Dietary Omega-3 Fatty Acids, Cyclooxygenase-2 Genetic Variation, and Aggressive Prostate Cancer Risk

Vincent Fradet,1,3 Iona Cheng,2,3 Graham Casey,4 and John S. Witte1,2,3

Abstract

Purpose: Dietary intake of long-chain ω-3 (LC n-3) polyunsaturated fatty acids may reduce inflammation and in turn decrease risk of prostate cancer development and progression. This potential effect may be modified by genetic variation in cyclooxygenase-2 (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 (SNP) were genotyped. We used logistic regression models to estimate odds ratios (OR) for association and interaction.

Results: Increasing intake of LC n-3 was strongly associated with a decreased risk of aggressive prostate cancer (P \text{trend} = 0.0001). The OR (95% confidence interval) for prostate cancer comparing the highest with the lowest quartile of n-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 (P \text{interaction} = 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% confidence interval, 1.80-16.7), which was reversed by increasing intake of LC n-3.

Conclusions: Dietary LC n-3 polyunsaturated fatty acids 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.

Prostate cancer is one of the most common cancers in men (1) and in 2008 is projected to account for ~ 30% of the new cancer diagnoses in the United States (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; proinflammatory 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, several nutritional factors may reduce the risk and progression of prostate cancer through antioxidant and anti-inflammatory effects (4). These include ω-3 (n-3) polyunsaturated fatty acids (PUFA), fish, selenium, vitamins D and E, and lycopene (4).

PUFAs are classified according to their molecular configuration: ω-6 (n-6) or n-3. The n-6 PUFAs, such as linoleic acid (LA) and arachidonic acid (AA), are metabolized into proinflammatory eicosanoids, including prostaglandin E2, 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 cyclooxygenase (COX) pathway (12), thus reducing the production of pro-inflammatory prostaglandins derived from AA. Long-chain n-3 (LC n-3) PUFAs (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 PUFAs play a role in prostate carcinogenesis. In animal studies, mice fed a n-3 versus a 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; ref. 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, 3 months of a low-fat, fish oil-supplemented diet decreased COX-2 expression in prostatic tissue in 4 of 7 men with untreated prostate cancer (15). COX, also known as prostaglandin H synthase, catalyzes the rate-limiting step in the formation of inflammatory prostaglandins. Whereas 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 associated with biological events such as injury, inflammation, and proliferation.

Some, although not all, epidemiologic 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), and 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 LC n-3 PUFAs) was inversely associated with prostate cancer risk [odds ratio (OR), 0.57; 95% confidence interval (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.

### Materials and 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, OH (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, tumor-node-metastasis stage ≥T3,c or prostate-specific antigen (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 examinations at the collaborating medical institutions. Controls had no diagnosis of prostate cancer or any other nonskin cancer. At study entry, all controls were screened with a serum PSA test. If their PSA value was >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 were 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 were collected using a validated food frequency questionnaire developed by the Nutrition Assessment Shared Resource of the Fred Hutchinson Cancer Research Center. Nutrient calculations were done using the Nutrient Data System for Research software version 2007 developed by the Nutrition Coordinating Center, University of Minnesota (Food and Nutrient Database version 2007; refs. 33–35). For these analyses, we excluded 68 subjects because of implausible values for total calorie intake (<500 or >5,000 kcal/d; ref. 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). 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), which 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 (Supplementary Table S1).

#### Statistical analysis.** We examined the association between dietary intake of fish, PUFA, and aggressive prostate cancer using unconditional logistic regression models. We evaluated the main effects of individual n-6 PUFA (LA and AA; g/d) and n-3 PUFAs and total LC n-3 (EPA, DHA, and DPA; g/d). All PUFAs were categorized into quartiles based on their distribution among controls. We also examined the
Table 1. Characteristics of prostate cancer cases and controls in study of aggressive disease

<table>
<thead>
<tr>
<th></th>
<th>Cases (n = 466)</th>
<th>Controls (n = 478)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y), mean ± SD</td>
<td>65.5 ± 8.1</td>
<td>65.7 ± 8.2</td>
</tr>
<tr>
<td>Ethnicity, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>76 (16.3)</td>
<td>481 (17.0)</td>
</tr>
<tr>
<td>Family history of prostate cancer, n (%)</td>
<td>390 (83.7)</td>
<td>397 (83.0)</td>
</tr>
<tr>
<td>Negative</td>
<td>438 (94.0)</td>
<td>473 (98.9)</td>
</tr>
<tr>
<td>Positive</td>
<td>28 (6.0)</td>
<td>5 (1.1)</td>
</tr>
<tr>
<td>Smoking (pack-years), n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>194 (41.7)</td>
<td>189 (39.9)</td>
</tr>
<tr>
<td>≤10</td>
<td>88 (18.9)</td>
<td>90 (19.0)</td>
</tr>
<tr>
<td>10-20</td>
<td>51 (11.0)</td>
<td>62 (13.1)</td>
</tr>
<tr>
<td>20-40</td>
<td>85 (18.3)</td>
<td>81 (17.1)</td>
</tr>
<tr>
<td>≥40</td>
<td>47 (10.1)</td>
<td>52 (10.9)</td>
</tr>
<tr>
<td>Body mass index (kg/m²), mean ± SD</td>
<td>27.7 ± 4.6</td>
<td>27.9 ± 4.7</td>
</tr>
<tr>
<td>Prior history of PSA test, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>99 (22.3)</td>
<td>104 (23.9)</td>
</tr>
<tr>
<td>Once</td>
<td>54 (12.1)</td>
<td>66 (15.2)</td>
</tr>
<tr>
<td>Twice or more</td>
<td>292 (65.6)</td>
<td>255 (60.9)</td>
</tr>
<tr>
<td>Serum PSA value (ng/mL), mean ± SD</td>
<td>13.5 ± 23.3</td>
<td>1.7 ± 1.7</td>
</tr>
<tr>
<td>Clinical stage, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1c</td>
<td>285 (64.0)</td>
<td></td>
</tr>
<tr>
<td>Any T2</td>
<td>133 (29.9)</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>27 (6.1)</td>
<td></td>
</tr>
<tr>
<td>Histologic tumor grade:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gleason score, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥6</td>
<td>75 (16.1)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>287 (61.6)</td>
<td></td>
</tr>
<tr>
<td>≥8</td>
<td>104 (22.3)</td>
<td></td>
</tr>
</tbody>
</table>

*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.

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 in 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 was significantly associated with a strong dose-response reduction in prostate cancer risk (P_trend ≤ 0.0001). For EPA, the adjusted OR (95% CI) for the second, third, and fourth quartiles in comparison with the first were 0.60 (0.42-0.86), 0.50 (0.35-0.71), and 0.35 (0.24-0.52), respectively. For DHA, the OR (95% CI) across low to high quartiles of intake were 0.71 (0.50-1.01), 0.45 (0.31-0.66), and 0.40 (0.27-0.59). For DHA, similar effects were also observed: 0.60 (0.40-0.86), 0.45 (0.31-0.65), and 0.36 (0.25-0.53). The associations observed were similar across ethnic group (African American or Caucasian; data not shown). We observed no significant association between aggressive prostate cancer and ALA or total n-6 PUFAs (Table 3). EPA was positively correlated with DPA and DHA (both r = 0.93); DPA was correlated with DHA (r = 0.96). The correlation between these LC n-3 and other PUFAs was lower: 0.17 (with ALA), 0.13 (LA), and 0.44 (AA). The observation of an inverse association between the higher quartiles of AA and aggressive prostate cancer may simply reflect its correlation with LC n-3 (r = 0.93). When we adjusted the AA effect for LC n-3, it was no longer associated with disease (fourth versus first quartile: OR, 0.85; 95% CI, 0.44). When we modeled 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 versus 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 one to three times per month had a 36% lower risk of prostate cancer in comparison with 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 with 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...
previously found an inverse association between NSAID use (OR, 0.42; 95% CI, 0.13-1.37).

With the variant and high intake of LC n-3 led to wide 95% CI
tion of LC n-3 by 0.5 g/d, although the small number of cases
association was essentially reversed with increasing consump-
tion of LC n-3 PUFAs resulting in an increased risk of aggressive
prostate cancer (OR, 5.49; 95% CI, 1.80-16.7). This positive
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), where
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.

Among men with the rs4648310 wild-type (AA), increasing LC
n-3 intake of LC n-3 PUFAs EPA, DPA, and DHA and aggressive
prostate cancer (OR, 0.67; 95% CI, 0.52-0.87).

and aggressive prostate cancer (OR, 0.67; 95% CI, 0.52-0.87).
Among NSAID users, the OR (95% CI) for the second, third,
and fourth quartiles of LC n-3 consumption in comparison
with the first were 0.87 (0.54-1.40), 0.60 (0.37-0.97), and
0.48 (0.30-0.80). In NSAID nonusers, the corresponding
OR (95% CI) were slightly lower: 0.44 (0.25-0.77), 0.39
(0.21-0.72), and 0.30 (0.16-0.56). Nevertheless, P values from
a formal test of interaction between the LC n-3 and NSAID
use did not appear to materially alter the interaction between
LC n-3 and rs4648310 (+8897 A>G) SNP (P = 0.02) or any other
COX2 SNP (not shown).

Discussion

We detected strong inverse associations between increasing
intake of LC n-3 PUFAs 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 with 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 cassette (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, the
main dietary effect was modified by the COX-2 SNP rs4648310
(+8897 A>G), whereby 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.

Table 2. Average intake of calories, total fat, PUFA, and fish in study population, stratified by prostate cancer status

<table>
<thead>
<tr>
<th>Dietary factor</th>
<th>Cases (n = 466), mean ± SD</th>
<th>Controls (n = 478), mean ± SD</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total calories (kcal/d)</td>
<td>2,282 ± 871</td>
<td>2,098 ± 785</td>
<td>0.0007</td>
</tr>
<tr>
<td>Total fat (g/d)</td>
<td>87.5 ± 43.9</td>
<td>78.8 ± 39.5</td>
<td>0.001</td>
</tr>
<tr>
<td>PUFA n-6 PUFAs (g/d)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LA 18:2</td>
<td>16.8 ± 8.6</td>
<td>15.3 ± 7.9</td>
<td>0.007</td>
</tr>
<tr>
<td>AA 20:4</td>
<td>0.175 ± 0.112</td>
<td>0.166 ± 0.090</td>
<td>0.20</td>
</tr>
<tr>
<td>PUFA n-3 PUFAs (g/d)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALA 18:3</td>
<td>1.73 ± 0.90</td>
<td>1.64 ± 0.83</td>
<td>0.10</td>
</tr>
<tr>
<td>EPA 20:5</td>
<td>0.072 ± 0.078</td>
<td>0.089 ± 0.075</td>
<td>0.0007</td>
</tr>
<tr>
<td>DPA 22:5</td>
<td>0.027 ± 0.028</td>
<td>0.033 ± 0.027</td>
<td>0.0008</td>
</tr>
<tr>
<td>DHA 22:6</td>
<td>0.147 ± 0.169</td>
<td>0.186 ± 0.175</td>
<td>0.0005</td>
</tr>
<tr>
<td>LC n-3 PUFAs</td>
<td>0.247 ± 0.270</td>
<td>0.309 ± 0.273</td>
<td>0.0005</td>
</tr>
<tr>
<td>Fish (servings/mo)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dark fish</td>
<td>1.11 ± 1.98</td>
<td>1.74 ± 2.64</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>White fish</td>
<td>1.72 ± 3.67</td>
<td>1.93 ± 2.58</td>
<td>0.31</td>
</tr>
<tr>
<td>Shellfish</td>
<td>0.57 ± 0.98</td>
<td>0.88 ± 1.48</td>
<td>&lt;0.0002</td>
</tr>
<tr>
<td>Tuna</td>
<td>2.02 ± 3.09</td>
<td>2.35 ± 3.64</td>
<td>0.14</td>
</tr>
<tr>
<td>Fried fish</td>
<td>1.43 ± 1.94</td>
<td>1.63 ± 2.17</td>
<td>0.15</td>
</tr>
</tbody>
</table>

*P values obtained from t tests comparing mean values between cases and controls.
†EPA + DPA + DHA.
‡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 cassette.
**Fried fish, fish sandwich, and fried shellfish (shrimp and oysters).
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 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; ref. 17). A study from the Swedish twin registry (26) found that individuals with a high fish intake had a 2-fold decrease in risk of prostate cancer and a 3-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 (40, 41). 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, whereas those of the Swedish (26, 27, 32) and Japanese (16) studies were much higher and those of the Dutch studies (24, 25) were 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 because 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 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 or methylmercury compounds contained in fish. Furthermore, 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, resulting in another source of potential confounding. Prospective studies where exposure is reassessed periodically, such as the Health Professionals’ 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 although 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 \( \times \) rs4648310 (+8897 A>G) interaction was similar across individual LC n-3 COX-2 SNP rs4648310 and rs5275 are located 2.4 kb apart and exhibit weak linkage disequilibrium in our population \( (r^2 = 0.01, \; \text{among Whites}) \). The functional effect of rs5275, an intronic SNP, and rs4648310, flanking the 3’ end of the 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 LC n-3 in NSAID nonusers, 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 NSAID nonusers or because of slightly different biological mechanisms. Both LC n-3 and NSAIDs compete with AA for binding to the COX active site, but the downstream effects appear different (42, 43). Our findings may support those at the cellular and molecular level of interrelated 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: NSAID use was protective for prostate cancer in carriers of the wild-type (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, in particular, salmon-type fish consumption (a proxy for LC n-3 intake) was protective only among men carrying the variant rs5275 genotypes \( (P_{\text{trend}} < 0.01) \). We did not observe a similar pattern of interaction with rs5275 in our study \( (P_{\text{interaction}} = 0.8) \). SNPs rs4648310 and rs5275 are located 2.4 kb apart and exhibit weak linkage disequilibrium in our population \( (r^2 = 0.01, \; \text{among Whites}) \). The functional effect of rs5275, an intronic variant, and rs4648310, flanking the 3’ end of the 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.

**Table 4. Association between dietary fish intake and aggressive prostate cancer**

<table>
<thead>
<tr>
<th>Fish</th>
<th>Frequency</th>
<th>Adjusted OR (95% CI)</th>
<th>COX-2 rs4648310 P&lt;sub&gt;trend&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Never (reference)</td>
<td>1-3/mo</td>
<td>≥1/wk</td>
</tr>
<tr>
<td>Dark fish &lt;sup&gt;1&lt;/sup&gt;</td>
<td>271/213</td>
<td>145/175</td>
<td>50/90</td>
</tr>
<tr>
<td>Cases/controls</td>
<td>1.0</td>
<td>0.64 (0.48-0.86)</td>
<td>0.43 (0.29-0.63)</td>
</tr>
<tr>
<td>Adjusted OR (95% CI) &lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White fish &lt;sup&gt;3&lt;/sup&gt;</td>
<td>192/165</td>
<td>205/225</td>
<td>69/88</td>
</tr>
<tr>
<td>Cases/controls</td>
<td>1.0</td>
<td>0.77 (0.58-1.03)</td>
<td>0.66 (0.45-0.96)</td>
</tr>
<tr>
<td>Adjusted OR (95% CI) &lt;sup&gt;4&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shellfish &lt;sup&gt;5&lt;/sup&gt;</td>
<td>296/265</td>
<td>112/120</td>
<td>58/93</td>
</tr>
<tr>
<td>Cases/controls</td>
<td>1.0</td>
<td>0.81 (0.59-1.11)</td>
<td>0.51 (0.35-0.74)</td>
</tr>
<tr>
<td>Adjusted OR (95% CI) &lt;sup&gt;6&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tuna &lt;sup&gt;6&lt;/sup&gt;</td>
<td>158/153</td>
<td>227/229</td>
<td>81/96</td>
</tr>
<tr>
<td>Cases/controls</td>
<td>1.0</td>
<td>0.92 (0.69-1.24)</td>
<td>0.75 (0.51-1.09)</td>
</tr>
<tr>
<td>Adjusted OR (95% CI) &lt;sup&gt;7&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fried fish &lt;sup&gt;8&lt;/sup&gt;</td>
<td>186/194</td>
<td>223/198</td>
<td>57/86</td>
</tr>
<tr>
<td>Cases/controls</td>
<td>1.0</td>
<td>1.10 (0.83-1.35)</td>
<td>0.56 (0.37-0.86)</td>
</tr>
<tr>
<td>Adjusted OR (95% CI) &lt;sup&gt;9&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Calculated with actual values as a continuous variable.

<sup>1</sup> Salmon, mackerel, and bluefish (broiled or baked).
<sup>2</sup> Adjusted for total fat intake, body mass index, smoking, PSA screening, and family history of prostate cancer did not materially alter our results.
<sup>3</sup> Sole, halibut, snapper, and cod (broiled or baked).
<sup>4</sup> Shrimp, lobster, crab, and oysters (not fried). Actual categories are never, once per month, and twice or more per month.
<sup>5</sup> Canned tuna, tuna salad, and tuna casserole.
<sup>6</sup> Fried fish, fish sandwich, and fried shellfish (shrimp and oysters).

<sup>7</sup> Calculated with actual values as a continuous variable.

*Calculated with actual values as a continuous variable.

<sup>1</sup> Salmon, mackerel, and bluefish (broiled or baked).
<sup>2</sup> Adjusted for total fat intake, body mass index, smoking, PSA screening, and family history of prostate cancer did not materially alter our results.
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<sup>5</sup> Canned tuna, tuna salad, and tuna casserole.
<sup>6</sup> Fried fish, fish sandwich, and fried shellfish (shrimp and oysters).

Dietary Omega-3, COX-2, and Prostate Cancer

Table 5. Association between LC n-3 PUFA and aggressive prostate cancer, stratified by COX-2 genotypes

<table>
<thead>
<tr>
<th>COX-2 SNP</th>
<th>Genotype</th>
<th>OR (^{1}) (95% CI)</th>
<th>LC n-3 PUFA*</th>
<th>OR (^{1}) (95% CI)</th>
<th>(P_{\text{trend}}) (^{3})</th>
<th>(P_{\text{interaction}}) (^{3})</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2745557</td>
<td>GG</td>
<td>1.0</td>
<td>173/120</td>
<td>119/119</td>
<td>95/119</td>
<td>79/120</td>
</tr>
<tr>
<td>(+201)</td>
<td>GA or AA</td>
<td>0.65 (0.49-0.86)</td>
<td>125/77</td>
<td>87/70</td>
<td>67/79</td>
<td>58/75</td>
</tr>
<tr>
<td>rs5277</td>
<td>CC</td>
<td>1.0</td>
<td>122/84</td>
<td>84/98</td>
<td>66/93</td>
<td>54/90</td>
</tr>
<tr>
<td>(+1225)</td>
<td>CG or GG</td>
<td>1.38 (1.03-1.86)</td>
<td>51/36</td>
<td>34/21</td>
<td>29/26</td>
<td>25/30</td>
</tr>
<tr>
<td>rs2206593</td>
<td>GG</td>
<td>1.0</td>
<td>161/108</td>
<td>110/103</td>
<td>88/97</td>
<td>71/104</td>
</tr>
<tr>
<td>(+6993)</td>
<td>GA or AA</td>
<td>0.53 (0.34-0.81)</td>
<td>12/11</td>
<td>9/16</td>
<td>7/22</td>
<td>8/16</td>
</tr>
<tr>
<td>rs899470</td>
<td>GG or GA</td>
<td>1.0</td>
<td>167/117</td>
<td>113/116</td>
<td>90/114</td>
<td>73/118</td>
</tr>
<tr>
<td>(+8346)</td>
<td>AA</td>
<td>2.23 (1.03-4.87)</td>
<td>6/2</td>
<td>5/3</td>
<td>5/5</td>
<td>6/2</td>
</tr>
<tr>
<td>rs4648310</td>
<td>AA</td>
<td>1.0</td>
<td>158/118</td>
<td>111/116</td>
<td>89/113</td>
<td>76/113</td>
</tr>
<tr>
<td>(+8897)</td>
<td>AG or GG</td>
<td>1.88 (1.04-3.40)</td>
<td>15/2</td>
<td>8/3</td>
<td>6/6</td>
<td>3/7</td>
</tr>
</tbody>
</table>

\(^{a}\)EPA + DPA + DHA.

\(^{1}\)For main genetic effect (ignoring PUFA intake). Adjusted for age, ethnicity, and institution.

\(^{3}\)Stratified by genotypes. From logistic model, with LC n-3 PUFAs as a continuous variable. ORs correspond to difference between median values of quartiles 1 and 4 (0.52 g/d) unit increase in PUFAs. Adjusted for total calorie intake, age, ethnicity, and institution.

\(^{\text{x}}\)Multiplicative interaction from cross-product term in logistic regression between PUFAs (continuous) and each COX-2 SNP.

Dietary Omega-3, COX-2, and Prostate Cancer

There are several 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 period before 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 because a majority of our cases were diagnosed by screening (45). 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, body mass index, 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 reported previously 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 shows that the dietary 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 LC n-3 and other factors involved with inflammation such as COX-2 genotypes affect aggressive prostate cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank the participants of this study, who have contributed to a better understanding of the genetic contributions to prostate cancer susceptibility.

References

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References


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Vincent Fradet, Iona Cheng, Graham Casey, et al.


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