

## Plasma Phospholipid Fatty Acids and Prostate Cancer Risk in the SELECT Trial

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**Background** Studies of dietary  $\omega$ -3 fatty acid intake and prostate cancer risk are inconsistent; however, recent large prospective studies have found increased risk of prostate cancer among men with high blood concentrations of long-chain  $\omega$ -3 polyunsaturated fatty acids ([LC $\omega$ -3PUFA] 20:5 $\omega$ 3; 22:5 $\omega$ 3; 22:6 $\omega$ 3). This case-cohort study examines associations between plasma phospholipid fatty acids and prostate cancer risk among participants in the Selenium and Vitamin E Cancer Prevention Trial.

**Methods** Case subjects were 834 men diagnosed with prostate cancer, of which 156 had high-grade cancer. The subcohort consisted of 1393 men selected randomly at baseline and from within strata frequency matched to case subjects on age and race. Proportional hazards models estimated hazard ratios (HR) and 95% confidence intervals (CI) for associations between fatty acids and prostate cancer risk overall and by grade. All statistical tests were two-sided.

**Results** Compared with men in the lowest quartiles of LC $\omega$ -3PUFA, men in the highest quartile had increased risks for low-grade (HR = 1.44, 95% CI = 1.08 to 1.93), high-grade (HR = 1.71, 95% CI = 1.00 to 2.94), and total prostate cancer (HR = 1.43, 95% CI = 1.09 to 1.88). Associations were similar for individual long-chain  $\omega$ -3 fatty acids. Higher linoleic acid ( $\omega$ -6) was associated with reduced risks of low-grade (HR = 0.75, 95% CI = 0.56 to 0.99) and total prostate cancer (HR = 0.77, 95% CI = 0.59 to 1.01); however, there was no dose response.

**Conclusions** This study confirms previous reports of increased prostate cancer risk among men with high blood concentrations of LC $\omega$ -3PUFA. The consistency of these findings suggests that these fatty acids are involved in prostate tumorigenesis. Recommendations to increase LC $\omega$ -3PUFA intake should consider its potential risks.

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Inflammation plays a role in the etiology of many cancers. The strongest evidence for an inflammatory component in prostate carcinogenesis is based on the characteristics of a precursor lesion, proliferative inflammatory atrophy, which is an area of highly proliferative but atrophic epithelial cells with notable inflammatory infiltrates (1,2). Considerable research has addressed whether factors that affect inflammation are associated with prostate cancer risk. With the exception of obesity, which is associated with increased inflammation and higher risks of high-grade prostate cancer (3,4) and prostate cancer death (5,6), studies on lifestyle factors associated with reduced inflammation, including use of aspirin (7,8) and nonsteroidal anti-inflammatory drugs (8) and statins (9) and consumption of long-chain  $\omega$ -3 fatty acids (10–12) (here defined as eicosapentaenoic, docosapentaenoic, and docosahexaenoic acids), have been inconsistent.

We recently reported, using data and serum collected in the Prostate Cancer Prevention Trial, that high concentration of serum phospholipid long-chain  $\omega$ -3 fatty acids, which is a

biomarker of usual  $\omega$ -3 fatty acid intake (13), was associated with a large increase in the risk of high-grade prostate cancer (14). We also found that high concentrations of trans-fatty acids, which are associated with increased inflammation (15,16), were associated with reduced risk of high-grade prostate cancer (14). These findings were counter to expectations but raised the possibility that high intakes of  $\omega$ -3 fatty acids, for example through use of fish oil supplements, could increase the risk of clinically significant, high-grade prostate cancer.

Here we replicate these analyses using data and plasma collected in the Selenium and Vitamin E Cancer Prevention Trial (SELECT; trial registration: [clinicaltrials.gov](http://clinicaltrials.gov) identifier NCT00006392). Given the widespread use of  $\omega$ -3 fatty acid supplements (17,18), an ongoing clinical trial testing  $\omega$ -3 fatty acid supplementation for cancer and cardiovascular disease prevention (19), and the purported health benefits of consuming fatty fish (20,21), it is important to further investigate whether high consumption of  $\omega$ -3 fatty acids could contribute to prostate cancer risk.

## Methods

### The Selenium and Vitamin E Cancer Prevention Trial

SELECT was a randomized, placebo-controlled trial that tested whether selenium and vitamin E, either alone or combined, reduced prostate cancer risk (22,23). Briefly, in 427 participating sites across the United States, Canada, and Puerto Rico, black men aged 50 years or older or men of all other races aged 55 years or older who had no history of prostate cancer and who had a serum prostate-specific antigen of 4 ng/mL or less and nonsuspicious digital rectal exam were eligible to participate. Between July 2001 and May 2004, 35 533 men were block-randomized by study site to one of four groups: selenium + vitamin E; vitamin E + placebo; selenium + placebo; or placebo + placebo. On September 15, 2008, the Data and Safety Monitoring Committee recommended the discontinuation of the trial supplements because of no observed evidence of a protective effect and no likelihood of an effect given current rates of cancer in each arm (22). In 2011, after an additional 54 464 person-years of follow-up, we reported that vitamin E, in contrast with a placebo, increased prostate cancer risk by 17% (24). All men provided written informed consent, and study procedures were approved by local institutional review boards for each participating study center.

### Case Subject and Subcohort Selection

This study is a case-cohort design nested within SELECT. Case subjects included in these analyses were men with baseline blood samples available for analysis who were diagnosed with incident, primary prostate cancers before July 31, 2009. Most cases (94.4%) were detected by prostate-specific antigen and/or digital rectal exam screening, which was suggested annually but not required. Screening procedures were reported annually. At each study contact, participants reported new cancer diagnoses to study staff, who then obtained pathology reports and, when possible, tissue. Almost all cases included in these analyses (92.6%; 842 of 909) were reviewed centrally for pathological confirmation and grading using the Gleason system (25). For 26 cases where tissue was not available, Gleason scores were abstracted from local pathology reports. High-grade tumors were defined as Gleason scores 8 to 10 and 7 (4 + 3); low-grade tumors were Gleason scores 2 to 6 and 7 (3 + 4).

A subcohort representative of SELECT participants was created a priori as the comparison group for this and other biomarker studies using the following approach. Men randomized into the study were stratified into nine age/race cohorts: aged less than 55 years (black men only), and aged 55 to 59 years, aged 60 to 64 years, aged 65 to 69 years, and aged 70 years or greater for both black men and men of all other races. Beginning in 2005 and annually until 2009, men with new diagnoses of prostate cancer had matching men randomly selected for the subcohort from the set of men with blood samples available within the same age-race stratum. A ratio of 1:3 was used for black men and 1:1.5 for men of other races.

Because of the high cost of phospholipid fatty acid assays, only the case subjects diagnosed through 2007 and their corresponding frequency-matched subcohort were planned for this analysis. This comprises 834 case subjects diagnosed during the first 6 years of the trial, plus 1393 men in the corresponding subcohort.

Twenty-nine men in the subcohort were diagnosed with prostate cancer. Subsequently, based on new findings of associations of fatty acids with high-grade cancer only (14), 75 additional men diagnosed with high-grade prostate cancer in years 8 and 9 of the trial were added to this study. Thus, three groups were evaluated in this analysis: 1) 834 men diagnosed with cancer before May 18, 2007, including 69 for whom grade was not available; 2) 684 men diagnosed with low-grade cancer before May 18, 2007; and 3) 156 men diagnosed with high-grade cancer before July 31, 2009.

### Data Collection and Laboratory Methods

Data on demographic and health-related characteristics were collected at baseline by self-administered questionnaire. Study staff measured height and weight, which were used to calculate body mass index (kg/m<sup>2</sup>). Venous blood samples were collected at baseline and refrigerated and shipped overnight to the specimen repository where the samples were combined, centrifuged, aliquoted, and stored at -70°C until analysis (26). Detailed methods for the phospholipid fatty acid assay have been published elsewhere (27). Briefly, total lipids were extracted from plasma, and phospholipids were separated from other lipids by one-dimensional thin-layer chromatography (28). Fatty acid methyl ester samples were prepared by direct transesterification and separated by gas chromatography (29). Fatty acid composition is expressed as the weight percentage of total phospholipid fatty acids. A lab quality control sample of pooled plasma from healthy volunteers was run with each batch of study samples. Samples from case subjects and the subcohort were analyzed annually in the same batches, and all laboratory personnel were blinded to the status of the samples. Coefficients of variation (standard deviation/mean) for fatty acids were as follows:  $\alpha$ -linolenic acid (ALA; 18:3 $\omega$ 3), 2.8%; eicosapentaenoic acid (EPA; 20:5 $\omega$ 3), 2.8%; docosapentaenoic acid (DPA; 22:5 $\omega$ 3), 1.4%; docosahexaenoic acid (DHA; 22:6 $\omega$ 3), 1.5%; linoleic acid (LA; 18:2 $\omega$ 6), and 0.6%; arachidonic acid (AA; 20:4 $\omega$ 6), 0.8%. Because of their low concentrations, trans-fatty acids were grouped as trans-16:1 (16:1 $\omega$ 7t + 16:1 $\omega$ 9t), trans-18:1 (18:1 $\omega$ 6t + 18:1 $\omega$ 7t + 18:1 $\omega$ 8t + 18:1 $\omega$ 9t + 18:1 $\omega$ 10-12t), and trans-18:2 (18:2 $\omega$ 6tt + 18:2 $\omega$ 6ct + 18:2 $\omega$ 6tc), with coefficients of variation of 5.4%, 4.3%, and 6.9%, respectively.

### Statistical Analysis

Total long-chain  $\omega$ -3 PUFA was calculated as the sum of EPA, DPA, and DHA. Fatty acids were categorized into quartiles based upon their distributions among the subcohort. Geometric means and 95% confidence intervals (CI) are given for each fatty acid, and weighted *t* tests were used to test differences between the case subject groups and noncase subjects.

Cox proportional hazards models were used to estimate hazard ratios (HRs) and 95% confidence intervals for the association between plasma phospholipids and risk of prostate cancer. Separate models were fit for all prostate cancers, low-grade prostate cancers and high-grade prostate cancers. The proportionality assumption in Cox proportional hazards models was tested by including an interaction term between each fatty acid and time. Of the 30 models, the assumption was violated in the following instances: ALA (high-grade), DPA (high-grade), trans-fatty acid (TFA) 18:2 (low-grade and high-grade) (Supplementary Methods, available online).

**Table 1.** Baseline demographic and health-related characteristics and cancer outcomes of SELECT participants, by prostate cancer grade (N = 2273)\*

Characteristic	Prostate cancer							
	No cancer (n = 1364)		Total (n = 834)		Low-grade (n = 684)		High-grade (n = 156)	
	No.	%	No.	%	No.	%	No.	%
Age, years†								
50–54	42	3.1	20	2.4	16	2.3	3	1.9
55–59	341	25.0	198	23.7	169	24.7	30	19.2
60–64	397	29.1	260	31.2	212	31.0	35	22.4
65–69	325	23.8	199	23.9	162	23.7	50	32.1
≥70	259	19.0	157	18.8	125	18.3	38	24.4
Race†								
Non-Hispanic white	985	72.2	670	80.3	558	81.6	129	82.7
Non-Hispanic black	274	20.1	118	14.1	91	13.3	21	13.5
Other	105	7.7	46	5.5	35	5.1	6	3.8
Education, years								
≤ High school graduate	341	25.0	165	19.8	136	19.9	29	18.6
Some college	349	25.6	203	24.3	161	23.5	39	25.0
≥ College graduate	662	48.5	459	55.0	381	55.7	87	55.8
Body mass index, kg/m <sup>2</sup>								
<25	278	20.4	154	18.5	134	19.6	21	13.5
25–<30	638	46.8	422	50.6	351	51.3	80	51.3
≥30	448	32.8	258	30.9	199	29.1	55	35.3
Pack-years smoking								
Nonsmokers	566	41.5	384	46.0	318	46.5	76	48.7
≤12.5 pack-years	296	21.7	190	22.8	155	22.7	27	17.3
12.5–25.0 pack-years	232	17.0	126	15.1	100	14.6	27	17.3
>25.0 pack-years	257	18.8	125	15.0	104	15.2	25	16.0
Alcohol consumption, drinks/day								
Nondrinkers	472	34.6	275	33.0	233	34.1	55	35.3
<1.0	509	37.3	308	36.9	248	36.3	66	42.3
1.0–1.9	162	11.9	124	14.9	97	14.2	18	11.5
≥2.0	148	10.9	99	11.9	82	12.0	12	7.7
Baseline PSA								
<1.0	556	40.8	48	5.8	34	5.0	18	11.5
1.0–1.9	497	36.4	183	21.9	148	21.6	41	26.3
2.0–2.9	211	15.5	260	31.2	215	31.4	50	32.1
≥3.0	100	7.3	343	41.1	287	42.0	47	30.1
Finasteride use								
No	1,285	94.2	772	92.6	634	92.7	143	91.7
Yes	79	5.8	62	7.4	50	7.3	13	8.3
Aspirin use								
No	805	59.0	511	61.3	416	60.8	84	53.8
Yes	559	41.0	323	38.7	268	39.2	72	46.2
History of diabetes								
No	1,190	87.2	772	92.6	641	93.7	140	89.7
Yes	174	12.8	62	7.4	43	6.3	16	10.3
First-degree relative with prostate cancer								
None	1,141	83.7	584	70.0	476	69.6	114	73.1
1	193	14.1	195	23.4	166	24.3	33	21.2
≥2	28	2.1	55	6.6	42	6.1	9	5.8
SELECT intervention assignment								
Vitamin E + selenium	333	24.4	202	24.2	162	23.7	39	25.0
Vitamin E alone	343	25.1	233	27.9	177	25.9	48	30.8
Selenium alone	351	25.7	200	24.0	172	25.1	32	20.5
Placebo	337	24.7	199	23.9	173	25.3	37	23.7
Clinical stage‡								
T1	—	—	593	71.1	493	72.1	111	71.2
T2	—	—	221	26.5	182	26.6	41	26.3
T3	—	—	1	0.1	0	0.0	3	1.9

\* PSA = prostate-specific antigen; SELECT = Selenium and Vitamin E Cancer Prevention Trial

† Stratification variable

‡ Clinical stage among prostate cancer cases according to the TNM Classification of Malignant Tumors staging system.

Associations are given for phospholipid fatty acids expressed as quartiles, contrasting quartiles 2, 3, and 4 with quartile 1, and additionally given as continuous hazard ratios. Because some fatty acids represent greater proportions of total weight than others, continuous hazard ratios have been adjusted to represent a 50% increase in fatty acid proportion. Tests for linear trend ( $P_{\text{trend}}$ ) across categories were calculated by treating categorical variables as continuous in regression models (30).

Additional covariables in multivariable regression models included education, history of diabetes, family history of prostate cancer, and SELECT intervention assignment. All statistical analyses were performed using SAS version 9.2 software (SAS Institute, Cary, NC). All statistical tests were two-sided, and  $P$  less than .05 was considered statistically significant.

### Meta-analysis

A meta-analysis was conducted to compare our results for individual and total long-chain  $\omega$ -3 PUFA with previous prospective biomarker studies of these fatty acids and prostate cancer risk overall and by grade. Associations between long-chain  $\omega$ -3 PUFA and prostate cancer stratified on grade in the Multiethnic Cohort (31) were provided by personal communication (S. Park, October 2012). Risk estimates and 95% confidence intervals contrasting highest with lowest quantiles of exposure to EPA, DHA, and total long-chain  $\omega$ -3 PUFA were abstracted from individual studies and combined under a fixed effects meta-analysis model (32) using STATA Release 12 (StataCorp LP, College Station, TX). Forest plots were used to display the results from individual studies and  $I^2$  statistics are given as measures of heterogeneity between studies.

### Results

Table 1 gives baseline demographic and other characteristics and cancer outcomes of the SELECT study population by Gleason grade. Low- and high-grade prostate cancer case subjects were

more educated, had a higher prostate-specific antigen score, and had a larger proportion of first-degree relatives with a history of prostate cancer compared with noncases. Low-grade case subjects were less likely to report a history of diabetes than noncase subjects. Body mass index, pack-years of smoking, or use of aspirin did not differ between case subjects and noncase subjects. Only four cancers, three high-grade and one unknown grade, were diagnosed with advanced stage disease (T3); approximately 26% were diagnosed with Stage T2, and the remainder were diagnosed at stage T1.

Table 2 gives age- and race-matched means of plasma  $\omega$ -3,  $\omega$ -6, and trans-fatty acids for cancer case subjects and the subcohort. The mean percentages of total long-chain  $\omega$ -3 PUFA were statistically significantly higher in total, low-, and high-grade prostate cancer case subjects compared with the subcohort. The percentages of the three individual long-chain  $\omega$ -3 fatty acids—EPA, DPA and DHA—were also higher but did not all reach statistical significance in the smaller group of high-grade prostate cancer case subjects. Mean percentages of each TFA (18:1, 18:2, and 16:1) were statistically significantly higher among total and low-grade prostate cancer case subjects compared with the subcohort, although differences were small. Mean TFA 16:1 was higher in the high-grade prostate cancer case subjects only. Mean proportions of ALA, LA, and AA were similar across groups.

Table 3 gives associations between plasma phospholipid fatty acids and risk of total, low-grade, and high-grade prostate cancer. Associations between fatty acids and prostate cancer risk did not differ by SELECT treatment assignment; therefore only combined analyses are presented. Higher total long-chain  $\omega$ -3 PUFA were associated with increased risks of total, low-, and high-grade cancer. Compared with men in the lowest quartile of total long-chain  $\omega$ -3 PUFA, men in the highest quartile had 44% (95% CI = 8% to 93%), 71% (95% CI = 0% to 194%), and 43% (95% CI = 9% to 88%) increased risks for low-grade, high-grade, and total cancer, respectively. In continuous models, each 50% increase in total

**Table 2.** Distribution of plasma phospholipid fatty acids among SELECT participants by prostate cancer grade (n=2273)\*

Fatty acid (% of total)	No cancer (n = 1364)		P†	Total cancer (n = 834)		P†	Low-grade cancer (n = 684)		P†	High-grade cancer (n = 156)		P†
	Mean (95% CI)†	Mean (95% CI)†		Mean (95% CI)‡	Mean (95% CI)†							
$\omega$ -3 fatty acids												
$\alpha$ -linolenic acid (18:3 $\omega$ 3)	0.13 (0.13 to 0.13)	0.13 (0.13 to 0.14)	.52	0.13 (0.13 to 0.14)	.32	0.14 (0.13 to 0.15)	.47					
EPA + DPA + DHA	4.48 (4.41 to 4.55)	4.66 (4.56 to 4.75)	.002	4.66 (4.56 to 4.77)	.002	4.71 (4.51 to 4.91)	.03					
EPA (20:5 $\omega$ 3)	0.61 (0.60 to 0.63)	0.65 (0.63 to 0.68)	.03	0.66 (0.63 to 0.68)	.02	0.65 (0.60 to 0.71)	.40					
DPA (22:5 $\omega$ 3)	0.86 (0.85 to 0.87)	0.90 (0.90, 0.91)	<.001	0.90 (0.89 to 0.92)	<.001	0.89 (0.86 to 0.92)	.16					
DHA (22:6 $\omega$ 3)	2.91 (2.86 to 2.96)	3.01 (2.95 to 3.08)	.006	3.01 (2.93 to 3.09)	.02	3.09 (2.95 to 3.23)	.009					
$\omega$ -6 fatty acids												
Linoleic acid (18:2 $\omega$ 6)	19.03 (18.88 to 19.18)	18.95 (18.76 to 19.14)	.17	18.91 (18.71 to 19.13)	.11	19.09 (18.66 to 19.54)	.81					
Arachidonic acid (20:4 $\omega$ 6)	11.40 (11.28 to 11.52)	11.20 (11.05 to 11.35)	.17	11.22 (11.06 to 11.39)	.26	11.33 (11.01 to 11.67)	.54					
Trans-fatty acids												
TFA 18:1	1.41 (1.38 to 1.44)	1.45 (1.41 to 1.50)	.048	1.46 (1.42 to 1.51)	.03	1.45 (1.35 to 1.55)	.64					
TFA 18:2	0.20 (0.20 to 0.21)	0.21 (0.20 to 0.21)	.08	0.21 (0.21 to 0.21)	.03	0.20 (0.19 to 0.21)	.32					
TFA 16:1	0.21 (0.21 to 0.21)	0.22 (0.21 to 0.22)	.002	0.22 (0.22 to 0.22)	<.001	0.22 (0.21 to 0.23)	.02					

\* CI = confidence interval; DHA = docosahexaenoic acid; DPA = docosapentaenoic acid; EPA = eicosapentaenoic acid; SELECT = Selenium and Vitamin E Cancer Prevention Trial; TFA = trans-fatty acid.

† Geometric means, frequency matched on age and race.

‡  $P$  value derived from  $t$  tests of means compared with the subcohort (restricted to noncase subjects).

**Table 3.** Associations between plasma phospholipid fatty acids and prostate cancer risk, by prostate cancer grade (n=2273)\*

Fatty acid (% of total)	Prostate cancer†					
	Total (n = 834)	Low-grade (n = 684)	High-grade (n = 156)	Total HR (95% CI)‡	Low-grade HR (95% CI)‡	High-grade HR (95% CI)‡
<b>ω-3 fatty acids</b>						
α-linolenic acid (18:3ω3)						
<0.10	205	166	36	1.00 (referent)	1.00 (referent)	1.00 (referent)
0.10–0.13	211	173	33	0.93 (0.71 to 1.22)	0.99 (0.74 to 1.32)	0.77 (0.46 to 1.29)
0.14–0.17	198	162	42	0.87 (0.66 to 1.15)	0.92 (0.69 to 1.24)	0.94 (0.57 to 1.54)
>0.17	220	183	45	0.87 (0.66 to 1.13)	0.91 (0.68 to 1.21)	0.95 (0.58 to 1.55)
<i>P</i> <sub>trend</sub>				.26	.44	.96
Continuous HR (95% CI)				0.98 (0.88 to 1.09)	1.00 (0.89 to 1.11)	1.05 (0.85 to 1.29)
Total long-chain ω-3						
PUFA (EPA + DPA + DHA)						
<3.68	176	146	26	1.00 (referent)	1.00 (referent)	1.00 (referent)
3.68–4.41	196	159	35	1.15 (0.87 to 1.51)	1.10 (0.82 to 1.47)	1.39 (0.79 to 2.44)
4.42–5.31	217	176	52	1.28 (0.97 to 1.69)	1.26 (0.94 to 1.68)	1.87 (1.11 to 3.15)
>5.31	245	203	43	1.43 (1.09 to 1.88)	1.44 (1.08 to 1.93)	1.71 (1.00 to 2.94)
<i>P</i> <sub>trend</sub>				.007	.009	.02
Continuous HR (95% CI)				1.23 (1.07 to 1.40)	1.24 (1.07 to 1.43)	1.24 (1.00 to 1.54)
EPA (20:5ω3)						
<0.43	183	146	33	1.00 (referent)	1.00 (referent)	1.00 (referent)
0.43–0.57	176	140	39	0.91 (0.68 to 1.20)	0.91 (0.67 to 1.23)	1.06 (0.63 to 1.77)
0.58–0.82	231	198	37	1.16 (0.89 to 1.52)	1.28 (0.97 to 1.70)	0.93 (0.55 to 1.56)
>0.82	244	200	47	1.18 (0.90 to 1.54)	1.22 (0.91 to 1.62)	1.30 (0.79 to 2.14)
<i>P</i> <sub>trend</sub>				.08	.048	.38
Continuous HR (95% CI)				1.06 (0.99 to 1.14)	1.07 (0.99 to 1.15)	1.05 (0.93 to 1.19)
DPA (22:5ω3)						
<0.76	154	113	34	1.00 (referent)	1.00 (referent)	1.00 (referent)
0.76–0.87	202	167	36	1.36 (1.02 to 1.80)	1.54 (1.13 to 2.09)	1.05 (0.63 to 1.76)
0.88–0.99	231	198	42	1.38 (1.04 to 1.82)	1.61 (1.19 to 2.18)	1.12 (0.67 to 1.85)
>0.99	247	206	44	1.38 (1.05 to 1.82)	1.56 (1.16 to 2.11)	1.15 (0.70 to 1.89)
<i>P</i> <sub>trend</sub>				.04	.008	.55
Continuous HR (95% CI)				1.23 (1.03 to 1.46)	1.30 (1.08 to 1.57)	1.20 (0.87 to 1.65)
DHA (22:6ω3)						
<2.33	193	159	29	1.00 (referent)	1.00 (referent)	1.00 (referent)
2.33–2.93	192	154	36	1.05 (0.80 to 1.38)	1.01 (0.76 to 1.35)	1.37 (0.81 to 2.33)
2.94–3.62	212	174	49	1.24 (0.95 to 1.63)	1.26 (0.95 to 1.68)	1.78 (1.08 to 2.94)
>3.62	237	197	42	1.39 (1.06 to 1.82)	1.42 (1.06 to 1.89)	1.46 (0.85 to 2.49)
<i>P</i> <sub>trend</sub>				.009	.008	.09
Continuous HR (95% CI)				1.21 (1.07 to 1.37)	1.21 (1.06 to 1.38)	1.26 (1.03 to 1.54)
<b>ω-6 fatty acids</b>						
Linoleic acid (18:2ω6)						
<17.36	229	192	37	1.00 (referent)	1.00 (referent)	1.00 (referent)
17.36–19.14	186	153	43	0.69 (0.52 to 0.90)	0.66 (0.50 to 0.89)	1.11 (0.68 to 1.82)
19.15–21.02	200	158	36	0.73 (0.56 to 0.96)	0.68 (0.51 to 0.91)	0.83 (0.49 to 1.39)
>21.02	219	181	40	0.77 (0.59 to 1.01)	0.75 (0.56 to 0.99)	0.92 (0.55 to 1.54)
<i>P</i> <sub>trend</sub>				.13	.09	.50
Continuous HR (95% CI)				0.74 (0.56 to 0.97)	0.70 (0.52 to 0.94)	0.85 (0.52 to 1.41)
Arachidonic acid (20:4ω6)						
<10.08	232	187	36	1.00 (referent)	1.00 (referent)	1.00 (referent)
10.08–11.58	236	192	50	1.04 (0.81 to 1.34)	1.06 (0.81 to 1.39)	1.49 (0.94 to 2.38)
11.59–13.00	167	138	40	0.82 (0.62 to 1.07)	0.83 (0.62 to 1.11)	1.34 (0.82 to 2.20)
>13.00	199	167	30	1.07 (0.81 to 1.41)	1.12 (0.84 to 1.51)	1.22 (0.71 to 2.08)
<i>P</i> <sub>trend</sub>				.81	.94	.50
Continuous HR (95% CI)				0.98 (0.80 to 1.19)	1.00 (0.80 to 1.24)	1.23 (0.86 to 1.76)
Trans-fatty acids						
TFA 18:1						
<1.06	182	148	33	1.00 (referent)	1.00 (referent)	1.00 (referent)
1.06–1.44	189	149	35	0.90 (0.68 to 1.20)	0.89 (0.66 to 1.20)	0.91 (0.54 to 1.54)
1.45–1.94	262	219	51	1.34 (1.02 to 1.75)	1.39 (1.05 to 1.85)	1.43 (0.87 to 2.36)
>1.94	201	168	37	1.05 (0.80 to 1.39)	1.10 (0.82 to 1.48)	0.93 (0.55 to 1.58)
<i>P</i> <sub>trend</sub>				.21	.11	.72
Continuous HR (95% CI)				1.05 (0.96 to 1.15)	1.07 (0.97 to 1.18)	1.00 (0.85 to 1.19)

(Table continues)

**Table 3 (Continued).**

Fatty acid (% of total)	Prostate cancer†					
	Total (n = 834)	Low-grade (n = 684)	High-grade (n = 156)	Total HR (95% CI)‡	Low-grade HR (95% CI)‡	High-grade HR (95% CI)‡
TFA 18:2						
<0.17	181	143	36	1.00 (referent)	1.00 (referent)	1.00 (referent)
0.17–0.20	229	187	42	1.21 (0.92 to 1.59)	1.23 (0.92 to 1.66)	1.22 (0.74 to 2.01)
0.21–0.24	195	164	43	1.05 (0.80 to 1.39)	1.11 (0.82 to 1.50)	1.27 (0.78 to 2.08)
>0.24	229	190	35	1.19 (0.90 to 1.56)	1.23 (0.92 to 1.65)	0.92 (0.55 to 1.55)
<i>P</i> <sub>trend</sub>				.41	.29	.79
Continuous HR (95% CI)				1.06 (0.93 to 1.22)	1.11 (0.96 to 1.28)	0.88 (0.69 to 1.13)
TFA 16:1						
<0.18	165	129	30	1.00 (referent)	1.00 (referent)	1.00 (referent)
0.18–0.22	208	170	32	1.09 (0.83 to 1.45)	1.11 (0.82 to 1.50)	0.94 (0.53 to 1.66)
0.23–0.25	218	180	38	1.13 (0.86 to 1.49)	1.16 (0.86 to 1.56)	1.05 (0.61 to 1.81)
>0.25	243	205	56	1.30 (0.99 to 1.70)	1.35 (1.00 to 1.80)	1.56 (0.94 to 2.60)
<i>P</i> <sub>trend</sub>				.06	.04	.05
Continuous HR (95% CI)				1.14 (0.98 to 1.32)	1.19 (1.02 to 1.40)	1.29 (0.95 to 1.75)

\* CI = confidence interval; DHA = docosahexaenoic acid; DPA = docosapentaenoic acid; EPA = eicosapentaenoic acid; HR = hazard ratio; PUFA = polyunsaturated fatty acid; TFA = trans-fatty acid.

† For all plasma phospholipids, the subcohort's distribution (n = 1393) is quartiles 1–3, n = 348; quartile 4, n = 349. The subcohort includes n = 29 cases (20 low-grade, 7 high-grade).

‡ Hazard ratios were adjusted for age and race (frequency matching variables), education, history of diabetes, family history of prostate cancer, and SELECT intervention arm and estimated with Cox proportional hazards models. All statistical tests were two-sided.

long-chain ω-3 PUFA was associated with a 22% to 25% increased cancer risk. Results for the individual long-chain ω-3 PUFA were similar, but effect sizes tended to be smaller and not all reached statistical significance. Of the major ω-6 PUFA, higher linoleic acid was associated with 25% (95% CI = 1% to 44%) and 23% (95% CI = -1% to 41%) reduced risks of low-grade and total cancer, respectively; however there was no dose response. Of the TFAs, there was weak evidence that higher TFA 16:1 was associated with increased risk, based on statistically significant or borderline significant trends across quartiles of exposure. However, none of the confidence intervals at the highest level of exposure excluded 1.00. The other classes of TFAs, ALA (a plant-based long-chain ω-3 PUFA), and AA (ω-6 PUFA) were not associated with risk.

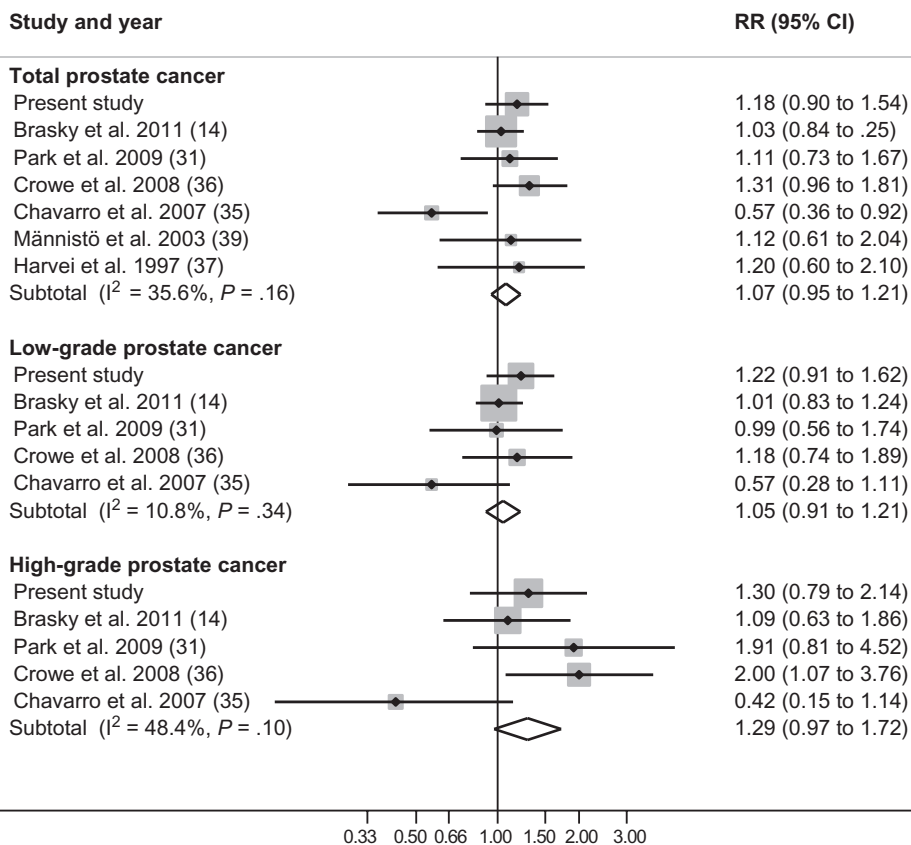
In a sensitivity analysis, we repeated the analyses using continuous measures of fatty acids after truncating the lowest and highest 5% of values to test whether associations were being driven by outliers. There were no appreciable differences when outliers were removed (data not shown). To address the potential for spurious associations to arise because of the measurement of fatty acids as proportion of total weight rather than absolute concentration (33) and to address the question of the importance of the ω-3/ω-6 ratio, long-chain ω-3 and ω-6 PUFA were included together in regression models predicting total, low-, and high-grade prostate cancer risk. Findings for total long-chain ω-3 PUFA adjusted for ω-6 PUFA were only slightly attenuated. The continuous multi-variable-adjusted hazard ratios predicting total, low-, and high-grade prostate cancer risk, respectively, were 1.16 (95% CI = 0.98 to 1.36), 1.15 (95% CI = 0.97 to 1.36), and 1.40 (95% CI = 1.03 to 1.92) (data not shown). The associations for LA were no longer statistically significant; however the point estimates were mostly unchanged (data not shown). Because it is possible that the positive association between long-chain ω-3 PUFA and prostate cancer risk is explained by increased cancer screening among high consumers

of ω-3 PUFA, in a separate sensitivity analysis, we censored noncase subjects at the date of their last screening test. Results were largely unchanged; hazard ratios for associations between total long-chain ω-3 PUFA and total, low-, and high-grade prostate cancer were 1.44 (95% CI = 1.09 to 1.90), 1.44 (95% CI = 1.07 to 1.94), and 1.67 (95% CI = 0.96 to 2.90), respectively.

## Discussion

In this large, prospective trial, high plasma phospholipid concentrations of long-chain ω-3 PUFA were associated with statistically significant increases in prostate cancer risk. These associations were similar for low- and high-grade disease and for EPA, DPA and DHA, which are anti-inflammatory, metabolically interrelated ω-3 fatty acids derived from oily fish and fish oil supplements. Findings for linoleic and arachidonic acids, the primary ω-6 fatty acids associated with increased inflammation (34), were inconsistent: concentrations of linoleic acid above the lowest quartile were associated with reduced cancer risk, with no evidence of a linear trend, and concentrations of arachidonic acid were not associated with cancer risk. Findings for TFA, which are also associated with increased inflammation (15,16), were similarly inconsistent: there were weak positive associations of TFA 16:1 fatty acids with cancer risk, and no associations of TFA 18:1 and 18:2 fatty acids with risk. Taken together, these findings contradict the expectation that high consumption of long-chain ω-3 fatty acids and low consumption of ω-6 fatty acids would reduce the risk of prostate cancer.

Figures 1 and 2 give results of our meta-analyses of studies reporting associations between EPA or DHA and prostate cancer risk, respectively (14,31,35–39). The two studies published before 2007 (37,39) contribute little to these meta-analyses because of their small sample sizes. Among the four large and more recent studies, three support the findings of a positive association of high



**Figure 1.** Meta-analysis of prospective biomarker studies examining associations between eicosapentaenoic acid (EPA) and total, low-, and high-grade prostate cancer risk. **Dots** and **horizontal lines** correspond to relative risks (RRs) and 95% confidence intervals (CIs), respectively, comparing the highest vs lowest quantile of EPA

measured in blood for each study. The size of the **shaded square** represents the study-specific weight in the meta-analysis. The **diamond** represents the meta-relative risk and 95% confidence interval. Relative risks estimated assuming fixed effects. All statistical tests were two-sided.

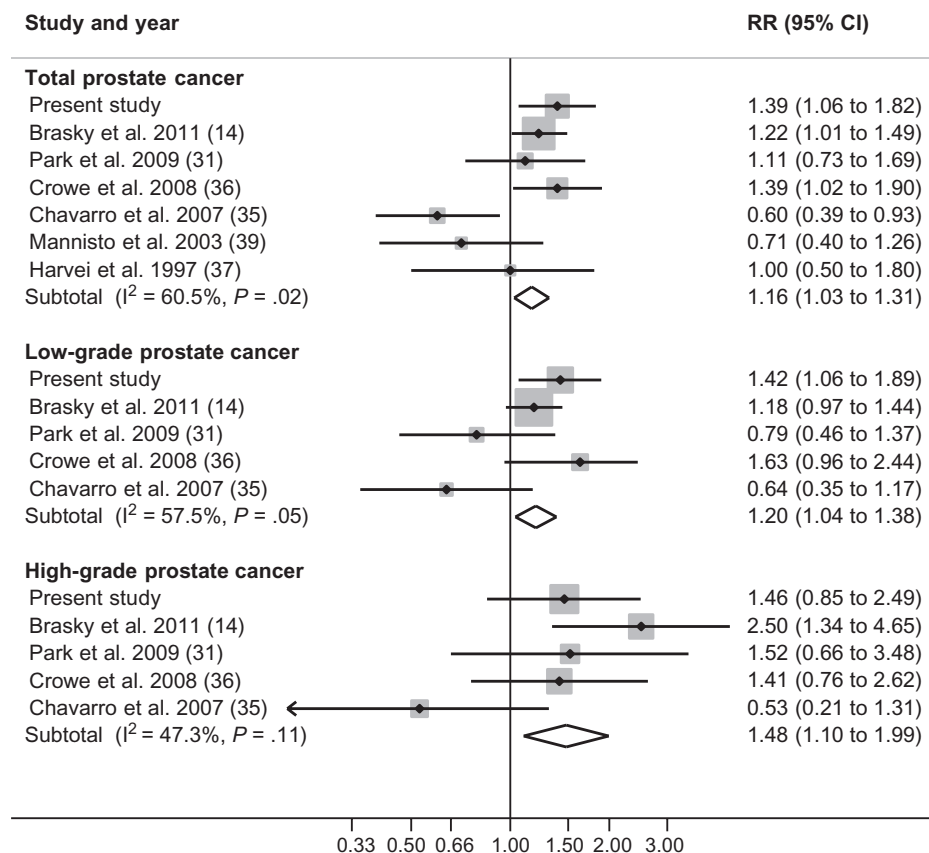
$\omega$ -3 fatty acids with risk reported here (14,31,40), albeit with some inconsistencies regarding cancer grade and/or specific  $\omega$ -3 fatty acid, and one study (35) reported inverse associations. For EPA, only the relative risk (RR) for high-grade cancer is notable, but it does not reach statistical significance (RR = 1.29, 95% CI = 0.97 to 1.72). For DHA, the summary relative risks for total (RR = 1.16, 95% CI = 1.03 to 1.31), low-grade (RR = 1.20, 95% CI = 1.04 to 1.38) and high-grade cancer (RR = 1.48, 95% CI = 1.10 to 1.99) are positive and statistically significant. Meta-analyses for total long-chain  $\omega$ -3 fatty acids are difficult to interpret because this measure is omitted from some reports and, when given, defined variously as 1) ALA, EPA plus DHA; 2) EPA, DPA plus DHA; or 3) EPA plus DHA. However, based on those studies that have reported this measure (14,31,35), the summary relative risks for total, low-, and high-grade cancer are 1.14 (95% CI = 0.99 to 1.32), 1.14 (95% CI = 0.98 to 1.33) and 1.51 (95% CI = 1.08 to 2.11), respectively (Figure 3).

We discuss findings for the remaining fatty acids briefly because this study contributes only modestly to this literature. Our finding of no association of ALA with prostate cancer risk is consistent with the majority of studies, which also reported no association (14,31,35,36,38,39). Results from previous studies that examined  $\omega$ -6 and TFA are inconsistent. Among the seven prospective studies of  $\omega$ -6 PUFA (14,31,35–39,41), two reported inverse associations for LA (35,38), and, similar to our study, all others reported

no associations. No studies have found associations of AA with risk. Three previous studies have examined the association between TFA and prostate cancer (14,42,43). No previous study found the increased risk of prostate cancer associated with TFA 16:1 reported here. One study reported statistically significant increases in non-aggressive cancer associated with TFA 18:1 and TFA 18:2 (42), and we have previously reported inverse associations of these fatty acids with risk (14). Given the inconsistency of these findings, we judge it unlikely that intakes of either  $\omega$ -6 PUFA or TFA are associated with prostate cancer risk.

Long-chain  $\omega$ -3 PUFA have many physiological effects. They are considered anti-inflammatory because of their multiple effects on inflammation pathways, such as inhibition of tumor necrosis factor alpha and modification of eicosanoid activity, and they also affect cell permeability, gene expression, and signal transduction (44). It is unclear why high levels of long-chain  $\omega$ -3 PUFA would increase prostate cancer risk, and further study will be needed to understand the mechanisms underlying the findings reported here.

This study had several strengths. Its design is prospective, and it is based on a large number of prostate cancer case subjects. The SELECT trial had near-complete (>95%) follow-up of participants, thereby minimizing the potential for attrition bias (22). Additionally, we were able to rigorously evaluate and rule out the potential for confounding by screening behavior. Lastly, we were



**Figure 2.** Meta-analysis of prospective biomarker studies examining associations between docosahexaenoic acid (DHA) and total, low-, and high-grade prostate cancer risk. **Dots** and **horizontal lines** correspond to relative risks (RRs) and 95% confidence intervals (CIs), respectively, for each study for comparisons of the highest vs lowest

quantile of DHA measured in blood. The size of the **shaded square** represents the study-specific weight in the meta-analysis. The **diamond** represents the meta-relative risk and 95% confidence interval. Relative risks estimated assuming fixed effects. All statistical tests were two-sided.

able to replicate major findings on long-chain  $\omega$ -3 PUFA from our prior work. There also were some limitations. Expressing fatty acids as weight proportions (33,45) could create spurious results because an increase in the percentage of one type of fatty acid requires a decrease in others (33); however, given the very low concentrations of  $\omega$ -3 PUFA, it is unlikely that their variability, which is strongly related to dietary intake, would be strongly affected by proportions of other phospholipid fatty acids. There is also an inverse association of  $\omega$ -3 with  $\omega$ -6 PUFA; however, in secondary analyses that controlled associations of long-chain  $\omega$ -3 with  $\omega$ -6 fatty acids, associations of  $\omega$ -3 fatty acids with prostate cancer risk were unchanged. Factors other than diet affect proportions of phospholipid essential fatty acids. For example, in feeding studies a low-fat diet modestly increases the blood proportion of long-chain  $\omega$ -3 PUFA (46); however, these effects are small compared with those due to supplementation (47). Lastly, model assumptions were violated for four models; however, assumptions were not violated in those measures for which we show statistically significant associations.

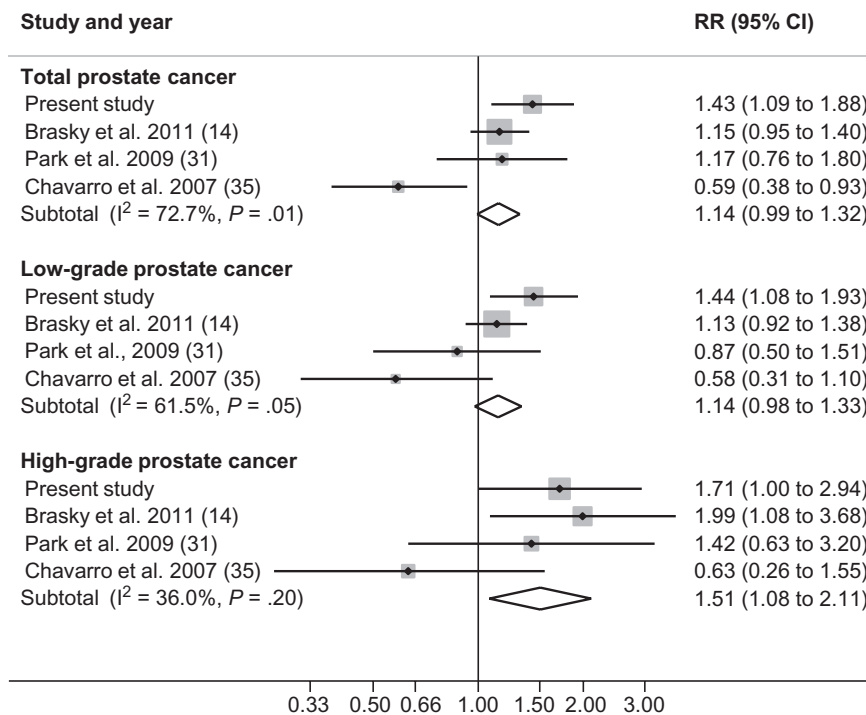
In conclusion, in this large, prospective study of plasma phospholipid fatty acids and prostate cancer risk, contrary to our expectations, we found that long-chain  $\omega$ -3 PUFA overall, and DPA and DHA in particular, were associated with strong, linear increases in prostate cancer risk. We note that this is not a novel finding because it has been reported previously in two other

prospective blood biomarker studies that have examined the associations between long-chain  $\omega$ -3 PUFA and prostate cancer risk. Whereas a lack of coherent mechanism has led authors of previous studies, including us, to consider these findings suspect, their replication here strongly suggests that long-chain  $\omega$ -3 PUFA do play a role in enhancing prostate tumorigenesis. As has been made evident from many other clinical trials of nutritional supplements and cancer risk, the associations of nutrients with chronic disease are complex and may affect diseases differently. Long-chain  $\omega$ -3 PUFA have been widely promoted for prevention of heart disease and cancer. Both this study and a recent meta-analysis of clinical trials showing no effects of long-chain  $\omega$ -3 PUFA supplementation on all-cause mortality, cardiac death, myocardial infarction, or stroke (48) suggest that general recommendations to increase long-chain  $\omega$ -3 PUFA intake should consider its potential risks.

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**Figure 3.** Meta-analysis of prospective biomarker studies examining associations between total long-chain  $\omega$ -3 polyunsaturated fatty acids and total, low-, and high-grade prostate cancer risk. **Dots and horizontal lines** correspond to relative risks (RRs) and 95% confidence intervals (CIs), respectively, for each study for comparisons of the highest vs

lowest quantile of long-chain  $\omega$ -3 polyunsaturated fatty acids measured in blood. The size of the **shaded square** represents the study-specific weight in the meta-analysis. The **diamond** represents the meta-relative risk and 95% confidence interval. Relative risks estimated assuming fixed effects. All statistical tests were two-sided.

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