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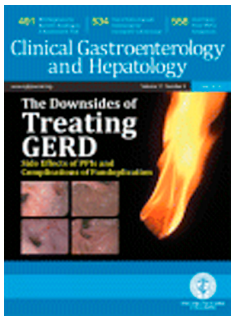


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# Consumption of Fish and Long-chain n-3 Polyunsaturated Fatty Acids is Associated With Reduced Risk of Colorectal Cancer in a Large European Cohort

**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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**Author contributions:** MJG, VC and NM conceived the study; CC and GN estimated dietary intake under the supervision of IH; VC supervised laboratory analyses and biomarkers data acquisition; EKA analysed the data under the supervision of VC and NM; VC provided guidance on data interpretation; EKA drafted the manuscript under the chaired supervision of VC, NM, MG and IH; TP, VF, MCBR, CCD, KO, AM, MW, BVG, GS, AJC, EW, HW provided critical appraisal of the draft. GS, MCBR, AF, VK, TK, AO, AT, CCD, KO, CL, AA, MJS, PA, JMH, EA, APC, AT, AK, GM, DP, VP, RT, AN, SP, BBM, AM, JWGD, SH, BO, MW, BVG, GS, MB, EW, AJC, HW, ER, TN, and MJG granted access to the EPIC cohort data and materials. All the co-authors provided edits and critiqued the manuscript for intellectual content.

**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 $\chi^2$ 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.

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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 $\chi^2$ 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) | *<0.001* | | 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) | *<0.001* | *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) | *<0.001* | *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.

Supplementary Table 1: Spearman rank correlation between fish, n-3 LC-PUFA intake and other covariates in EPIC, EPIC cohort study, 1992-2014

| | Total fish | Fatty fish | Lean fish | EPA* | DPA* | DHA* | n-3 LC-PUFA* | n-6/n-3 LC-PUFA* | BMI | Alcohol | Red and processed meat | Physical activity | Education |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Total fish | 1 | | | | | | | | | | | | |
| Fatty fish | 0.738 | 1 | | | | | | | | | | | |
| Lean fish | 0.728 | 0.478 | 1 | | | | | | | | | | |
| EPA* | 0.855 | 0.789 | 0.490 | 1 | | | | | | | | | |
| DPA* | 0.817 | 0.715 | 0.446 | 0.904 | 1 | | | | | | | | |
| DHA* | 0.890 | 0.789 | 0.531 | 0.980 | 0.913 | 1 | | | | | | | |
| n-3 LC-PUFA* | 0.881 | 0.790 | 0.514 | 0.991 | 0.929 | 0.996 | 1 | | | | | | |
| n-6/n-3 LC-PUFA* | -0.675 | -0.609 | -0.456 | -0.784 | -0.603 | -0.768 | -0.761 | 1 | | | | | |
| BMI | 0.067 | 0.032 | 0.048 | 0.080 | 0.028 | 0.081 | 0.077 | -0.087 | 1 | | | | |
| Alcohol | 0.049 | 0.113 | -0.039 | 0.124 | 0.135 | 0.110 | 0.120 | -0.003 | -0.013 | 1 | | | |
| Red and processed meat | 0.142 | 0.172 | 0.093 | 0.217 | 0.292 | 0.216 | 0.230 | 0.081 | 0.160 | 0.234 | 1 | | |
| Physical activity | 0.006 | 0.038 | 0.031 | 0.049 | 0.060 | 0.035 | 0.042 | 0.001 | -0.119 | 0.104 | 0.063 | 1 | |
| Education | -0.047 | -0.023 | -0.056 | -0.083 | -0.011 | -0.088 | -0.080 | 0.135 | -0.292 | 0.134 | -0.102 | 0.071 | 1 |
| Smoking | 0.053 | 0.068 | 0.026 | 0.067 | 0.098 | 0.069 | 0.072 | -0.012 | -0.023 | 0.183 | 0.118 | 0.050 | 0.073 |

*Dietary estimates

All p-values were significant, due to large sample size

Supplementary table 1: Hazard ratios (HRs)* and 95% confidence intervals (95% CI) for colorectal cancer risk associated with shellfish and combined shellfish and fish intake (quintiles and tertiles and continuous) , EPIC cohort study, 1992-2014

| | Quintiles of shellfish and fish intake and tertiles of shellfish intake | | | | | | |
|---|---|---|---|---|---|---|---|
| | Q1 | Q2 | Q3 | Q4 | Q5 | $P_{trend}$ | Continuous, per 100g/day |
| **Total fish and shellfish intake, g/day** | <10.4 | 10.4-<21.2 | 21.2-<34.2 | 34.2-56.1 | >56.1 | | |
| Colorectal cancer cases | 1148 | 1181 | 1262 | 1361 | 1339 | | |
| Colorectal cancer | 1.00 (Ref.) | 0.95 (0.87-1.03) | 0.92 (0.84-1.00) | 0.89 (0.81-0.97) | 0.87 (0.79-0.96) | *0.003* | 0.90 (0.82-0.98) |
| Colon cancer | 1.00 (Ref.) | 1.00 (0.90-1.11) | 0.91 (0.82-1.02) | 0.89 (0.80-1.00) | 0.89 (0.79-1.00) | *0.014* | 0.90 (0.80-1.01) |
| Proximal colon cancer | 1.00 (Ref.) | 0.98 (0.83-1.15) | 0.94 (0.79-1.11) | 0.85 (0.71-1.01) | 0.92 (0.77-1.11) | *0.165* | 0.90 (0.76-1.07) |
| Distal colon cancer | 1.00 (Ref.) | 1.09 (0.93-1.26) | 0.93 (0.79-1.09) | 0.98 (0.83-1.15) | 0.91 (0.76-1.09) | *0.173* | 0.95 (0.80-1.12) |
| Rectal cancer | 1.00 (Ref.) | 0.89 (0.77-1.03) | 0.96 (0.83-1.12) | 0.91 (0.78-1.06) | 0.88 (0.75-1.05) | *0.240* | 0.91 (0.77-1.07) |
| | Tertile 1 | Tertile 2 | Tertile 3 | | | | |
| **Shellfish intake, g/day** | 0 | >0-2.94 | >2.95 | | | | |
| Colorectal cancer cases | 1883 | 2320 | 2088 | - | - | | |
| Colorectal cancer | 1.00 (Ref.) | 1.01 (0.94-1.10) | 1.00 (0.93-1.08) | - | - | *0.950* | 1.23 (0.76-1.99) |
| Colon cancer | 1.00 (Ref.) | 1.06 (0.96-1.17) | 1.02 (0.93-1.12) | - | - | *0.801* | 1.22 (0.67-2.22) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Proximal colon cancer | 1.00 (Ref.) | 1.09 (0.94-1.27) | 1.06 (0.92-1.22) | - | - | *0.572* | 1.22 (0.49-3.05) |
| Distal colon cancer | 1.00 (Ref.) | 1.03 (0.89-1.19) | 1.04 (0.91-1.20) | - | - | *0.543* | 1.67 (0.73-3.80) |
| Rectal cancer | 1.00 (Ref.) | 0.94 (0.82-1.09) | 0.97 (0.85-1.11) | - | - | *0.790* | 1.23 (0.54-2.80) |

* 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