

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

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**Abstract Purpose:** Dietary intake of long-chain  $\omega$ -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 ( $P_{\text{trend}} \leq 0.0001$ ). 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 intra-epithelial 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  $\omega$ -3 (n-3) polyunsaturated fatty acids (PUFA), fish, selenium, vitamins D and E, and lycopene (4).

PUFAs are classified according to their molecular configuration:  $\omega$ -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  $E_2$ , which has been linked to carcinogenesis in studies of prostate and other tumors (10, 11). In contrast, the n-3 PUFAs, such as  $\alpha$ -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

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Received 9/28/08; revised 1/17/09; accepted 1/25/09; published OnlineFirst 3/24/09.

**Grant support:** NIH grants CA88164, CA94211, and CA98683; Laval University McLaughlin dean's grant (V. Fradet); and NIH R25T training grant CA112355 (I. Cheng).

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**Note:** Supplementary data for this article are available at Clinical Cancer Research Online (<http://clincancerres.aacrjournals.org/>).

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doi:10.1158/1078-0432.CCR-08-2503

### Translational Relevance

By aiming to understand 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 *cyclooxygenase-2* (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.

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  $\geq 7$ , tumor-node-metastasis stage  $\geq T_{2c}$ , 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).<sup>5</sup> 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 PUFAs, fish, and aggressive prostate cancer using unconditional logistic regression models. We evaluated the main effects of individual n-6 PUFAs (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

<sup>5</sup> <http://gvs.gs.washington.edu/GVS/>

**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)	481 (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	88 (18.9)	90 (19.0)
10-20	51 (11.0)	62 (13.1)
20-40	85 (18.3)	81 (17.1)
≥40	47 (10.1)	52 (10.9)
Body mass index (kg/m <sup>2</sup> ), 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 (%)		
T <sub>1c</sub>	285 (64.0)	
Any T <sub>2</sub>	133 (29.9)	
T <sub>3</sub>	27 (6.1)	
Histologic tumor grade: Gleason score, n (%)		
≤6	75 (16.1)	
7	287 (61.6)	
≥8	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.

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 (shrimp, lobster, and oysters; not fried), tuna (canned tuna, tuna salad, and tuna casserole), and fried fish (fried fish, fish sandwich, and fried shellfish). Fish intake variables (except shellfish) were categorized into never, one to three 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.  $P_{\text{trend}}$  values were calculated with the PUFA/fish variable modeled continuously across all quartiles.

To investigate potential modification of the PUFA 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 reported previously (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), body mass index (kg/m<sup>2</sup>), 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 (always resulting in a <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 nonsteroidal anti-inflammatory drug (NSAID) use (ever versus never). All  $P$  values are two-sided, and all analyses were undertaken with SAS software (version 9.1; SAS Institute).

## 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 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 were significantly associated with a strong dose-response reduction in prostate cancer risk ( $P_{\text{trend}} \leq 0.0001$ ). 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 DPA, 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 ( $r = 0.44$ ). 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, 0.52-1.40). 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 ( $P = 0.0023$  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  $P_{\text{trend}} < 0.0001$ ). Such an

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

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

\*P values obtained from t tests comparing mean values between cases and controls.

<sup>†</sup>EPA + DPA + DHA.

<sup>‡</sup>Salmon, mackerel, and bluefish (broiled or baked).

<sup>§</sup>Sole, halibut, snapper, and cod (broiled or baked).

<sup>||</sup>Shrimp, lobster, crab, and oysters (not fried).

<sup>¶</sup>Canned tuna, tuna salad, and tuna casserole.

<sup>\*\*</sup>Fried fish, fish sandwich, and fried shellfish (shrimp and oysters).

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 wild-type (AA), increasing LC n-3 consumption by 0.5 g/d 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 0.5 g/d, although the small number of cases with the variant and high intake of LC n-3 led to wide 95% CI (OR, 0.42; 95% CI, 0.13-1.37).

To investigate whether these findings were modified by NSAIDs, we stratified the analyses by NSAID use. Note that we previously found an inverse association between NSAID use

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 NSAIDs were relatively large (>0.20). Adjusting the models for NSAID use did not appear to materially alter the interaction between LC n-3 and rs4648310 (+8897 A>G) COX-2 genotype ( $P_{\text{interaction}} = 0.02$ ) 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 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, 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.

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; 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

**Table 3.** Association between dietary PUFA and aggressive prostate cancer

PUFA	Quartile of PUFA intake				P <sub>trend</sub> *
	1 (reference)	2	3	4	
<b>n-6 PUFAs</b>					
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 (95% CI) ‡	1.0	0.78 (0.52-1.16)	0.88 (0.57-1.35)	0.97 (0.56-1.67)	0.84
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 (95% CI) ‡	1.0	1.00 (0.69-1.44)	0.64 (0.43-0.97)	0.59 (0.38-0.93)	0.37
<b>n-3 PUFAs</b>					
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 (95% CI) ‡	1.0	0.83 (0.56-1.23)	0.71 (0.46-1.09)	0.81 (0.48-1.35)	0.11
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 (95% CI) ‡	1.0	0.60 (0.42-0.86)	0.50 (0.35-0.71)	0.35 (0.24-0.52)	<0.0001
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 (95% CI) ‡	1.0	0.71 (0.50-1.01)	0.45 (0.31-0.66)	0.40 (0.27-0.59)	<0.0001
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 (95% CI) ‡	1.0	0.60 (0.42-0.86)	0.45 (0.31-0.65)	0.36 (0.25-0.53)	<0.0001
LC n-3 PUFAs§					
Level † (g)	0.067	0.167	0.297	0.588	
Cases/controls	173/120	119/119	95/119	79/120	
Adjusted OR (95% CI) ‡	1.0	0.61 (0.43-0.87)	0.47 (0.32-0.68)	0.37 (0.25-0.54)	<0.0001
Total n-3 to total n-6 ratio					
Level †	0.096	0.118	0.136	0.165	
Cases/controls	160/119	146/120	92/119	68/120	
Adjusted OR (95% CI) ‡	1.0	0.94 (0.67-1.33)	0.60 (0.42-0.86)	0.41 (0.28-0.60)	0.0002
LC n-3 to total n-6 ratio					
Level †	0.004	0.011	0.022	0.047	
Cases/controls	173/120	140/119	88/120	65/119	
Adjusted OR (95% CI) ‡	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.

† Midpoint of quartile.

‡ Adjusted for calories, age, ethnicity, and institution (n = 944). Adjustment for total fat intake, body mass index, smoking, PSA screening, and family history of prostate cancer did not materially affect the results.

§EPA + DPA + DHA.

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

Fish	Frequency			<i>P</i> <sub>trend</sub> *
	Never (reference)	1-3/mo	≥1/wk	
Dark fish †				
Cases/controls	271/213	145/175	50/90	
Adjusted OR (95% CI) ‡	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 (95% CI) ‡	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 (95% CI) ‡	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 (95% CI) ‡	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 (95% CI) ‡	1.0	1.10 (0.83-1.47)	0.56 (0.37-0.86)	0.03

\*Calculated with actual values as a continuous variable.  
†Salmon, mackerel, and bluefish (broiled or baked).  
‡Adjusted for calories, age, ethnicity, and institution (*n* = 944). Adjustment for total fat intake, body mass index, smoking, PSA screening, and family history of prostate cancer did not materially alter our results.  
§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).

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,<sup>6</sup> 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 × rs4648310 (+8897 A>G) interaction was similar across individual LC n-3 (EPA, DPA, and DHA) and dark fish (*P*<sub>interaction</sub> = 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 effect of fish

intake on prostate cancer (*P*<sub>interaction</sub> < 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*<sub>trend</sub> < 0.01). We did not observe a similar pattern of interaction with rs5275 in our study (*P*<sub>interaction</sub> = 0.8). SNPs rs4648310 and rs5275 are located 2.4 kb apart and exhibit weak linkage disequilibrium in our population (*r*<sup>2</sup> = 0.01, 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.

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 risk only 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,

<sup>6</sup> Cancer Statistics in Japan. <http://ganjoho.ncc.go.jp/public/statistics/backnumber/2007.en.html>. Accessed August 2008.

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

COX-2 SNP		LC n-3 PUFA*							
rs no. (position)	Genotype	OR <sup>†</sup> (95% CI)	Cases/controls, quartile of intake				OR <sup>‡</sup> (95% CI)	P <sub>trend</sub> <sup>‡</sup>	P <sub>interaction</sub> <sup>§</sup>
			1	2	3	4			
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	GG	1.0	161/108	110/103	88/97	71/104	0.53 (0.39-0.71)	<0.0001	0.14
(+6993)	GA or AA	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	

\*EPA + DPA + DHA.

<sup>†</sup> For main genetic effect (ignoring PUFA intake). Adjusted for age, ethnicity, and institution.<sup>‡</sup> 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.<sup>§</sup> Multiplicative interaction from cross-product term in logistic regression between PUFAs (continuous) and each COX-2 SNP.

potentially affecting different subfunctions of the enzyme, may have different effects on prostate carcinogenesis: rs2745557 appears more important to the pharmacogenetics of NSAIDs, whereas 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, NSAID use was shown to be a modifier in addition to fish intake (44). The interaction between COX-1 genetic variation and fish intake was statistically significant ( $P = 0.04$ ) only when NSAID 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.

We observed that African Americans, on average, report lower total calorie intake and ALA, more AA, but similar LC n-3 than Caucasians (not shown). Moreover, there were some differences in genotype by ethnicity, and the main SNP of interest, rs4648310 (+8897 A>G), was only observed once among African Americans. Stratifying our analyses by ethnic group, the magnitude of the dietary effects was not materially changed, but the trend tests remained statistically significant only among Caucasians (not shown). This likely reflects the smaller sample size of African American men in our study. The association of rs4648310 with prostate cancer was slightly weaker when restricted to Caucasians ( $P = 0.07$ ), although the gene  $\times$  LC n-3 interaction was unchanged. Of course, residual ethnic confounding of the genetic associations and interaction remains possible. Nevertheless, by matching cases and controls on ethnic group, the likelihood that population stratification leads to substantial bias in the results is low. This is further supported by our observation of consistent COX-2 gene effects among both African Americans and Caucasians (36).

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

1. World Cancer Research Fund/American Institute for Cancer Research. Food, nutrition, and physical activity, and the prevention of cancer: a global perspective. Washington (DC): American Institute for Cancer Research; 2007.
2. Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2008. *CA Cancer J Clin* 2008;58:71–96.
3. De Marzo AM, Platz EA, Sutcliffe S, et al. Inflammation in prostate carcinogenesis. *Nat Rev Cancer* 2007;7:256–69.
4. Chan JM, Gann PH, Giovannucci EL. Role of diet in prostate cancer development and progression. *J Clin Oncol* 2005;23:8152–60.
5. Dennis LK, Dawson DV. Meta-analysis of measures of sexual activity and prostate cancer. *Epidemiology* 2002;13:72–9.
6. Taylor ML, Mainous AG III, Wells BJ. Prostate cancer and sexually transmitted diseases: a meta-analysis. *Fam Med* 2005;37:506–12.
7. Urisman A, Molinaro RJ, Fischer N, et al. Identification of a novel Gammaretrovirus in prostate tumors of patients homozygous for R462Q RNASEL variant. *PLoS Pathog* 2006;2:e25.
8. Kirby RS, Lowe D, Bultitude MI, Shuttleworth KE. Intra-prostatic urinary reflux: an aetiological factor in abacterial prostatitis. *Br J Urol* 1982;54:729–31.
9. Persson BE, Sjoman M, Niklasson F, Ronquist G. Uridine, xanthine and urate concentrations in prostatic fluid and seminal plasma of patients with prostatitis. *Eur Urol* 1991;19:253–6.
10. Rose DP. Dietary fatty acids and prevention of hormone-responsive cancer. *Proc Soc Exp Biol Med* 1997;216:224–33.
11. Terry PD, Rohan TE, Wolk A. Intakes of fish and marine fatty acids and the risks of cancers of the breast and prostate and of other hormone-related cancers: a review of the epidemiologic evidence. *Am J Clin Nutr* 2003;77:532–43.
12. McEntee MF, Whelan J. Dietary polyunsaturated fatty acids and colorectal neoplasia. *Biomed Pharmacother* 2002;56:380–7.
13. Kelavkar UP, Hutzley J, Dhir R, Kim P, Allen KG, McHugh K. Prostate tumor growth and recurrence can be modulated by the  $\omega$ -6: $\omega$ -3 ratio in diet: athymic mouse xenograft model simulating radical prostatectomy. *Neoplasia* 2006;8:112–24.
14. McEntee MF, Ziegler C, Reel D, et al. Dietary n-3 polyunsaturated fatty acids enhance hormone ablation therapy in androgen-dependent prostate cancer. *Am J Pathol* 2008;173:229–41.
15. Aronson WJ, Glaspay JA, Reddy ST, Reese D, Heber D, Bagga D. Modulation of  $\omega$ -3/ $\omega$ -6 polyunsaturated ratios with dietary fish oils in men with prostate cancer. *Urology* 2001;58:283–8.
16. Allen NE, Sauvaget C, Roddam AW, et al. A prospective study of diet and prostate cancer in Japanese men. *Cancer Causes Control* 2004;15:911–20.
17. Augustsson K, Michaud DS, Rimm EB, et al. A prospective study of intake of fish and marine fatty acids and prostate cancer. *Cancer Epidemiol Biomarkers Prev* 2003;12:64–7.
18. Giovannucci E, Rimm EB, Colditz GA, et al. A prospective study of dietary fat and risk of prostate cancer. *J Natl Cancer Inst* 1993;85:1571–9.
19. Le Marchand L, Kolonel LN, Wilkens LR, Myers BC, Hirohata T. Animal fat consumption and prostate cancer: a prospective study in Hawaii. *Epidemiology* 1994;5:276–82.
20. Leitzmann MF, Stampfer MJ, Michaud DS, et al. Dietary intake of n-3 and n-6 fatty acids and the risk of prostate cancer. *Am J Clin Nutr* 2004;80:204–16.
21. Mills PK, Beeson WL, Phillips RL, Fraser GE. Cohort study of diet, lifestyle, and prostate cancer in Adventist men. *Cancer* 1989;64:598–604.
22. Park SY, Murphy SP, Wilkens LR, Henderson BE, Kolonel LN. Fat and meat intake and prostate cancer risk: the Multiethnic Cohort Study. *Int J Cancer* 2007;121:1339–45.
23. Pham TM, Fujino Y, Kubo T, et al. Fish intake and the risk of fatal prostate cancer: findings from a cohort study in Japan. *Public Health Nutr*. Published online by Cambridge University Press 29 July 2008 doi: 10.1017/S1368980008003182.
24. Schuurman AG, van den Brandt PA, Dorant E, Brants HA, Goldbohm RA. Association of energy and fat intake with prostate carcinoma risk: results from The Netherlands Cohort Study. *Cancer* 1999;86:1019–27.
25. Schuurman AG, van den Brandt PA, Dorant E, Goldbohm RA. Animal products, calcium and protein and prostate cancer risk in The Netherlands Cohort Study. *Br J Cancer* 1999;80:1107–13.
26. Terry P, Lichtenstein P, Feychting M, Ahlbom A, Wolk A. Fatty fish consumption and risk of prostate cancer. *Lancet* 2001;357:1764–6.
27. Wallstrom P, Bjartell A, Gullberg B, Olsson H, Wirfalt E. A prospective study on dietary fat and incidence of prostate cancer (Malmö, Sweden). *Cancer Causes Control* 2007;18:1107–21.
28. Meyer F, Bairati I, Shadmani R, Fradet Y, Moore L. Dietary fat and prostate cancer survival. *Cancer Causes Control* 1999;10:245–51.
29. Veierod MB, Laake P, Thelle DS. Dietary fat intake and risk of prostate cancer: a prospective study of 25,708 Norwegian men. *Int J Cancer* 1997;73:634–8.
30. Astorg P. Dietary n-6 and n-3 polyunsaturated fatty acids and prostate cancer risk: a review of epidemiological and experimental evidence. *Cancer Causes Control* 2004;15:367–86.
31. Brouwer IA, Katan MB, Zock PL. Dietary  $\alpha$ -linolenic acid is associated with reduced risk of fatal coronary heart disease, but increased prostate cancer risk: a meta-analysis. *J Nutr* 2004;134:919–22.
32. Hedelin M, Chang ET, Wiklund F, et al. Association of frequent consumption of fatty fish with prostate cancer risk is modified by COX-2 polymorphism. *Int J Cancer* 2007;120:398–405.
33. Schakel SF. Maintaining a nutrient database in a changing marketplace: Keeping pace with changing food products: a research perspective. *J Food Compos Anal* 2001;14:315–22.
34. Schakel SF, Buzzard IM, Gebhardt SE. Procedures for estimating nutrient values for food composition databases. *J Food Compos Anal* 1997;10:102–14.
35. Schakel SF, Sievert YA, Buzzard IM. Sources of data for developing and maintaining a nutrient database. *J Am Diet Assoc* 1988;88:1268–71.
36. Cheng I, Liu X, Plummer SJ, Krumroy LM, Casey G, Witte JS. COX2 genetic variation, NSAIDs, and advanced prostate cancer risk. *Br J Cancer* 2007;97:557–61.
37. Altshuler D, Brooks LD, Chakravarti A, Collins FS, Daly MJ, Donnelly P. A haplotype map of the human genome. *Nature* 2005;437:1299–320.
38. Panguluri RC, Long LO, Chen W, et al. COX-2 gene promoter haplotypes and prostate cancer risk. *Carcinogenesis* 2004;25:961–6.
39. Shahedi K, Lindstrom S, Zheng SL, et al. Genetic variation in the COX-2 gene and the association with prostate cancer risk. *Int J Cancer* 2006;119:668–72.
40. Brawley OW. Prostate carcinoma incidence and patient mortality: the effects of screening and early detection. *Cancer* 1997;80:1857–63.
41. Ung JO, Richie JP, Chen MH, Renshaw AA, D'Amico AV. Evolution of the presentation and pathologic and biochemical outcomes after radical prostatectomy for patients with clinically localized prostate cancer diagnosed during the PSA era. *Urology* 2002;60:458–63.
42. Smith WL, DeWitt DL, Garavito RM. Cyclooxygenases: structural, cellular, and molecular biology. *Annu Rev Biochem* 2000;69:145–82.
43. Massaro M, Habib A, Lubrano L, et al. The  $\omega$ -3 fatty acid docosahexaenoate attenuates endothelial cyclooxygenase-2 induction through both NADP(H) oxidase and PKC $\epsilon$  inhibition. *Proc Natl Acad Sci U S A* 2006;103:15184–9.
44. Poole EM, Bigler J, Whitton J, et al. Genetic variability in prostaglandin synthesis, fish intake and risk of colorectal polyps. *Carcinogenesis* 2007;28:1259–63.
45. Rothman KJ. *Epidemiology: an introduction*. New York: Oxford University Press; 2002. p. 202–3.



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*Clin Cancer Res* 2009;15:2559-2566.

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