, at levels recommended by World Health Organization, with reduced risk of colorectal cancer. The potential effect of fish consumption on colorectal tumorigenesis might be mediated by specific fatty acids in seafood. There might be differences in effect on risk in different regions of the colon.

Implications for patient care: Consumption of fish appears to reduce the risk of colorectal cancer and should be encouraged as part of a healthy diet.

Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer globally with an estimated 1.8 million new cases in 2018[1]. Established lifestyle and dietary risk factors for CRC include smoking, alcohol consumption, obesity, physical inactivity, high red and processed meat consumption, and low intake of fibre[2]. The World Cancer Research Fund (WCRF) concluded, based on a meta-analysis of eighteen prospective studies, that there was *"limited but suggestive"* evidence that fish decreases CRC risk[3]. Nevertheless, there is still uncertainty whether fish consumption is beneficial for CRC prevention and how consumption of different fish types (e.g. fatty/oily, white/lean) relates to CRC risk.

Fatty/oily fish is the near exclusive dietary source of long-chain n-3 polyunsaturated fatty acids (n-3 LC-PUFA). In animal[4] and *in vitro*[5] models, n-3 LC-PUFAs have been shown to have pro-apoptotic and anti-proliferative properties on colon tumour cells. Human studies that have investigated the association between dietary intake of n-3 LC-PUFA and CRC risk have generally shown inverse relationships with possible differences by sex, study population, duration of follow-up, and tumour characteristics including location, stage and molecular features[6-11]. Two meta-analyses of prospective studies showed an inverse association between n-3 LC-PUFA intake and CRC in men, in proximal colon cancer, and with extended follow-up period whereas null or even positive associations were observed for distal colon cancer and in Asian men[6, 7]. Dietary n-3 LC-PUFA has also been inversely associated with risk of microsatellite instability (MSI)-high CRC but not with microsatellite stable tumors[9]. In addition, the association of marine n-3 LC-PUFA with CRC risk has been shown to vary depending on the presence of tumor-infiltrating T-cells[12].

For circulating biomarker studies, the associations of plasma levels of n-3 LC-PUFA with CRC have shown inconsistent results, ranging from null[13, 14] to weak inverse associations[15, 16] that were statistically significant in men and for studies with longer follow-

up periods[15]. Alternatively, it has been proposed that the balance between n-6 and n-3 PUFA may be more relevant for health outcomes than the absolute intake of n-3 LC-PUFA, as a consequence of their divergent metabolic effects on inflammation[17]. Overall, previous studies on the role of n-3 LC-PUFA and CRC incidence remain inconclusive. Thus, further prospective studies in different populations are needed to clarify the association between n-3 LC-PUFAs, their relative balance with n-6 LC-PUFA, their metabolism, and CRC risk.

In this study, we undertook a comprehensive investigation of how fish consumption, and dietary and circulating levels of n-3 LC-PUFA as well as n-6:n-3 LC-PUFA ratio were associated with CRC risk in the European Prospective Investigation into Cancer and Nutrition (EPIC), a large multi-country prospective cohort with over 520,000 participants and wide variation in fish intake. A prior analysis conducted within EPIC reported inverse associations between fish consumption and CRC risk[18]. Here, we performed additional analyses that included both dietary and circulating n-3 LC-PUFA, with an additional 11 years of follow-up and almost 5-fold higher number of incident cases.

Methods

Study participants

EPIC is a prospective cohort of 521,324 participants, recruited between 1992 and 2000 in 23 centres located in 10 European countries (Denmark, France, Germany, Greece, Italy, Netherlands, Norway, Spain, Sweden, UK)[19]. Anthropometric measures, lifestyle and dietary intake were collected at recruitment. Blood samples were also collected and stored at the International Agency for Research on Cancer (IARC), or in local biobanks. Ethical approval was obtained from the review boards pertaining to IARC and to the respective recruiting centres. Informed consent was obtained from all the participants. Our analysis excluded participants missing follow-up (n=4,148), diagnosed with cancer prior recruitment

(n=25,184), missing dietary data (n=6,259), or within 1% highest/lowest energy intake vs requirement (n=9,573). Our final cohort analysis included 476,160 participants (142,241 men and 333,919 women).

Lifestyle, anthropometry and diet

Body weight and height were measured by a trained nurse in the majority of EPIC centres or were self-reported. Questionnaires were used to obtain information on education, smoking and physical activity. Dietary intake was assessed at recruitment by validated centre-specific questionnaires. Fish and fish products (excluding fish oil supplements) included fatty/oily (fat>4%/weight; e.g. salmon) and lean/white fish (fat≤4%/weight; e.g. cod). Shellfish (e.g. prawn) intake was considered separately or combined with fish as "*total fish and shellfish*". Dietary intakes of LC-PUFAs were estimated using the United States Department of Agriculture (USDA) Nutrient Database, Release 20 (https://ndb.nal.usda.gov/ndb/). The USDA database was previously matched with the EPIC food list to expand the EPIC Nutrient Database (ENDB) with extra food components. We also estimated total n-3 LC-PUFA (sum of eicosapentaenoic, EPA; docosapentaenoic, DPA; and docosahexaenoic, DHA) and n-6:n-3 LC-PUFA ratio (arachidonic+di-homo-γ-linolenic/n-3 LC-PUFA).

Follow-up and vital status

Incident CRC cases were identified through regional cancer registries or via a combination of methods, including health insurance records, pathology registries, and active follow-up of participants and relatives. CRC cases were defined according to the International Classification of Diseases for Oncology (ICD-O): proximal colon (C18.0-C18.5: cecum, appendix, ascending colon, hepatic flexure, transverse colon and splenic flexure), distal colon

(C18.6-C18.7: descending and sigmoid colon), rectum (C19: recto-sigmoid junction, C20: rectum).

Sub-study of circulating PUFAs and CRC

Pre-diagnostic plasma samples from 461 incident CRC cases and 461 matched controls from seven countries were included in a nested case-control analysis of circulating n-3 LC-PUFAs and CRC. Controls were selected by incidence density sampling from all cohort members alive and free of cancer at the time of diagnosis of the index case. Cases and controls were matched by centre, sex, blood collection details including time (±2-4 hours interval), age (±6 months-<±2 years), fasting status (<3/3-6 hours) and among women by menopausal status, and among premenopausal women, by phase of menstrual cycle and hormone replacement therapy use.

Measurements of plasma phospholipid fatty acids

Plasma phospholipid levels of LC-PUFAs were determined by gas chromatography using a method previously described[20]. Briefly, total lipids were extracted from plasma samples by chloroform-methanol 2:1 (v/v). Phospholipids were purified by adsorption chromatography on silica tubes. Fatty acid methyl esters (FAMEs) were formed by transmethylation with Methyl-Prep II (Alltech, Deerfield, USA). Analyses were carried out on the gas chromatograph 7890A (Agilent Technologies, USA). The individual LC-PUFAs were separated and identified by comparison of their respective retention time with those of purchased standard methyl ester fatty acids. Plasma phospholipid LC-PUFAs were expressed as percentages of total fatty acids. The ratio of circulating n-6:n-3 LC-PUFA was also calculated.

Statistical analyses

Full prospective cohort

Socio-demographic and dietary intake variables in the EPIC population are presented separately for cases and non-cases, and compared using Wilcoxon rank-sum and χ^2 tests for continuous and categorical variables, respectively. Supplementary Table 1 presents Spearman correlation matrix for fish intake, fatty acids and other potential confounding variables. Cox proportional hazards regression was used to estimate hazard ratios (HRs) and 95% confidence intervals (CI) for the association between fish intake, dietary n-3 LC-PUFA, and CRC risk in the full EPIC cohort. Time at study entry was age at recruitment and exit time was age at whichever of the following came first: CRC diagnosis, death, emigration, or completed follow-up. Models were stratified by age at recruitment (1-year categories), sex, and centre. Analyses were run with fish and dietary n-3 LC-PUFA intakes in quintiles or as continuous variables for intakes of 100g/day of fish[3], 100mg/day of n-3 LC-PUFA, and 5-point increment of n-6:n-3 LC-PUFA. The distribution of shellfish consumption did not allow the categorisation by quintiles, but by tertiles. We additionally evaluated the association with CRC risk considering the recommendation by the World Health Organisation which is to consume 1-2 servings (100-150g/serving) of fish weekly[21]. For all the analyses, proportionality was evaluated using the slope of Schoenfeld residuals over time, which showed no deviation from the proportional hazards assumption. All the models were adjusted for risk factors *a priori* associated with CRC: as continuous variables, body mass index (BMI), height, intakes of alcohol, red and processed meat, fibre, dairy products, and as categorical variables (Table 1) physical activity, smoking, and education. Variables with missing data (<5%) were coded as distinct categories. Trends tests were performed using median values of categories as continuous. Multiplicative interaction was assessed by including a cross-product term in the model, the statistical significance of which was

evaluated using the Wald test. Separate analyses were also conducted by sex, and anatomical subtypes of CRC. To evaluate the possible impact of reverse causation, we re-ran the analyses with cases diagnosed within the first two years of follow-up excluded.

Nested case-control biomarker sub-study

In the sub-study of circulating n-3 LC-PUFAs and CRC risk, multivariable conditional logistic regression was used to compute odds ratios (OR) and 95%CI for the associations between circulating levels of n-3 LC-PUFAs and CRC. Participants were divided into quartiles based on the distributions in the control group. Analyses were adjusted for the same covariates as in the analyses for dietary intakes. Subsite analyses were run for proximal and distal colon, but not for rectum, due to few number of cases (n=5). Two-sided *P*-values <0.05 were considered statistically significant.

Results

After a median follow-up time of 14.9 years, 6,291 incident cases of CRC (2,719 men and 3,572 women) were diagnosed. Of these cases, 4,197 were colon cancers whereas 2,094 cases were rectal cancer cases. Compared to non-cases, cases were more likely to be current or former smokers, and higher consumers of red and processed meats and alcohol (Table 1).

Dietary fish consumption and CRC

Table 2 summarizes the associations between fish intake and the risk for CRC. Overall, total fish intake was inversely associated with CRC (HR comparing extreme quintiles $HR_{Q5vs.Q1}$=0.88, 95%CI=0.80-0.96, P_{trend}=0.005) and particularly colon cancer ($HR_{Q5vs.Q1}$=0.89, 95%CI=0.79-1.00, P_{trend}=0.024). The inverse associations were observed for total fish intake with both distal and proximal colon cancers risk, but the risk estimates did

not reach the threshold of significance (Table 2). Both fatty fish and lean fish intakes were inversely associated with CRC and specifically, colon cancer (Table 2). By anatomic location, there was no difference between men and women in the association between fish intake and the risk for CRC (*P* for heterogeneity>0.05) (Supplementary figure 1). Shellfish intake was not associated with CRC risk, but total fish intake combined with shellfish intake was inversely associated with the risk for CRC (Supplementary Table 2). Compliance with WHO's recommendation for fish intake (1-2 servings/week of 100g each) was associated with a 7% lower risk of CRC, compared to <1 serving/week (Supplementary Figure 2). There was no overall difference in the association of fish intake and CRC by country ($P_{heterogeneity}$=0.12) (Supplementary Figure 3).

Dietary n-3 LC-PUFA intake and CRC

Dietary intake of total n-3 LC-PUFA was inversely associated with the risk for CRC ($HR_{Q5vs.Q1}$=0.86, 95%CI=0.78-0.95, P_{trend}=0.010) and specifically colon ($HR_{Q5vs.Q1}$=0.85, 95%CI=0.75-0.96, P_{trend}=0.038), but not rectal cancer (Table 3). All individual n-3 LC-PUFA (EPA, DPA, and DHA) were significantly inversely associated with CRC risk (Table 3). The n-6:n-3 LC-PUFA ratio was associated with higher CRC risk ($HR_{Q5vs.Q1}$=1.31, 95%CI=1.18-1.45, P_{trend}<0.001), colon ($HR_{Q5vs.Q1}$=1.32, 95%CI=1.17-1.50, P_{trend}<0.001), and rectal cancer ($HR_{Q5vs.Q1}$=1.24, 95%CI=1.04-1.48, P_{trend}=0.020). Although no significant differences in the associations between estimates of EPA, DPA, DHA and total n-3 LC-PUFA, and CRC was observed between men and women (*P* for heterogeneity >0.05), the risk estimates only reached statistical significance in women (Supplementary Figure 4). In sensitivity analyses excluding cases diagnosed during the first 2 years of follow-up (n=781 cases excluded for the analysis), the results were generally unchanged (data not shown). Similar associations between dietary intakes of fish and CRC risk were observed across strata of BMI, alcohol

consumption, red and processed meats, or physical activity (data not shown, all *P* for interactions>0.05).

Sub-study of circulating PUFAs and CRC

The associations between plasma phospholipid EPA, DPA, and DHA, total n-3 LC-PUFA, n-6:n-3 LC-PUFA and CRC risk were not statistically significant (Table 4). However, an inverse trend was observed for proximal (OR quantile 4 *vs* 1 of n-3 LC-PUFA levels $OR_{Q4vs.Q1}$=0.55, 95%CI=0.27-1.11) compared to distal colon cancer ($OR_{Q4vs.Q1}$=1.54, 95%CI=0.77-3.08) ($P_{heterogeneity}$=0.026). The results did not change by BMI, or smoking status, or when cases diagnosed within 2 years of follow-up were excluded (data not shown).

Discussion

In this prospective analysis of approximately half a million participants, we found that intakes total fish including fatty fish, lean fish and shellfish were inversely associated with CRC risk. Overall, weekly intake of 100-200g of fatty or lean fish was associated with a 7% lower CRC risk. Similarly, dietary intakes of all n-3 LC-PUFA were inversely associated with the risk for CRC while the n-6:n-3 LC-PUFA ratio was positively associated with CRC. On the other hand, circulating levels of n-3 LC-PUFA were not associated with CRC risk in a sub-study.

Our observed inverse association between fish consumption and CRC is consistent with the WCRF meta-analysis that reported that 100g/day increment intake of total fish was associated with an 11% lower risk of CRC (HR=0.89, 95%CI=0.80-0.99)[3]. However, in that meta-analysis, the inverse association was only apparent in men (HR=0.83, 95%CI=0.71-0.98) and not in women (HR=0.96, 95%CI=0.82-1.12). We found inverse associations between both fatty and lean fish intakes and CRC risk, which suggests that fish consumption in general (independent of the type) may be beneficial against the development of CRC.

The biological mechanisms through which fish consumption potentially lowers CRC risk are not fully understood. Fatty/oily fish are primary sources of n-3 LC-PUFAs which may inhibit cancer development through the production of eicosanoids that possess anti-inflammatory properties[17]. Although fat content is lower in lean/white fish compared to fatty fish, lean fish could be a non-negligible source of n-3 LC-PUFAs. In fact, the overall composition of fish with respect to n-3 LC-PUFA content depends not only on the amount of total fat, but also on the percentage of fatty acids; for example sole-like lean fish with less than 1.7% total fat has approximately 24.6% (as a proportion of total fatty acids) of EPA and DHA, while herring which contains 12.7% of total fat has 12% of EPA and DHA[22]. The n-3 LC-PUFAs produce anti-inflammatory five-series leukotrienes and three-series prostaglandins, and act as competitive inhibitors of the actions of the n-6 LC-PUFAs; the latter lead to the production of four-series leukotrienes and two-series prostaglandins and promote the synthesis of pro-inflammatory interleukins and tumour necrosis factor[17]. In agreement with this hypothesis, our study showed that the n-6:n-3 LC-PUFA ratio in the diet is positively associated with CRC risk. We additionally observed that fatty fish intake was significantly inversely associated with proximal colon cancer, whereas lean fish intake tended to be inversely associated with distal colon cancer. In addition to exposure to n-3 LC-PUFAs, the associations we observed for both fatty and lean fish and CRC may be due to a combination of diverse nutritional factors derived from fish in general, including vitamins D and B_{12}, selenium, or particular amino-acids[23].

In our population we observed 14% lower CRC risk comparing those in the lowest vs highest quintiles of intake of n-3 LC-PUFA. The inverse association between dietary n-3 LC-PUFAs and CRC risk observed in our study did not differ between men and women, albeit the risk estimates only attained statistical significance in women (potentially due to the higher number of women in our analysis); thus our study provided additional evidence that high

dietary intake of n-3 LC-PUFAs might decrease the risk of CRC, regardless of sex. Of note, we did not find any association between circulating n-3 LC-PUFAs and the risk for CRC.

Interestingly, we observed an inverse trend between circulating n-3 LC-PUFA and risk for proximal colon cancer compared with distal colon cancer, which is in agreement with previous findings[7]. Since the proximal and distal colon have different embryologic origins, divergent functions and invariably display distinct molecular features[9], it has been hypothesized that cancers that arise across the sub-locations could have different aetiologies. At a physiological level, as faecal matter moves from the proximal colon towards the distal colon and rectum, the concentration of electrolytes, bile acids and other residues of digestion changes with continuous absorption of water, which influences the diversity and genus of microbes along the colon. Elevated levels of n-3 LC-PUFA in the proximal colon may stimulate increased production of short-chain fatty acids, which have been suggested to decrease the risk for CRC through lowering of inflammation in the colon[24]. Further experimental research is needed to investigate why the effects of n-3 LC-PUFA may differ on the proximal vs distal colon.

The current analysis represents the largest study to date to comprehensively investigate the association between fish and n-3 LC-PUFA intakes and CRC risk. The large number of incident CRC cases allowed analyses by sex and tumour location, and the detailed phenotypic information collected from all participants permitted careful adjustment for known CRC risk factors. A limitation of our study is that dietary intake information was only available from baseline (recruitment) while dietary habits of the EPIC participants may have changed over the follow-up period. Nevertheless, intakes of fish and other food items reported at recruitment were generally reliable over time, when compared with two repeated dietary questionnaires and 12 consecutive monthly 24-hour dietary recalls administered to a sub-sample of EPIC participants[25]. Another limitation is that our data did not include

information on fish oil supplement intake. An investigation of a subgroup of EPIC participants showed that use of vitamin and micronutrient supplements was common[26]. Fish oil use was not specifically explored; hence unmeasured effects of supplementation may have influenced the risk for CRC in our analysis. Finally, although we adjusted for a comprehensive set of covariates, and we conducted numerous sensitivity analyses, potential unmeasured and residual confounding cannot be excluded.

In conclusion, our data suggest that fish intake, and dietary intake of individual and total n-3 LC-PUFA may lower the risk for CRC. Finally, this study showed that an imbalanced ratio of n-6:n-3 LC-PUFA from the diet was associated with an increased risk of CRC. Our analysis makes a substantial contribution to the growing body of evidence linking fish consumption to potentially lower risk of CRC.

References

1. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018. Epub 2018/09/13.

2. WCRF. Cancer Prevention and Survival: Summary of global evidence on diet, weight, physical activity and what increases or decreases your risk of cancer London2017; Available from: https://www.wcrf.org/sites/default/files/Summary-third-expert-report.pdf.

3. World Cancer Research Fund/American Institute for Cancer Research. CUP Expert Report 2018. Diet, nutrition, physical activity and colorectal cancer. 2018.

4. Fukunaga K, Hossain Z, Takahashi K. Marine phosphatidylcholine suppresses 1,2-dimethylhydrazine-induced colon carcinogenesis in rats by inducing apoptosis. Nutrition research (New York, NY). 2008;28(9):635-40. Epub 2008/12/17.

5. Zhang C, Yu H, Ni X, et al. Growth inhibitory effect of polyunsaturated fatty acids (PUFAs) on colon cancer cells via their growth inhibitory metabolites and fatty acid composition changes. PloS one. 2015;10(4):e0123256. Epub 2015/04/18.

6. Shen XJ, Zhou JD, Dong JY, et al. Dietary intake of n-3 fatty acids and colorectal cancer risk: a meta-analysis of data from 489 000 individuals. The British journal of nutrition. 2012;108(9):1550-6. Epub 2012/08/22.

7. Chen GC, Qin LQ, Lu DB, et al. N-3 polyunsaturated fatty acids intake and risk of colorectal cancer: meta-analysis of prospective studies. Cancer causes & control : CCC. 2015;26(1):133-41. Epub 2014/11/25.

8. Song M, Chan AT, Fuchs CS, et al. Dietary intake of fish, omega-3 and omega-6 fatty acids and risk of colorectal cancer: A prospective study in U.S. men and women. International journal of cancer. 2014;135(10):2413-23. Epub 2014/04/08.

9. Song M, Nishihara R, Wu K, et al. Marine omega-3 polyunsaturated fatty acids and risk of colorectal cancer according to microsatellite instability. Journal of the National Cancer Institute. 2015;107(4). Epub 2015/03/27.

10. Hall MN, Chavarro JE, Lee IM, et al. A 22-year prospective study of fish, n-3 fatty acid intake, and colorectal cancer risk in men. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2008;17(5):1136-43. Epub 2008/05/17.

11. Butler LM, Wang R, Koh WP, et al. Marine n-3 and saturated fatty acids in relation to risk of colorectal cancer in Singapore Chinese: a prospective study. International journal of cancer. 2009;124(3):678-86. Epub 2008/11/01.

12. Song M, Nishihara R, Cao Y, et al. Marine omega-3 Polyunsaturated Fatty Acid Intake and Risk of Colorectal Cancer Characterized by Tumor-Infiltrating T Cells. JAMA oncology. 2016;2(9):1197-206. Epub 2016/05/06.

13. Butler LM, Yuan J-M, Huang JY, et al. Plasma fatty acids and risk of colon and rectal cancers in the Singapore Chinese Health Study. npj Precision Oncology. 2017;1(1):38.

14. Hall MN, Campos H, Li H, et al. Blood levels of long-chain polyunsaturated fatty acids, aspirin, and the risk of colorectal cancer. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2007;16(2):314-21. Epub 2007/02/16.

15. Kojima M, Wakai K, Tokudome S, et al. Serum levels of polyunsaturated fatty acids and risk of colorectal cancer: a prospective study. American journal of epidemiology. 2005;161(5):462-71. Epub 2005/02/19.

16. Hodge AM, Williamson EJ, Bassett JK, et al. Dietary and biomarker estimates of fatty acids and risk of colorectal cancer. International journal of cancer. 2015;137(5):1224-34. Epub 2015/02/17.

17. DiNicolantonio JJ, O'Keefe JH. Importance of maintaining a low omega-6/omega-3 ratio for reducing inflammation. Open heart. 2018;5(2):e000946. Epub 2018/12/20.

18. Norat T, Bingham S, Ferrari P, et al. Meat, fish, and colorectal cancer risk: the European Prospective Investigation into cancer and nutrition. Journal of the National Cancer Institute. 2005;97(12):906-16. Epub 2005/06/16.

19. Riboli E, Hunt KJ, Slimani N, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. Public Health Nutr. 2002;5(6b):1113-24. Epub 2003/03/18.

20. Chajes V, Thiebaut AC, Rotival M, et al. Association between serum trans-monounsaturated fatty acids and breast cancer risk in the E3N-EPIC Study. American journal of epidemiology. 2008;167(11):1312-20. Epub 2008/04/09.

21. WHO. Diet, nutrition and the prevention of chronic diseases: Report of a Joint WHO/FAO Expert Consultation Geneva: World Health Organisation 2003.

22. Strobel C, Jahreis G, Kuhnt K. Survey of n-3 and n-6 polyunsaturated fatty acids in fish and fish products. Lipids in health and disease. 2012;11:144. Epub 2012/11/01.

23. Song M, Garrett WS, Chan AT. Nutrients, foods, and colorectal cancer prevention. Gastroenterology. 2015;148(6):1244-60.e16. Epub 2015/01/13.

24. Yang J, Yu J. The association of diet, gut microbiota and colorectal cancer: what we eat may imply what we get. Protein & cell. 2018;9(5):474-87. Epub 04/30.

25. Kaaks R, Slimani N, Riboli E. Pilot phase studies on the accuracy of dietary intake measurements in the EPIC project: overall evaluation of results. European Prospective Investigation into Cancer and Nutrition. International journal of epidemiology. 1997;26 Suppl 1:S26-36. Epub 1997/01/01.

26. Skeie G, Braaten T, Hjartaker A, et al. Use of dietary supplements in the European Prospective Investigation into Cancer and Nutrition calibration study. Eur J Clin Nutr. 2009;63 Suppl 4:S226-38. Epub 2009/11/06.

Table 1: Selected baseline demographic and lifestyle characteristics of study participants by colorectal cancer status, EPIC cohort study, 1992-2014

	Colorectal cancer cases (n=6291)	Non-cases (n=469 869)	*P-value**
Men, %	43.2	29.7	<0.001
Age at recruitment, years, mean±SD	57.3±7.87	51.2±9.95	<0.001
Follow-up, years, mean±SD	9.22±4.73	14.0±4.0	<0.001
Age at diagnosis, years, mean±SD	66.5±10.2	-	-
Anthropometry			
Body mass index, kg/m^2, mean±SD	26.4±4.26	25.4±4.30	<0.001
Socio-economic status and lifestyle			
Education status			<0.001
None	4.72	4.45	
Primary school	32.1	25.9	
Technical or professional	25.2	22.5	
Secondary school	15.6	20.8	
Higher education	19.0	24.2	
Smoking status			<0.001
Never	37.2	43.2	
Current, 1 to <16cigarettes/day	11.0	11.6	
Current, 16-<26 cigarettes/day	6.29	6.23	
Current, >26 cigarettes/day	1.72	1.82	
Former, quit <10 years	10.6	9.53	

Former, quit 11-<20 years	10.1	8.14	
Former, quit >20 years	11.8	7.83	
Current, pipe-cigar-occasional	8.28	8.42	
Physical activity status			<0.001
Inactive	24.9	20.9	
Moderately inactive	32.5	32.9	
Moderately active	22.5	26.4	
Active	18.4	17.9	
Alcohol consumption			<0.001
None	6.39	5.67	
<5 g/day	35.4	41.9	
5 to <14.9 g/day	25.7	27.0	
15.0 to <29.9 g/day	14.7	13.8	
>30 g/day	17.8	12.0	
Dietary intake, g/day, mean±SD			
Red and processed meat	83.3±56.3	74.9±52.7	<0.001
Fibre	22.7±8.04	22.9±8.14	0.107
Dairy products	333.7±245.1	326.5±235.4	0.166
Total fish and shellfish	39.0±35.3	37.1±35.7	<0.001
Total fish	35.1±33.6	33.6±34.6	<0.001
Fatty fish	13.2±16.7	11.8±15.6	<0.001
Lean fish	18.0±23.6	17.3±24.6	<0.001
Shellfish	3.13±5.61	3.03±5.57	<0.001
Dietary energy, kcal/day, mean±SD	2105.0±613.8	2074.7±619.3	<0.001

n-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA)			
Dietary intakes, mg/day, mean±SD			
Eicosapentaenoic acid (EPA)	129±160	114±152	<0.001
Docosapentaenoic acid (DPA)	30±29	29.0±30.2	<0.001
Docosahexaenoic acid (DHA)	196±228	178±163.5	<0.001
n-3 LC-PUFA (EPA+DPA+DHA)	355±413	321±401	<0.001
Ratio n-6:n-3 LC-PUFA	0.26±0.40	0.26±1.29	0.022
Plasma phospholipid, % of total fatty acids[†]	n=461	n=461	
Eicosapentaenoic acid (EPA)	0.92 (0.87-0.96)	0.93 (0.88-0.97)	0.731
Docosapentaenoic acid (DPA)	0.90 (0.89-0.92)	0.91 (0.89-0.93)	0.738
Docosahexaenoic acid (DHA)	4.53 (4.41-4.66)	4.58 (4.45-4.70)	0.778
n-3 LC-PUFA (EPA+DPA+DHA)	6.55 (6.38-6.72)	6.61 (6.45-6.78)	0.626
Ratio n-6:n-3 LC-PUFA	2.42 (2.35-2.50)	2.43 (2.35-2.50)	0.925

Frequencies may not add up to 100% due to missing data

* Using Wilcoxon rank-sum and χ^2 tests

[†]Geometric means (95% confidence intervals)

Table 2: Hazard ratios (HRs)* and 95% confidence intervals (95%CI) for colorectal cancer risk associated with dietary fish intake (quintiles and continuous), EPIC cohort study, 1992-2014

	Quintiles of fish intake							
	Q1	Q2	Q3	Q4	Q5	P_{trend}	$P_{heterogeneity}$	Continuous[§]
Total fish, g/day	<9.07	9.07-<19.0	19.0-<30.9	30.9-51.3	>51.3			
Colorectal cancer								
Cases	1178	1129	1271	1364	1349			
HR(95%CI)	1.00	0.92 (0.85-1.00)	0.93 (0.85-1.01)	0.88 (0.80-0.96)	0.88 (0.80-0.96)	*0.005*		0.90 (0.82-0.98)
Colon cancer								
Cases	751	762	813	884	870			
HR(95%CI)	1.00	0.96 (0.87-1.06)	0.92 (0.83-1.03)	0.89 (0.80-0.99)	0.89 (0.79-1.00)	*0.024*	*0.506*[†]	0.90 (0.80-1.01)
Proximal colon cancer								
Cases	359	368	353	409	388			
HR(95%CI)	1.00	1.02 (0.88-1.18)	0.91 (0.78-1.07)	0.93 (0.80-1.10)	0.93 (0.79-1.11)	*0.295*	*0.350*[‡]	0.90 (0.76-1.07)

Distal colon cancer								
Cases	315	306	365	358	399			
HR(95%CI)	1.00	0.91 (0.77-1.06)	0.96 (0.82-1.13)	0.84 (0.71-0.99)	0.89 (0.75-1.07)	*0.145*		0.95 (0.80-1.12)
Rectal cancer								
Cases	399	349	436	452	458			
HR(95%CI)	1.00	0.87 (0.75-1.01)	0.98 (0.84-1.13)	0.87 (0.75-1.02)	0.88 (0.75-1.04)	*0.181*		0.91 (0.77-1.07)
Fatty fish, g/day	<1.0	1.0-<4.36	4.36-<9.13	9.13-17.7	>17.7			
Colorectal cancer								
Cases	1165	1076	1241	1358	1451			
HR(95%CI)	1.00	1.00 (0.92-1.09)	0.95 (0.88-1.04)	0.95 (0.88-1.04)	0.90 (0.82-0.98)	*0.009*		0.84 (0.71-1.00)
Colon cancer								
Cases	768	693	816	875	928			
HR(95%CI)	1.00	0.99 (0.89-1.10)	0.94 (0.85-1.05)	0.92 (0.83-1.03)	0.89 (0.80-0.99)	*0.022*	*0.199*[†]	0.88 (0.71-1.09)
Proximal colon cancer								

Cases	386	310	386	408	387			
HR(95%CI)	1.00	0.96 (0.82-1.12)	0.95 (0.82-1.09)	0.93 (0.80-1.08)	0.81 (0.70-0.95)	*0.018*	*0.096*[‡]	0.76 (0.55-1.04)
Distal colon cancer								
Cases	307	298	336	361	441			
HR(95%CI)	1.00	1.07 (0.91-1.26)	0.98 (0.84-1.15)	0.95 (0.80-1.11)	1.03 (0.87-1.21)	*0.856*		1.11 (0.83-1.50)
Rectal cancer								
Cases	373	358	402	464	497			
HR(95%CI)	1.00	1.04 (0.89-1.20)	0.99 (0.86-1.14)	1.05 (0.91-1.21)	0.91 (0.78-1.06)	*0.330*		0.80 (0.59-1.07)
Lean fish, g/day	<0.74	0.74-<6.45	6.45-<13.9	13.9-26.5	>26.5			
Colorectal cancer								
Cases	1148	1144	1260	1426	1313			
HR(95%CI)	1.00	0.99 (0.91-1.09)	0.93 (0.85-1.02)	0.91 (0.83-0.99)	0.91 (0.83-1.00)	*0.016*		0.92 (0.80-1.05)
Colon cancer								
Cases	742	761	804	914	859			
HR(95%CI)	1.00	1.01 (0.91-1.13)	0.90 (0.81-1.01)	0.89 (0.80-0.99)	0.90 (0.80-1.01)	*0.019*	*0.766*[†]	0.90 (0.76-1.06)

Proximal colon cancer								
Cases	355	343	360	416	403			
HR(95%CI)	1.00	1.00 (0.85-1.18)	0.91 (0.77-1.07)	0.88 (0.76-1.03)	0.95 (0.80-1.12)	*0.263*	*0.902*[‡]	1.00 (0.78-1.26)
Distal colon cancer								
Cases	322	335	329	392	365			
HR(95%CI)	1.00	1.08 (0.91-1.28)	0.89 (0.75-1.06)	0.93 (0.79-1.09)	0.85 (0.71-1.01)	*0.038*		0.80 (0.61-1.03)
Rectal cancer								
Cases	383	364	434	480	433			
HR(95%CI)	1.00	0.97 (0.83-1.13)	1.01 (0.87-1.18)	0.96 (0.82-1.11)	0.96 (0.82-1.13)	*0.555*		0.98 (0.78-1.24)

*Adjusted for BMI, height, physical activity, smoking, education, and intakes of energy, alcohol, red and processed meat, fibre, dairy products and stratified by age, sex, and centre

[†]Colon vs rectum

[‡]Proximal vs distal colon

[§]100g/day increment

Table 3: Hazard ratios (HRs)* and 95% confidence intervals (CI) for colorectal cancer risk associated with dietary n-3 long-chain polyunsaturated fatty acids estimates (quintiles and continuous), EPIC cohort study, 1992-2014

	Quintiles of n-3 long-chain polyunsaturated fatty acids intake (n-3 LC-PUFA)							
	Q1	Q2	Q3	Q4	Q5	P_{trend}	$P_{heterogeneity}$	Continuous[§]
Eicosapentaenoic acid (EPA), mg/day	<23.5	23.5-<49.0	49.0-<84.5	84.5-164.6	>164.6			
Colorectal cancer								
Cases	1161	1129	1082	1299	1620			
HR(95%CI)	1.00	0.93 (0.86-1.02)	0.88 (0.80-0.96)	0.92 (0.84-1.01)	0.86 (0.78-0.95)	*0.008*		0.97 (0.95-0.99)
Colon cancer								
Cases	753	747	704	850	1026			
HR(95%CI)	1.00	0.94 (0.85-1.05)	0.86 (0.77-0.97)	0.93 (0.83-1.04)	0.87 (0.77-0.98)	*0.033*	*0.189*[†]	0.97 (0.95-0.99)
Proximal colon cancer								
Cases	359	345	333	404	436			
HR(95%CI)	1.00	0.96 (0.82-1.12)	0.93 (0.79-1.09)	1.02 (0.87-1.21)	0.84 (0.70-1.01)	*0.190*	*0.258*[‡]	0.96 (0.93-1.00)

Distal colon cancer								
Cases	317	305	297	343	481			
HR(95%CI)	1.00	0.92 (0.78-1.08)	0.83 (0.70-0.98)	0.87 (0.73-1.03)	0.94 (0.78-1.13)	*0.435*		0.99 (0.96-1.03)
Rectal cancer								
Cases	385	355	360	430	564			
HR(95%CI)	1.00	0.91 (0.79-1.06)	0.91 (0.78-1.06)	0.93 (0.79-1.09)	0.87 (0.74-1.04)	*0.212*		0.98 (0.95-1.02)
Docosapentaenoic acid (DPA), mg/day	<9.30	9.30-<16.6	16.6-<25.3	25.3-41.3	>41.3			
Colorectal cancer								
Cases	1039	1241	1348	1327	1336			
HR(95%CI)	1.00	0.96 (0.88-1.05)	0.95 (0.87-1.04)	0.91 (0.82-1.00)	0.83 (0.75-0.92)	*<0.001*		0.84 (0.76-0.94)
Colon cancer								
Cases	674	838	891	821	856			
HR(95%CI)	1.00	0.98 (0.88-1.09)	0.94 (0.84-1.06)	0.87 (0.78-0.98)	0.83 (0.73-0.94)	*<0.001*	*0.061*[†]	0.83 (0.73-0.95)

						1		
Proximal colon cancer								
Cases	320	386	422	367	382			
HR(95%CI)	1.00	0.97 (0.83-1.14)	0.97 (0.82-1.14)	0.90 (0.76-1.08)	0.85 (0.71-1.03)	*0.069*	*0.398*[‡]	0.82 (0.67-1.00)
Distal colon cancer								
Cases	276	360	366	349	392			
HR(95%CI)	1.00	0.94 (0.80-1.11)	0.88 (0.74-1.05)	0.81 (0.68-0.97)	0.82 (0.68-1.00)	*0.017*		0.92 (0.76-1.12)
Rectal cancer								
Cases	341	381	434	486	452			
HR(95%CI)	1.00	0.94 (0.81-1.10)	0.98 (0.84-1.15)	1.00 (0.85-1.18)	0.84 (0.71-1.01)	*0.172*		0.86 (0.72-1.04)
Docosahexaenoic acid (DHA), mg/day	<42.1	42.1-<84.0	84.0-<140	140-264	>264			
Colorectal cancer								
Cases	1141	1109	1145	1350	1546			
HR(95%CI)	1.00	0.91 (0.83-0.99)	0.90 (0.83-0.99)	0.92 (0.84-1.01)	0.87 (0.78-0.96)	*0.020*		0.98 (0.97-1.00)

Colon cancer								
Cases	731	730	762	884	973			
HR(95%CI)	1.00	0.92 (0.83-1.03)	0.92 (0.82-1.03)	0.94 (0.84-1.06)	0.87 (0.77-0.99)	*0.084*	*0.261*[†]	0.98 (0.96-1.00)
Proximal colon cancer								
Cases	358	338	354	408	419			
HR(95%CI)	1.00	0.93 (0.79-1.08)	0.94 (0.80-1.10)	1.02 (0.86-1.21)	0.89 (0.74-1.06)	*0.450*	*0.189*[‡]	0.97 (0.95-1.00)
Distal colon cancer								
Cases	303	294	327	370	449			
HR(95%CI)	1.00	0.88 (0.74-1.04)	0.91 (0.77-1.09)	0.88 (0.74-1.05)	0.89 (0.74-1.08)	*0.353*		1.00 (0.97-1.02)
Rectal cancer								
Cases	383	359	361	448	543			
HR(95%CI)	1.00	0.90 (0.78-1.05)	0.89 (0.76-1.04)	0.91 (0.77-1.07)	0.87 (0.73-1.04)	*0.201*		0.99 (0.97-1.01)
n-3 LC-PUFA (EPA+DPA+DHA), mg/day	<77.3	77.3-<151	151-<250	250-470	>470			

Colorectal cancer								
Cases	1150	1116	1128	1321	1576			
HR(95%CI)	1.00	0.91 (0.84-1.00)	0.89 (0.81-0.97)	0.91 (0.83-1.00)	0.86 (0.78-0.95)	*0.010*		0.99 (0.98-1.00)
Colon cancer								
Cases	746	727	740	874	993			
HR(95%CI)	1.00	0.90 (0.81-1.01)	0.89 (0.80-1.00)	0.93 (0.83-1.04)	0.85 (0.75-0.96)	*0.038*	*0.142*[†]	0.99 (0.98-1.00)
Proximal colon cancer								
Cases	358	335	353	409	422			
HR(95%CI)	1.00	0.93 (0.79-1.08)	0.96 (0.81-1.12)	1.04 (0.88-1.23)	0.86 (0.72-1.04)	*0.386*	*0.236*[‡]	0.99 (0.97-1.00)
Distal colon cancer								
Cases	316	296	308	357	466			
HR(95%CI)	1.00	0.84 (0.71-0.99)	0.84 (0.71-1.00)	0.82 (0.69-0.98)	0.86 (0.72-1.04)	*0.182*		1.00 (0.98-1.01)
Rectal cancer								
Cases	377	348	381	434	554			
HR(95%CI)	1.00	0.94 (0.81-1.09)	0.91 (0.78-1.06)	0.90 (0.76-1.06)	0.91 (0.77-1.08)	*0.277*		0.99 (0.98-1.01)

n-6:n-3 LC-PUFA	<0.05	0.05-<0.10	0.10-<0.18	0.18-0.36	>0.36			
Colorectal cancer								
Cases	1306	1322	1213	1180	1270			
HR(95%CI)	1.00	1.13 (1.04-1.23)	1.19 (1.09-1.30)	1.20 (1.09-1.32)	1.31 (1.18-1.45)	*<0.00 1*		1.06 (1.04-1.09)
Colon cancer								
Cases	746	727	740	874	993			
HR(95%CI)	1.00	1.14 (1.03-1.26)	1.23 (1.10-1.37)	1.21 (1.08-1.37)	1.32 (1.17-1.50)	*<0.00 1*	*0.991*[†]	1.06 (1.03-1.10)
Proximal colon cancer								
Cases	358	335	353	409	422			
HR(95%CI)	1.00	1.14 (0.97-1.33)	1.22 (1.03-1.45)	1.32 (1.11-1.58)	1.39 (1.15-1.68)	*<0.00 1*	*0.046*[‡]	1.08 (1.04-1.13)
Distal colon cancer								
Cases	316	296	308	357	466			
HR(95%CI)	1.00	1.07 (0.92-1.24)	1.13 (0.96-1.34)	1.03 (0.86-1.24)	1.14 (0.94-1.39)	*0.320*		1.02 (0.98-1.07)

Rectal cancer							
Cases	377	348	381	434	554		
HR(95%CI)	1.00	1.09 (0.95-1.26)	1.12 (0.96-1.31)	1.17 (0.99-1.38)	1.24 (1.04-1.48)	*0.020*	1.05 (1.01-1.09)

*Adjusted for BMI, height, physical activity, smoking, education, and intakes of energy, alcohol, red and processed meat, fibre, dairy products and stratified by age, sex, and centre

[†]Colon vs rectum

[‡]Proximal vs distal colon

[§]100mg/day increment except for n-6:n-3 LC-PUFA (per 5-units)

Table 4: Odds ratios* and 95% confidence intervals (CI) for colorectal cancer risk associated with plasma phospholipid n-3 long-chain polyunsaturated fatty acids (Quantiles and continuous), EPIC cohort study, 1992-2014

	Quantiles of plasma phospholipid of n-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA)						
	Q1	Q2	Q3	Q4	P_{trend}	$P_{heterogeneity}$ †	Continuous, per unit increase
Eicosapentaenoic acid (EPA)							
Colorectal cancer							
Cases	124	105	124	108			
OR(95%CI)	1.00	0.79 (0.53-1.18)	0.92 (0.62-1.37)	0.89 (0.59-1.35)	*0.745*		0.93 (0.71-1.23)
Colon cancer							
Cases	122	103	124	106			
OR(95%CI)	1.00	0.78 (0.53-1.17)	0.94 (0.63-1.40)	0.89 (0.59-1.35)	*0.762*		0.93 (0.70-1.22)
Proximal colon cancer							

Cases	54	45	41	45			
OR(95%CI)	1.00	0.89 (0.46-1.70)	0.74 (0.38-1.42)	0.79 (0.41-1.50)	*0.403*	*0.146*	0.88 (0.57-1.36)
Distal colon cancer							
Cases	52	51	70	49			
OR(95%CI)	1.00	0.75 (0.40-1.41)	1.31 (0.68-2.52)	1.00 (0.50-2.00)	*0.580*		1.03 (0.65-1.64)
Docosapentaenoic acid (DPA)							
Colorectal cancer							
Cases	131	101	105	124			
OR(95%CI)	1.00	0.70 (0.46-1.07)	0.82 (0.54-1.24)	1.18 (0.73-1.91)	*0.542*		0.99 (0.49-2.00)
Colon cancer							
Cases	129	100	103	123			
OR(95%CI)	1.00	0.72 (0.47-1.10)	0.83 (0.55-1.26)	1.18 (0.73-1.92)	*0.545*		0.97 (0.48-1.97)

Proximal colon cancer							
Cases	55	39	33	58			
OR(95%CI)	1.00	0.73 (0.36-1.49)	0.48 (0.23-1.02)	0.99 (0.44-2.22)	*0.700*	*0.176*	0.85 (0.27-2.68)
Distal colon cancer							
Cases	56	51	60	55			
OR(95%CI)	1.00	1.21 (0.63-2.33)	1.62 (0.86-3.05)	1.75 (0.83-3.68)	*0.080*		1.35 (0.44-4.15)
Docosahexaenoic acid (DHA)							
Colorectal cancer							
Cases	126	104	118	113			
OR(95%CI)	1.00	1.11 (0.75-1.61)	1.02 (0.68-1.52)	1.19 (0.76-1.85)	*0.573*		1.03 (0.60-1.75)
Colon cancer							
Cases	124	103	118	110			
OR(95%CI)	1.00	1.10 (0.75-1.61)	1.02 (0.68-1.53)	1.19 (0.76-	*0.579*		1.03 (0.60-1.77)

				1.86)			
Proximal colon cancer							
Cases	52	40	48	45			
OR(95%CI)	1.00	0.65 (0.35-1.21)	0.81 (0.40-1.62)	0.75 (0.37-1.53)	*0.528*	*0.050*	0.78 (0.32-1.87)
Distal colon cancer							
Cases	59	49	60	54			
OR(95%CI)	1.00	1.71 (0.93-3.13)	1.89 (1.01-3.55)	1.92 (0.93-3.94)	*0.058*		1.64 (0.72-3.78)
n-3 LC-PUFA (EPA+DPA+DHA)							
Colorectal cancer cases							
Cases	135	93	120	113			
OR(95%CI)	1.00	0.74 (0.50-1.09)	0.98 (0.66-1.48)	0.94 (0.61-1.44)	*0.999*		0.98 (0.56-1.72)
Colon cancer							

Cases	133	92	119	111			
OR(95%CI)	1.00	0.72 (0.49-1.07)	0.97 (0.64-1.46)	0.94 (0.61-1.44)	*0.999*		0.98 (0.56-1.72)
Proximal colon cancer							
Cases	56	37	46	46			
OR(95%CI)	1.00	0.44 (0.23-0.85)	0.66 (0.33-1.34)	0.55 (0.27-1.11)	*0.195*	*0.026*	0.76 (0.31-1.82)
Distal colon cancer							
Cases	65	40	63	54			
OR(95%CI)	1.00	0.86 (0.46-1.58)	1.55 (0.83-2.90)	1.54 (0.77-3.08)	*0.122*		1.59 (0.64-3.95)
n-6:n-3 LC-PUFA‡							
Colorectal cancer							
Cases	119	120	105	117			
OR(95%CI)	1.00	0.92 (0.62-1.37)	0.86 (0.56-1.32)	0.87 (0.55-1.36)	*0.516*		0.88 (0.55-1.40)

Colon cancer							
Cases	117	120	105	113			
OR(95%CI)	1.00	0.93 (0.62-1.38)	0.85 (0.56-1.31)	0.86 (0.55-1.35)	*0.479*		0.88 (0.55-1.40)
Proximal colon cancer							
Cases	48	52	44	41			
OR(95%CI)	1.00	0.78 (0.39-1.54)	0.77 (0.37-1.60)	0.74 (0.33-1.64)	*0.498*	*0.633*	0.97 (0.45-2.09)
Distal colon cancer							
Cases	57	61	47	57			
OR(95%CI)	1.00	1.21 (0.66-2.22)	0.69 (0.35-1.35)	0.69 (0.35-1.36)	*0.150*		0.63 (0.30-1.32)

*Adjusted for BMI, height, physical activity, smoking, education, and intakes of energy, alcohol, red and processed meat, fibre, dairy products

[†]Proximal vs distal colon

[‡](arachidonic+di-homo-γ-linolenic)/(EPA+DPA+DHA)

Supplementary figures

S1: Hazard ratios, per 100 g/day increment (continuous), and 95% confidence interval for colorectal cancer risk associated with fish intake, by sex

Risk associations were estimated by multivariate Cox proportional hazard models. No heterogeneity was observed between men and women, fatty fish and lean fish intake, or colorectal cancer subtypes.

S2: Hazard ratios, per servings/week of types of fish, and 95% confidence interval for colorectal cancer risk associated with recommended intakes of fish

Risk associations were estimated by multivariate Cox proportional hazard models. The intake of 1 to 2 servings of fish/week as recommended by WHO, was associated with a decrease in colorectal cancer risk.

S3: Hazard ratios and 95% confidence interval for colorectal cancer risk, by EPIC country

Hazard ratios per colorectal cancer risk were estimated for each EPIC participating country, using multivariate Cox proportional hazard models. No heterogeneity was observed for the colorectal cancer risk between countries ($P_{heterogeneity}$=0.12).

S4: Hazard ratios and 95% confidence interval for colorectal cancer risk associated with dietary n-3 LC-PUFA, by sex

Hazard ratios for colorectal cancer risk, per 100 mg per day increment for individual and grouping of n-3 LC-PUFA and 5-unit increment in n-6:n-3 LC-PUFA, were estimated by multivariate Cox proportional hazard models. No heterogeneity was observed between men and women, fatty fish and lean fish intake, or colorectal cancer subtypes, although the associations reached significance in women.