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# Dietary Omega-3 Fatty Acids, *COX-2* Genetic Variation, and Aggressive Prostate Cancer Risk

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## Abstract

**Purpose—**Dietary intake of long-chain omega-3 polyunsaturated fatty acids (LC n-3) may reduce inflammation and in turn decrease risk of prostate cancer development and progression. This potential effect may be modified by genetic variation in *COX-2*, a key enzyme in fatty acid metabolism and inflammation.

**Experimental Design—**We used a case-control study of 466 men diagnosed with aggressive prostate cancer and 478 age- and ethnicity-matched controls. Diet was assessed with a semiquantitative food frequency questionnaire, and nine *COX-2* tag single nucleotide polymorphisms (SNPs) were genotyped. We used logistic regression models to estimate odds ratios (ORs) for association and interaction.

**Results—**Increasing intake of LC n-3 was strongly associated with a decreased risk of aggressive prostate cancer (trend p<=0.0001). The OR (95% CI) for prostate cancer comparing the highest to the lowest quartile of omega-3 intake was of 0.37 (0.25 – 0.54). The LC n-3 association was modified by SNP rs4648310 (+8897 A/G), flanking the 3′ region of *COX-2* (interaction p=0.02). In particular, the inverse association was even stronger among men with this variant SNP. This reflected the observation that men with low LC n-3 intake and the variant rs4648310 SNP had an increased risk of disease (OR = 5.49; 95% CI: 1.80-16.7), which was reversed by increasing intake of LC n-3.

**Conclusions—**Dietary LC n-3 PUFAs appear protective for aggressive prostate cancer, and this effect is modified by the *COX-2* SNP rs4648310. Our findings support the hypothesis that LC n-3 may impact prostate inflammation and carcinogenesis through the COX-2 enzymatic pathway.

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**Statement of Translational Relevance:** By aiming to understanding the clinical and mechanistic effect of modifiable risk factors—dietary fatty acids—on the most commonly diagnosed cancer in men, this topic is of high relevance to the scientific community. In fact, our findings suggest that by consuming high amount of long chain omega-3 fatty acids – mainly dark fish and shellfish – men can lower their risk of prostate cancer. Importantly, this protective effect is even stronger in men carrying a *COX-2* gene variant (rs4648310), a risk factor for prostate cancer, or is independent of genetic variation at other *COX-2* single nucleotide polymorphisms. *COX-2* is the main enzyme involved in the metabolism of fatty acids and plays a key role in chronic inflammation that may lead to prostate carcinogenesis. Genetic variation of *COX-2* is associated with prostate cancer. Our findings suggest that the dietary recommendation may be appropriate for all men, independently of their genetic background.

**Keywords**

prostatic neoplasms; diet; polyunsaturated fatty acids; omega-3 fatty acids; cyclooxygenase 2; gene; genetic variation; single nucleotide polymorphism

## Introduction

Prostate cancer is one of the most common cancers in men[1] and in 2008 is projected to account for almost 30% of the new cancer diagnoses in the U.S.[2] Identifying risk factors for prostate cancer is critically important to develop potential interventions and to expand our understanding of the biology of this disease. Increasing evidence supports the existence of risk factors involved with inflammation; pro-inflammatory mediators within the prostate can lead to a state of chronic inflammation resulting in lesions of proliferative inflammatory atrophy that may transition to prostatic intraepithelial neoplasia and eventually prostate adenocarcinoma.[3]

Several sources of inflammation may influence the risk of prostate cancer, including diet,[4] bacterial[5, 6] and viral[7] infections, and intraprostatic urine reflux.[8, 9] With regard to diet, a number of nutritional factors may reduce the risk and progression of prostate cancer through anti-oxidant and anti-inflammatory effects.[4] These include omega-3 (n-3) polyunsaturated fatty acids (PUFAs), fish, selenium, vitamins D and E, and lycopene.[4]

PUFAs are classified according to their molecular configuration: omega-6 (n-6) or omega-3 (n-3). The n-6 PUFAs, such as linoleic acid (LA) and arachidonic acid (AA), are metabolized into pro-inflammatory eicosanoids, including prostaglandin $E_2$–which has been linked to carcinogenesis in studies of prostate and other tumors.[10, 11] In contrast, the n-3 PUFAs, such as α-linolenic acid (ALA) 18:3, eicosapentaenoic acid (EPA) 20:5, docosahexaenoic acid (DHA) 22:6 and docosapentaenoic acid (DPA) 22:5 exhibit anti-inflammatory properties by competitively inhibiting the AA cascade, mainly at the coclooxygenase (COX) pathway,[12] thus reducing the production of pro-inflammatory prostaglandins derived from arachidonic acid. The n-3 PUFAs are. Long chain n-3 PUFAs (LC n-3), EPA, DPA and DHA, appear the most potent at this enzyme inhibition. The main sources of LC n-3 in the typical 'Western diet' are dark fish and shellfish.

Multiple lines of evidence suggest that polyunsaturated acids (PUFAs) play a role in prostate carcinogenesis. In animal studies, mice fed an n-3 versus an n-6 PUFA diet exhibit a decreased expression of *COX-2* in their implanted prostate tumors as well as a decreased rate of prostate cancer tumor recurrence after excision (mimicking radical prostatectomy).[13] Mice fed an EPA-rich diet have higher LC n-3 content in their implanted prostate tumor and a better response to hormone ablation.[14] In humans, three months of a low-fat, fish oil supplemented diet decreased *COX-2* expression in prostatic tissue in four of seven men with untreated prostate cancer.[15] Cyclooxygenase (COX), also known as prostaglandin H synthase (PTGS) or prostaglandin-endoperoxide synthase, catalyzes the rate-limiting step in the formation of inflammatory prostaglandins. While the first form of the enzyme (*COX-1*) is involved in production of prostaglandins for cellular housekeeping functions, the second form (*COX-2*) is inducible and is associated with biologic events such as injury, inflammation, and proliferation.

Some, though not all, epidemiological studies of fish and/or n-3 PUFA intake and prostate cancer have observed inverse associations.[16-26] Of seven prospective studies of fish intake and prostate cancer risk, three reported an inverse association with high intake of fish,[17, 23, 26] one reported a positive association,[16] while four were equivocal.[19, 21, 22, 24] Similarly, some prospective studies of LC n-3, EPA, DPA or DHA and prostate cancer have detected inverse

associations with increased intake,[17, 20, 22] although another study found a positive association, [27] and three other studies observed no association.[24, 28, 29] These somewhat inconsistent findings might reflect the heterogeneity of prostate cancer; in fact, the potential protective effect of fish and LC n-3 appears strongest for aggressive and metastatic disease, and for death caused by prostate cancer.[17, 23, 26] Note also that ALA and total n-6 PUFA intake have been associated with increased risk of prostate cancer.[11, 30, 31]

Another possible explanation for the slightly equivocal inverse associations observed for n-3 PUFAs is effect modification by genotype. A recent study of Swedish men found that frequent consumption of fatty fish—a proxy for long-chain n-3 PUFAs—was inversely associated with prostate cancer risk (OR= 0.57; 95% CI: 0.43-0.76); moreover, this effect was modified by the rs5275 (+6364 A>G) single nucleotide polymorphism (SNP) in *COX-2*, whereby only men carrying the variant allele maintained a strong inverse association between fatty fish intake and prostate cancer.[32] This suggests that the potential protective effect of long-chain PUFAs on prostate cancer may be modified by *COX-2*.

In light of these findings, and the potential stronger protective effect of LC n-3 and dark fish on aggressive disease, we investigate here the influence of n-3 PUFAs and dark fish on risk of aggressive prostate cancer. Furthermore, we examine whether such associations are modified by *COX-2* variants.

## Methods

### Study Subjects

Between 2001 and 2004, we recruited 506 aggressive incident prostate cancer cases and 506 controls from the major medical institutions in Cleveland, Ohio (The Cleveland Clinic, University Hospitals of Cleveland, and their affiliates). Aggressive prostate cancer cases were confirmed histologically and defined as having a Gleason score ≥7, TNM stage ≥ T2c, or PSA at diagnosis >10 ng/ml. Cases were contacted shortly following diagnosis with a median time between diagnosis and recruitment of 4.7 months. To help ensure that the controls were representative of the source population of the cases, controls were men who underwent standard annual medical exams at the collaborating medical institutions. Controls had no diagnosis of prostate cancer or any other non-skin cancer. At study entry, all controls were screened with a serum PSA test. If their PSA value was higher than 4.0 ng/mL, patients underwent a formal prostate cancer evaluation and biopsy. Follow-up on the 50 patients having PSA > 4 ng/mL led to the diagnosis of 2 new prostate cancer cases. Both patients met our criteria for aggressive disease and were subsequently included as cases in our study. Controls were frequency matched to cases by age (within 5 years), ethnicity, and medical institution. Data was collected on various clinical, anthropometric and demographic factors during an in-person computer-aided interview.

Institutional Review Board approval was obtained from the participating medical institutions. Informed consent was obtained from all study participants.

### Nutritional assessment

Nutrient data was collected using a validated food frequency questionnaire developed by the Nutrition Assessment Shared Resource (NASR) of the Fred Hutchinson Cancer Research Center. Nutrient calculations were performed using the Nutrient Data System for Research (NDSR) software version 2007 developed by the Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN (Food and Nutrient Database version 2007).[33-35] For these analyses, we excluded 68 subjects because of implausible values for total calorie intake (<500 kcal/day or >5000 kcal/day).[32]

### *COX-2* tag SNP selection

We previously reported detailed methods of SNP selection and genotyping of *COX-2* in our study.[36] Briefly, we evaluated the genetic structure of *COX-2* using information from the International HapMap project,[37] Perlegen, and the Seattle SNP projects (National Heart, Lung, and Blood Institute Genome Variation Server; http://gvs.gs.washington.edu/GVS/). We identified seven tag SNPs that could be successfully genotyped and two other common *COX-2* SNPs, +8365 C>T (rs689470) and -899 G>C (rs20417) that were previously associated with prostate cancer.[38, 39] These nine SNPs were genotyped in our case-control population: rs689466, rs20417, rs2745557, rs5277, rs2066826, rs5275, rs2206593, rs689470 and rs4648310 (Supplemental Table 1).

### Statistical analysis

We examined the association between dietary intake of PUFAs, fish, and aggressive prostate cancer using unconditional logistic regression models. We evaluated the main effects of individual n-6 PUFAs (i.e., LA and AA; grams/day) and n-3 PUFAs and total LC n-3 (i.e., EPA, DHA, DPA; grams/day). All PUFAs were categorized into quartiles based on their distribution among controls. We also examined the intake of the following fish: dark fish (such as salmon, mackerel, and bluefish; boiled or baked), white fish (such as sole, halibut, snapper and cod; boiled or baked), shellfish (i.e. shrimp, lobster and oysters; not fried), tuna (i.e. canned tuna, tuna salad and tuna casserole), and fried fish (i.e. fried fish, fish sandwich and fried shellfish). Fish intake variables (except shellfish) were categorized into never, 1-3 times per month, and once or more per week. Because of lower intake, shellfish intake was categorized in never, once per month, and twice or more per month. The trend p-values were calculated with the PUFA/fish variable modeled continuously across all quartiles.

To investigate potential modification of the PUFAs effects by *COX-2* genotypes, we focused on overall LC n-3 consumption and the five SNPs with statistically significant associations with prostate cancer, using dominant or recessive coding as previously reported.[36] Here, we first stratified the logistic regression analyses of LC n-3 (continuous) by COX-2 genotypes. Then we extended the unconditional logistic regression model to include LC n-3 PUFAs, *COX-2* genotype, and their interaction.

All logistic regression models adjusted for the matching variables – age, ethnicity and institution – and total calorie intake. To evaluate potential confounding due to lifestyle factors associated with healthy behavior and prostate screening, we examined the following covariates: smoking (pack years), BMI ($kg/m^2$), prior history of PSA testing for prostate cancer (never/once/twice or more), and family history of prostate cancer (two or more first-degree relative per family, or one first-degree and two or more second-degree relatives). None of these covariates materially influenced the main-effect logistic regression coefficients (i.e., always resulting in a less than 10% change in the regression coefficients), and are thus excluded from our final models. We also examined the potential modification of the associations evaluated here by NSAIDs use (ever versus never). All p-values are two-sided, and all analyses were undertaken with SAS software (version 9.1; SAS Institute Inc., Cary, North Carolina).

## Results

The demographic and clinical characteristics of the study subjects are presented in Table 1. Cases reported a higher frequency of family history of prostate cancer and previous history of PSA testing than controls. The average PSA at diagnosis for cases was 13.4 ng/mL and 84% of the cases had a Gleason score ≥7. Mean dietary intake of total calories, fat, and LA was statistically significantly higher in cases than controls (Table 2). In contrast, mean intake of

EPA, DHA, and DPA was significantly lower in cases than controls. In addition, the mean intake of dark fish and shellfish was significantly lower in cases than in controls (Table 2).

The associations between dietary PUFAs and aggressive prostate cancer are presented in Table 3. Higher intake of any and total LC n-3s were significantly associated with a strong dose-response reduction in prostate cancer risk (p for trend ≤0.0001). For EPA, the adjusted odds ratios (OR) and 95% confidence intervals (CIs) for the second, third, and fourth quartiles in comparison to the first were 0.60 (95% CI: 0.42 – 0.86), 0.50 (95% CI: 0.35 – 0.71), and 0.35 (95% CI: 0.24 – 0.52), respectively. For DPA, the ORs and 95% CIs across low to high quartiles of intake were 0.71 (95% CI: 0.50 – 1.01), 0.45 (95% CI: 0.31 – 0.66), and 0.40 (95% CI: 0.27 – 0.59). For DHA, similar effects were also observed : 0.60 (95% CI: 0.40 – 0.86), 0.45 (95% CI: 0.31 – 0.65), and 0.36 (95% CI: 0.25 – 0.53). EPA was positively correlated with DPA and DHA (both r = 0.93); DPA was correlated with DHA (r = 0.96). The associations observed were similar across ethnic group (African American or Causasian; data not shown). We observed no significant association between aggressive prostate cancer and ALA or total n-6 PUFA (Table 3). The correlation coefficient (r) between LC n-3 and these PUFAs is 0.17, 0.13 and 0.44 respectively for ALA, LA and AA. The observation of an inverse association between the higher quartiles of arachidonic acid and aggressive prostate cancer may simply reflect its correlation with LC n-3 (r=0.44). When we adjusted the arachidonic effect for long chain n-3 fatty acids, it was no longer associated with disease (fourth versus first quartile OR=0.85, 95% CI: 0.52 - 1.40). Modeling a ratio of LC n-3 to n-6 did not materially change the results, but significance of the association was lower than that for LC n-3 (p=0.0023 vs p<0.0001) (Table 3).

The associations between fish types and aggressive prostate cancer risk are shown in Table 4. Higher intake of dark fish was associated with a significantly decreased risk of prostate cancer. Men who ate dark fish 1-3 times per month had a 36% lower risk of prostate cancer in comparison to men who never ate dark fish (OR = 0.64; 95% CI: 0.48-0.86). Furthermore, those who ate dark fish more than once per week had an even larger reduction in risk in comparison to those who never ate dark fish (OR = 0.43; 95% CI: 0.29-0.63). A similar dose-response reduction in risk of aggressive prostate cancer was found for shellfish intake (both p trend <0.0001). Such an association pattern or significance level was not observed with other fish types (Table 4).

Results from the LC n-3 analyses stratified by *COX-2* genotypes are given in Table 5. The main effects for the five nominally significant SNPs are listed first, followed by the stratified case / control counts and total LC n-3 associations. Stratification by most of the SNPs did not materially alter the LC n-3 associations (Table 5). However, the inverse association between LC n-3 and aggressive prostate cancer was even stronger among men with the variant (AG or GG) rs4648310 (+8897 A>G) genotype (Table 5). This reflects the larger variation in case-control counts across quartiles of LC n-3 intake among men with the variant genotype: in the lowest quartile there are substantially more cases than controls, whereas the opposite is observed in the highest quartile (Table 5).

This difference was also supported by the interaction models, which gave a nominally significant interaction between LC n-3 and the rs4648310 (+8897 A>G) SNP in *COX-2* (p = 0.02). Among men with the rs4648310 wildtype (AA), increasing LC n-3 consumption by a half-gram per day was inversely associated with prostate cancer at a similar level as suggested from the overall main effects for LC n-3 (OR = 0.61; 95% CI: 0.46-0.81). However, for men with the rs4648310 variant, low consumption of LC n-3 PUFAs resulted in an increased risk of aggressive prostate cancer (OR = 5.49; 95% CI: 1.80-16.7). This positive association was essentially reversed with increasing consumption of LC n-3 by a half-gram per day, although

the small number of cases with the variant and high intake of LC n-3 led to wide confidence intervals (OR = 0.42; 95% CI: 0.13-1.37).

To investigate whether these findings were modified by NSAIDs, we stratified the analyses by NSAIDs use. We previously reported[40] an inverse association between NSAIDs use and aggressive prostate cancer (OR = 0.67, 95%CI: 0.52 – 0.87). Among NSAIDs users, the odds ratios for the second, third and forth quartiles of long chain omega-3 polyunsaturated fatty acid consumption in comparison to the first were: 0.87 (95% CI: 0.54 – 1.40), 0.60 (95% CI: 0.37 – 0.97), and 0.48 (95% CI: 0.30 – 0.80). In NSAIDs non-users, the corresponding odds ratios were slightly lower: 0.44 (95% CI: 0.25 – 0.77), 0.39 (95% CI: 0.21 – 0.72), and 0.30 (95% CI: 0.16 – 0.56). Nevertheless, p-values from a formal test of interaction between the fatty acids and NSAIDs were relatively large (>0.20). Adjusting the models for NSAIDs use did not appear to materially alter the interaction between long chain polyunsaturated fatty acids and rs4648310 (+8897 A>G) *COX-2* genotype (interaction p=0.02) or any other *COX-2* SNP (not shown).

## Discussion

We detected strong inverse associations between increasing intake of long-chain omega-3 PUFAs (LC n-3) EPA, DPA and DHA and aggressive prostate cancer. The decreased risk followed a clear dose-response pattern across increasing levels of LC n-3 intake, whereby men in the highest quartile of consumption had less than half the risk of aggressive disease in comparison to men in the lowest quartile. Similar inverse associations were observed for increasing intake of dark fish and shellfish, the two main sources of LC n-3. Tuna, another source of LC n-3 that was measured here including tuna casserole—rich in other kinds of fat—is also expected to be inversely associated with prostate cancer in our model. This was found weakly, probably because of confounding by other kinds of fat. In addition, this inverse association was modified by the *COX-2* SNP rs4648310 (+8897 A>G), and men with the variant genotype (AG or GG) and low intake of LC n-3 had a much higher risk than men with the variant genotype but a high intake of LC n-3.

Our findings for the main effects of PUFAs are consistent with previous reports. Despite mixed results for overall prostate cancer, LC n-3 appears to be more strongly associated with more aggressive prostate cancers. The Health Professionals Follow-Up Study found a weak inverse association between high fish consumption—and high LC n-3 consumption—and prostate cancer risk. The association was stronger and statistically significant only for metastatic prostate cancer (OR = 0.56, 95% CI: 0.37 – 0.86).[17] A study from the Swedish twin registry[26] found that individuals with a high fish intake had a two-fold decrease in risk of prostate cancer and a three-fold decrease in death from prostate cancer. In another Swedish study,[32] high fatty fish consumption was associated with a 2-fold decrease in prostate cancer risk. This effect estimate was stable across disease stages, but the study population was composed of an advanced cancer sample: primarily men unscreened for prostate cancer with 41% having metastatic disease.

These results—and or observations—suggest that LC n-3 may have a more pronounced effect on biologically aggressive tumors or on their progression, and less on carcinogenesis of more benign or earlier stage tumors often detected by screening.[41, 42] This appears to be true across varying baseline population levels of fish and LC n-3 intake. The fish/LC n-3 levels of the Health Professionals Follow-Up Study[17, 20] are similar to that of our study, while those of the Swedish[26, 27, 32] and Japanese[16] studies were much higher, and those of the Dutch studies[24, 25], lower, with a narrow range of variation making association patterns more difficult to isolate.

In most of the studies reporting no association, PUFAs or fish were measured only once in the 1980s or early 1990s and the fish type was not differentiated,[19, 21, 22, 25] or the different PUFAs were not distinguished but rather evaluated overall.[24, 28, 29] This might explain the absence of association. Moreover, two of the negative studies were on cohorts with short follow-up, which might be problematic for prostate cancer since it is a relatively latent disease generally occurring later in life.[18, 21]

Two studies reported a positive association between LC n-3/fish intake and prostate cancer, although they were both undertaken in populations with much higher fish intake than our study —Sweden[27] and Japan[16]—and they did not differentiate type of fish consumed. As noted by the authors of these studies, the positive association could be confounded by environmental toxins, such as polychlorinated biphenyls (PCBs) or methylmercury compounds contained in fish. In the Japanese study,[16] the exposure was defined in the 1960s and 1970s with follow-up until late 1990s, during which dietary patterns have changed [Cancer Statistics in Japan. http://ganjoho.ncc.go.jp/public/statistics/backnumber/2007_en.html (accessed August 2008)], resulting in another source of potential confounding. Prospective studies where exposure is reassessed periodically, such as the Health Professional Follow-up Study,[17, 20] provide better measures of adult dietary intake and have shown negative associations.

Our findings of an interaction between LC n-3 and the *COX-2* SNP rs4648310 suggest that while carriers of the variant SNP had an overall increased risk of aggressive prostate cancer, this deleterious effect was found only in men consuming low levels of LC n-3. Moreover, this association was reversed by high consumption of LC n-3. The diet × rs4648310 (+8897 A>G) interaction was similar across individual LC n-3 (EPA, DPA and DHA) and dark fish (interaction p = 0.002, data not shown)—the main source of the PUFAs.

These results are in general agreement with those previously reported in a Swedish study[32]. Although rs4648310 (+8897 A>G) was not genotyped in their study, they found that another *COX-2* SNP (rs5275, +6364 A>G) modified the impact of fish intake on prostate cancer (p interaction < 0.01). In particular, Salmon-type fish consumption—a proxy for LC n-3 intake —was protective only among men carrying the variant rs5275 genotypes (p trend < 0.01). We did not observe a similar pattern of interaction with rs5275 in our study (p interaction = 0.8). SNPs rs4648310 and rs5275 are located 2.4 kilobases apart and exhibit weak linkage disequilibrium in our population ($r^2$=0.01, among whites). The functional impact of rs5275, an intronic variant, and rs4648310, flanking the 3′ *COX-2* gene, on *COX-2* activity is not yet known. It is possible that either of these polymorphisms, or another linked variant, may have biological effects on *COX-2* activity. Collectively, the combined findings of our study and that of the Swedish population support the overall hypothesis that LC n-3 modifies prostate inflammation through the *COX-2* enzymatic pathway.

NSAIDs are one of the most frequently used inhibitors of the *COX-2* enzyme, one of the most important enzymes involved in the metabolism of the n-3 PUFAs. Although we observed a stronger reduction of prostate cancer risk by LCn-3 in NSAIDs non users, formal testing of interaction between the fatty acids and NSAIDs was not significant. This could be due to lack of power to show a stronger effect in NSAIDs non-users, or because of slightly different biological mechanisms. Both long chain omega-3 polyunsaturated fatty acids and NSAIDs compete with arachidonic acid for binding to the cyclooxygenase active site, but the downstream effects appear different.[43, 44] Our findings may support those at the cellular and molecular level of inter-related but slightly different mechanisms of action between n-3 PUFA and NSAIDs. In fact, we previously published[36] that *COX-2* SNP rs2745557 appeared to modify the NSAIDs effect: NSAIDs use was protective for prostate cancer risk only in carriers of the wildtype (GG) rs2745557 (OR 0.58; 95%CI: 0.42-0.79) but in carriers of at least one variant allele (GA/AA), no association was observed (OR 0.86; 95% CI: 0.55-1.35). Thus,

*COX-2* genetic variation at different areas, potentially affecting different sub-functions of the enzyme, may have different effects on prostate carcinogenesis: rs2745557 appears more important to the pharmacogenetics of NSAIDs, while rs4648310 appears more relevant to the metabolism of n-3 PUFAs. In contrast, in another study about fish intake—a proxy for LC n-3—and colon cancer, NSAIDs use was shown to be a modifier in addition to fish intake.[45] The interaction between COX-1 genetic variation and fish intake was statistically significant (p=0.04) only when NSAIDs use was also taken into account. Thus NSAIDs and LC n-3 may act synergistically in colon cancer and the same could be true for prostate cancer, despite our statistically negative findings in this regard.

To investigate ethnic differences in the observed associations, we first note descriptive information about the differences in dietary intake and *COX-2* polymorphisms by ethnic group. Here, we observed that African-Americans on average report lower total calorie intake and ALA, more AA, but similar LC n-3. Of course, any differences due to variation in caloric intake are adjusted for in our analyses. With regard to genotype, as expected, there are some differences by ethnicity, and in particular the main SNP of interest here, rs4648310, was only observed once among African-Americans. Secondly, the associations were examined stratified by ethnic group. The dietary effects appeard similar between the two ethnic groups: the OR comparing the highest to the lowest quartile of intake were similar across ethnic groups; but, while the significance of the trend test remained unchanged in Caucasians (p=0.0001), it dropped in African Americans (p=0.30), likely because of smaller sample size. In Caucasians, the significance of SNP rs4648310 effect was slightly lower at p=0.07, but the gene × LC n-3 interaction significance was unchanged (p=0.02). The interaction appears constant across ethnic groups, but could be driven by the effect in Caucasians, since our power for such stratified analysis is limited, especially in the African American group. Residual ethnic confounding of the genetic associations and interaction remains possible. Although, by matching cases and controls on ethnic group and medical institution, the likelihood that population stratification may have biased our results is low. Moreover we observed consistent *COX-2* gene effects among both African Americans and Caucasians[36], indicating that large-scale bias due to underlying population structure is unlikely.

There are a number of potential limitations to our study that merit consideration. First, our study has a limited sample size to detect gene-diet interactions. On the other hand, this speaks to the strength of the observed association. Second, by using a case-control design, we cannot completely exclude recall bias. Yet, when subjects were recruited into this study, there was little information to suggest that food elements rich in LC n-3 were protective against prostate cancer. In addition, cases were recruited into the study shortly following diagnosis, and asked to recall their dietary intake in the time period prior to diagnosis. Therefore, a differential recall of food intake between cases and controls explaining the observed association appears unlikely.

Third, prognostic selection bias in our study cannot be completely excluded since a majority of our cases were diagnosed by screening.[46] Screening, a health-conscious behavior, may be associated with the consumption of a healthier diet, fish, and LC n-3. We attempted to address this issue by adjusting for variables associated with health-seeking behaviors: smoking, BMI, previous prostate cancer screening with PSA, and total dietary fat intake.[30] Controlling for these factors did not materially modify our findings. In addition, we required that our controls also be PSA screened. Another group has previously reported that adjusting for PSA screening did not affect the association of n-3 PUFA and aggressive prostate cancer.[17] In addition, any potential confounding due to the cases having more healthy behaviors than controls is unlikely to explain the relatively large protective effect of n-3 PUFA we observed.

In summary, our study demonstrates that the dietary long-chain omega-3 fatty acids (LC n-3), EPA, DPA and DHA, are inversely associated with aggressive prostate cancer. This potential

protective effect may be modified by genetic variation in *COX-2*, whereby the deleterious effect of one SNP (rs4648310, +8897 A>G) was reversed by the LC n-3 effect. Furthermore, our study provides additional support for the role of inflammation in prostate cancer susceptibility and progression. More clinical and biological studies are needed to decipher how dietary long-chain omega-3 fatty acids and other factors involved with inflammation such as *COX-2* genotypes affect aggressive prostate cancer.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Abbreviations

**AA**
: Arachidonic Acid 20 4 (n-6)

**ALA**
: α- Linolenic Acid 18 3 (n-3)

**BMI**
: Body Mass Index

**COX-2**
: Cyclooxygenase enzyme, type 2, also know as prostaglandin H synthase type 2

**DHA**
: Docosahexaenoic Acid 22 6 (n-3)

**DPA**
: Docosapentaenoic Acid 22 5 (n-3)

**EPA**
: Eicosapentaenoic Acid 20 5 (n-3)

**LA**
: Linoleic Acid 18 2 (n-6)

**LC n-3**
: Long Chain Omega-3 Polyunsaturated Fatty Acid

**n-3**
: Omega-3 Polyunsaturated Fatty Acid

**n-6**
: Omega-6 Polyunsaturated Fatty Acid

**OR**
: Odds Ratio

**PSA**
: Prostate Specific Antigen

**PUFA**

Polyunsaturated Fatty Acid

**SNP**

Single Nucleotide Polymorphism

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**Table 1**

Characteristics of prostate cancer cases and controls in study of aggressive disease.

| | Cases (n=466) | Controls (n=478) |
|---|---|---|
| Age, y | | |
| Mean +/- SD | 65.5 +/- 8.1 | 65.7 +/- 8.2 |
| Ethnicity: n (%) | | |
| African American | 76 (16.3) | 81 (17.0) |
| Caucasian | 390 (83.7) | 397 (83.0) |
| Family history of prostate cancer: n (%) | | |
| Negative | 438 (94.0) | 473 (98.9) |
| Positive* | 28 (6.0) | 5 (1.1) |
| Smoking† (pack years): n (%) | | |
| Never | 194 (41.7) | 189 (39.9) |
| 10 or less | 88 (18.9) | 90 (19.0) |
| 10 – 20 | 51 (11.0) | 62 (13.1) |
| 20 – 40 | 85 (18.3) | 81 (17.1) |
| 40 or more | 47 (10.1) | 52 (10.9) |
| Body Mass Index ($kg/m^2$) | | |
| Mean +/- SD | 27.7 +/- 4.6 | 27.9 +/- 4.7 |
| Prior history of PSA test†: n (%) | | |
| Never | 99 (22.3) | 104 (23.9) |
| Once | 54 (12.1) | 66 (15.2) |
| Twice or more | 292 (65.6) | 265 (60.9) |
| Serum PSA value (ng/mL) | | |
| Mean +/- SD | 13.5 +/- 23.3 | 1.7 +/- 1.7 |
| Clinical stage†: n (%) | | |
| T1c | 285 (64.0) | |
| any T2 | 133 (29.9) | |
| T3 | 27 (6.1) | |
| Histologic tumor grade: Gleason score: n (%) | | |
| 6 or less | 75 (16.1) | |
| 7 | 287 (61.6) | |
| 8 or more | 104 (22.3) | |

*Family history of prostate cancer was defined as two or more first-degree relative per family, or one first-degree and two or more second-degree relatives.

†Numbers do not always add to 100% because of missing data.

**Table 2**

Average intake of calories, total fat, polyunsaturated fatty acids (PUFAs), and fish in study population, stratified by prostate cancer status.

| | Cases (N=466) | | Controls (N=478) | | |
|---|---|---|---|---|---|
| **Dietary Factor** | **Mean** | **SD** | **Mean** | **SD** | **p-value*** |
| Total Calories (kcal / day) | 2282 | 871 | 2098 | 785 | 0.0007 |
| Total Fat (g / day) | 87.5 | 43.9 | 78.8 | 39.5 | 0.001 |
| Polyunsaturated Fatty Acids | | | | | |
| Omega-6 PUFAs (g / day) | | | | | |
| Linoleic acid [LA] 18:2 | 16.8 | 8.6 | 15.3 | 7.9 | 0.007 |
| Arachidonic acid [AA] 20:4 | 0.175 | 0.112 | 0.166 | 0.090 | 0.20 |
| Omega-3 PUFAs (g / day) | | | | | |
| Alpha-Linolenic acid [ALA] 18:3 | 1.73 | 0.90 | 1.64 | 0.83 | 0.10 |
| Eicosapentaenoic acid [EPA] 20:5 | 0.072 | 0.078 | 0.089 | 0.075 | 0.0007 |
| Docosapentaenoic acid [DPA] 22:5 | 0.027 | 0.028 | 0.033 | 0.027 | 0.0008 |
| Docosahexaenoic acid [DHA] 22:6 | 0.147 | 0.169 | 0.186 | 0.175 | 0.0005 |
| Long-Chain Omega-3 PUFAs† | 0.247 | 0.270 | 0.309 | 0.273 | 0.0005 |
| Fish (servings / month) | | | | | |
| Dark fish‡ | 1.11 | 1.98 | 1.74 | 2.64 | <0.0001 |
| Whitefish§ | 1.72 | 3.67 | 1.93 | 2.58 | 0.31 |
| Shellfish// | 0.57 | 0.98 | 0.88 | 1.48 | 0.0002 |
| Tuna¶ | 2.02 | 3.09 | 2.35 | 3.64 | 0.14 |
| Fried Fish** | 1.43 | 1.94 | 1.63 | 2.17 | 0.15 |

*p-values obtained from t-tests comparing mean values between cases and controls.

†Eicosapentaenoic acid + Docosapentaenoic acid + Docosahexaenoic acid.

‡Salmon, mackerel and bluefish (broiled or baked).

§Sole, halibut, snapper and cod (broiled or baked).

//Shrimp, lobster, crab and oysters (not fried).

¶Canned tuna, tuna salad and tuna casserole.

**Fried fish, fish sandwich and fried shellfish (shrimp and oysters).

**Table 3**

Association between dietary polyunsaturated fatty acids and aggressive prostate cancer

| Polyunsaturated Fatty Acid | Quartile of PUFA Intake | | | | P-trend* |
|---|---|---|---|---|---|
| | 1 (reference) | 2 | 3 | 4 | |
| Omega-6 PUFAs | | | | | |
| Linoleic Acid (LA) 18:2 | | | | | |
| Level† (g) | 7.53 | 11.73 | 16.19 | 24.22 | |
| Cases / controls | 104 / 120 | 91 / 119 | 116 / 120 | 115 / 119 | |
| Adjusted OR‡ | 1.0 | 0.78 (0.52 – 1.16) | 0.88 (0.57 – 1.35) | 0.97 (0.56 – 1.67) | 0.84 |
| Arachidonic Acid (AA) 20:4 | | | | | |
| Level† (g) | 0.075 | 0.126 | 0.177 | 0.280 | |
| Cases / controls | 111 / 119 | 135 / 120 | 105 / 120 | 115 / 119 | |
| Adjusted OR‡ | 1.0 | 1.00 (0.69 – 1.44) | 0.64 (0.43 - 0.97) | 0.59 (0.38 – 0.93) | 0.37 |
| Omega-3 PUFAs | | | | | |
| α-Linolenic Acid (ALA) 18:3 | | | | | |
| Level† (g) | 0.79 | 1.27 | 1.75 | 2.55 | |
| Cases / controls | 108 / 120 | 106 / 119 | 103 / 120 | 149 / 119 | |
| Adjusted OR‡ | 1.0 | 0.83 (0.56 - 1.23) | 0.71 (0.46 - 1.09) | 0.81 (0.48 – 1.35) | 0.11 |
| Eicosapentaenoic acid (EPA) 20:5 | | | | | |
| Level† (g) | 0.020 | 0.051 | 0.090 | 0.167 | |
| Cases / controls | 176 / 119 | 113 / 120 | 103 / 120 | 74 / 119 | |
| Adjusted OR‡ | 1.0 | 0.60 (0.42 - 0.86) | 0.50 (0.35 - 0.71) | 0.35 (0.24 - 0.52) | <0.0001 |
| Docosapentaenoic acid (DPA) 22:5 | | | | | |
| Level† (g) | 0.008 | 0.020 | 0.034 | 0.061 | |
| Cases / controls | 164 / 120 | 131 / 119 | 89 / 120 | 82 / 119 | |
| Adjusted OR‡ | 1.0 | 0.71 (0.50 – 1.01) | 0.45 (0.31 - 0.66) | 0.40 (0.27 - 0.59) | <0.0001 |
| Docosahexaenoic acid (DHA) 22:6 | | | | | |
| Level† (g) | 0.037 | 0.097 | 0.180 | 0.368 | |
| Cases / controls | 175 / 120 | 112 / 119 | 92 / 120 | 77 / 119 | |
| Adjusted OR‡ | 1.0 | 0.60 (0.42 – 0.86) | 0.45 (0.31 - 0.65) | 0.36 (0.25 - 0.53) | <0.0001 |
| Long Chain Omega-3 PUFAs§ | | | | | |

| Polyunsaturated Fatty Acid | Quartile of PUFA Intake | | | | P-trend* |
|---|---|---|---|---|---|
| | 1 (reference) | 2 | 3 | 4 | |
| Level† (g) | 0.067 | 0.167 | 0.297 | 0.588 | |
| Cases / controls | 173 / 120 | 119 / 119 | 95 / 119 | 79 / 120 | |
| Adjusted OR‡ | 1.0 | 0.61 (0.43 – 0.87) | 0.47 (0.32 – 0.68) | 0.37 (0.25 – 0.54) | <0.0001 |
| Total Omega-3 to Total Omega-6 Ratio | | | | | |
| Level† | 0.096 | 0.118 | 0.136 | 0.165 | |
| Cases / controls | 160 / 119 | 146 / 120 | 92 / 119 | 68 / 120 | |
| Adjusted OR‡ | 1.0 | 0.94 (0.67 - 1.33) | 0.60 (0.42 - 0.86) | 0.41 (0.28 - 0.60) | 0.0002 |
| Long Chain Omega-3 to Total Omega-6 Ratio | | | | | |
| Level† | 0.004 | 0.011 | 0.022 | 0.047 | |
| Cases / controls | 173 / 120 | 140 / 119 | 88 / 120 | 65 / 119 | |
| Adjusted OR‡ | 1.0 | 0.83 (0.59 - 1.16) | 0.54 (0.38 - 0.78) | 0.41 (0.28 - 0.60) | 0.0023 |

*Calculated with actual values as a continuous variable.

†Mid-point of quartile.

‡Adjusted for calories, age, ethnicity, and institution, N=944. Adjustment for total fat intake, BMI, smoking, PSA screening, and family history of prostate cancer did not materially affect the results.

§Eicosapentaenoic acid + Docosapentaenoic acid + Docosahexaenoic acid. PUFAs= Polyunsaturated Fatty Acids

**Table 4**

Association between dietary fish intake and aggressive prostate cancer

| Fish | Frequency | | | P-trend* |
|---|---|---|---|---|
| | Never (reference) | 1-3/month | ≥ 1 / week | |
| Dark Fish‡ | | | | |
| Cases / controls | 271 / 213 | 145 / 175 | 50 / 90 | |
| Adjusted OR† | 1.0 | 0.64 (0.48 – 0.86) | 0.43 (0.29 – 0.63) | <0.0001 |
| White Fish§ | | | | |
| Cases / controls | 192 / 165 | 205 / 225 | 69 / 88 | |
| Adjusted OR† | 1.0 | 0.77 (0.58 – 1.03) | 0.66 (0.45 – 0.96) | 0.32 |
| Shellfish// | | | | |
| Cases / controls | 296 / 265 | 112 / 120 | 58 / 93 | |
| Adjusted OR† | 1.0 | 0.81 (0.59 – 1.11) | 0.51 (0.35 – 0.74) | <0.0001 |
| Tuna¶ | | | | |
| Cases / controls | 158 / 153 | 227 / 229 | 81 / 96 | |
| Adjusted OR† | 1.0 | 0.92 (0.69 – 1.24) | 0.75 (0.51 – 1.09) | 0.04 |
| Fried Fish** | | | | |
| Cases / controls | 186 / 194 | 223 / 198 | 57 / 86 | |
| Adjusted OR† | 1.0 | 1.10 (0.83 – 1.47) | 0.56 (0.37 – 0.86) | 0.03 |

*Calculated with actual values as a continuous variable.

†Adjusted for calories, age, ethnicity, and institution, N=944. Adjustment for total fat intake, BMI, smoking, PSA screening, and family history of prostate cancer did not materially alter our results.

‡Salmon, mackerel and bluefish (broiled or baked).

§Sole, halibut, snapper and cod (broiled or baked).

//Shrimp, lobster, crab and oysters (not fried). Actual categories are never, once per month and twice or more per month.

¶Canned tuna, tuna salad and tuna casserole.

**Fried fish, fish sandwich and fried shellfish (shrimp and oysters).

**Table 5**

Association between long chain omega-3 polyunsaturated fatty acids and aggressive prostate cancer, stratified by *COX-2* genotypes.

| ***COX-2* SNP** | | | **Long Chain Omega-3 PUFA*** | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | **Cases / controls Quartile of Intake** | | | | | | |
| **rs number (position)** | **Genotype** | **OR† (95% CI)** | **1** | **2** | **3** | **4** | **OR‡ (95% CI)** | **P-trend‡** | **P-Interaction§** |
| All subjects | | | 173 / 120 | 119 / 119 | 95 / 119 | 79 / 120 | 0.61 (0.46-0.81) | | |
| rs2745557 | GG | 1.0 | 125 / 77 | 87 / 70 | 67 / 79 | 58 / 75 | 0.59 (0.42-0.83) | 0.002 | 0.72 |
| (+201) | GA or AA | 0.65 (0.49-0.86) | 48 / 43 | 32 / 49 | 28 / 40 | 21 / 45 | 0.51 (0.32-0.84) | 0.007 | |
| rs5277 | CC | 1.0 | 122 / 84 | 84 / 98 | 66 / 93 | 54 / 90 | 0.60 (0.44-0.82) | 0.001 | 0.75 |
| (+1225) | CG or GG | 1.38 (1.03-1.86) | 51 / 36 | 34 / 21 | 29 / 26 | 25 / 30 | 0.48 (0.27-0.85) | 0.012 | |
| rs2206593 | CC | 1.0 | 161 / 108 | 110 / 103 | 88 / 97 | 71 / 104 | 0.53 (0.39-0.71) | <0.0001 | 0.14 |
| (+6993) | CT or TT | 0.53 (0.34-0.81) | 12 / 11 | 9 / 16 | 7 / 22 | 8 / 16 | 0.96 (0.47-1.95) | 0.91 | |
| rs689470 | GG or GA | 1.0 | 167 / 117 | 113 / 116 | 90 / 114 | 73 / 118 | 0.56 (0.43-0.75) | <0.0001 | 0.98 |
| (+8364) | AA | 2.23 (1.03-4.87) | 6 / 2 | 5 / 3 | 5 / 5 | 6 / 2 | 0.16 (0.01-2.01) | 0.15 | |
| rs4648310 | AA | 1.0 | 158 / 118 | 111 / 116 | 89 / 113 | 76 / 113 | 0.61 (0.47-0.81) | 0.0006 | 0.02 |
| (+8897) | AG or GG | 1.88 (1.04-3.40) | 15 / 2 | 8 / 3 | 6 / 6 | 3 / 7 | 0.07 (0.01-0.41) | 0.003 | |

*Eicosapentaenoic acid + Docosapentaenoic acid + Docosahexaenoic acid.

†For main genetic effect (ignoring PUFA intake). Adjusted for age, ethnicity and institution.

‡Stratified by genotypes. From logistic model, with long chain omega-3 PUFAs as a continuous variable. ORs correspond to difference between median values of quartiles 1 and 4 (0.52 g/day) unit increase in PUFAs. Adjusted for total calorie intake, age, ethnicity and institution.

§Multiplicative interaction from cross-product term in logistic regression between PUFAs (continuous) and each *COX-2* SNP.