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## Original Contribution

# Serum Phospholipid Fatty Acids and Prostate Cancer Risk: Results From the Prostate Cancer Prevention Trial

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Inflammation may be involved in prostate cancer development and progression. This study examined the associations between inflammation-related phospholipid fatty acids and the 7-year-period prevalence of prostate cancer in a nested case-control analysis of participants, aged 55–84 years, in the Prostate Cancer Prevention Trial during 1994–2003. Cases ($n = 1,658$) were frequency matched to controls ($n = 1,803$) on age, treatment, and prostate cancer family history. Phospholipid fatty acids were extracted from serum, and concentrations of ω-3, ω-6, and *trans*-fatty acids (TFAs) were expressed as proportions of the total. Logistic regression models estimated odds ratios and 95% confidence intervals of associations of fatty acids with prostate cancer by grade. No fatty acids were associated with low-grade prostate cancer risk. Docosahexaenoic acid was positively associated with high-grade disease (quartile 4 vs. 1: odds ratio (OR) = 2.50, 95% confidence interval (CI): 1.34, 4.65); TFA 18:1 and TFA 18:2 were linearly and inversely associated with risk of high-grade prostate cancer (quartile 4 vs. 1: TFA 18:1, OR = 0.55, 95% CI: 0.30, 0.98; TFA 18:2, OR = 0.48, 95% CI: 0.27, 0.84). The study findings are contrary to those expected from the pro- and antiinflammatory effects of these fatty acids and suggest a greater complexity of effects of these nutrients with regard to prostate cancer risk.

fatty acids; histology; inflammation; phospholipids; prostatic neoplasms; serum

Abbreviations: CI, confidence interval; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; OR, odds ratio; RR, relative risk; TFA, *trans*-fatty acid.

Many lines of evidence support an important role for inflammation in the pathogenesis of prostate cancer (1, 2). Proliferative inflammatory atrophy of the prostate may be the precursor lesion for prostate cancer (1, 3), and both epidemiologic and animal experimental models report inverse associations between nonsteroidal antiinflammatory drugs and prostate cancer (4–7). Dietary compounds can also influence inflammation. Both in vitro and human studies have found ω-6 and *trans*-fatty acids (TFAs) to be proinflammatory and long-chain ω-3 fatty acids to be antiinflammatory (8, 9). However, results from the few studies that have examined associations of these fatty acids in blood with prostate cancer risk have been inconsistent (10–18).

Here, we examine the associations of inflammation-related serum phospholipid fatty acids with prostate cancer risk in a case-control study nested within the Prostate Cancer Prevention Trial. We hypothesized that ω-6 and TFAs would be positively and ω-3 fatty acids inversely associated with risk. Several aspects of the Prostate Cancer Prevention Trial are unique: The presence or absence of prostate cancer was determined by biopsy, and cancer grade was determined by centralized, uniform pathology. Thus, although almost all prostate cancer cases were diagnosed as local stage, detection bias was minimized, and pathologic grading of cases was rigorous. Results from this study can help to inform whether these fatty acids should be further investigated for prostate cancer prevention.

**Table 1.** Baseline Characteristics of Participants, Stratified by Prostate Cancer Grade, in the Prostate Cancer Prevention Trial, 1994–2003

| Characteristic | Low-Grade Cases ($n$ = 1,533) | | | High-Grade Cases ($n$ = 125) | | | Controls ($n$ = 1,803) | | |
|---|---|---|---|---|---|---|---|---|---|
| | No. | % | Mean (SD) | No. | % | Mean (SD) | No. | % | Mean (SD) |
| Age, years[a] | | | 63.6 (5.5) | | | 65.0** (5.9) | | | 63.6 (5.6) |
| 55–59 | 479 | 26.6 | | 23 | 18.4* | | 479 | 26.6 | |
| 60–64 | 584 | 32.4 | | 42 | 33.6 | | 584 | 32.4 | |
| 65–69 | 444 | 24.6 | | 29 | 23.2 | | 444 | 24.6 | |
| ≥70 | 296 | 16.4 | | 31 | 24.8 | | 296 | 16.4 | |
| Education, years | | | | | | | | | |
| ≤12 | 257 | 16.8** | | 24 | 19.2 | | 349 | 19.4 | |
| 13–15 | 413 | 27.0 | | 36 | 28.8 | | 542 | 30.1 | |
| ≥16 | 862 | 56.3 | | 65 | 52.0 | | 911 | 50.6 | |
| Race[b] | | | | | | | | | |
| White | 1,435 | 79.6*** | | 109 | 87.2* | | 1,435 | 79.6 | |
| Black | 175 | 9.7 | | 11 | 8.8 | | 175 | 9.7 | |
| Other | 193 | 10.7 | | 5 | 4.0 | | 193 | 10.7 | |
| Physical activity | | | | | | | | | |
| Sedentary | 261 | 17.1 | | 20 | 16.0 | | 311 | 17.3 | |
| Light | 631 | 41.3 | | 53 | 42.4 | | 741 | 41.3 | |
| Moderate | 508 | 33.3 | | 45 | 36.0 | | 555 | 30.9 | |
| Active | 128 | 8.4 | | 7 | 5.6 | | 188 | 10.5 | |
| Smoking status | | | | | | | | | |
| Never | 550 | 35.9 | | 46 | 36.8 | | 620 | 34.4 | |
| Former | 881 | 57.5 | | 68 | 54.4 | | 1,045 | 58.0 | |
| Current | 102 | 6.7 | | 11 | 8.8 | | 138 | 7.7 | |
| Alcohol consumption, g/day[c] | | | 12.8 (17.3) | | | 14.3 (16.3) | | | 11.8 (14.6) |
| Nondrinker | 341 | 22.2 | | 31 | 24.8 | | 415 | 23.0 | |
| >0–<30 | 1,050 | 68.5 | | 80 | 64.0 | | 1,234 | 68.4 | |
| ≥30 | 143 | 9.3 | | 14 | 11.2 | | 154 | 8.5 | |
| Body mass index, kg/m$^2$ | | | 27.4 (4.0) | | | 28.1 (4.0) | | | 27.6 (4.0) |
| <25.0 | 435 | 29.7 | | 26 | 20.8 | | 449 | 25.1 | |
| 25.0–29.9 | 775 | 51.1 | | 62 | 49.6 | | 944 | 52.8 | |
| ≥30.0 | 308 | 20.3 | | 37 | 29.6 | | 394 | 22.1 | |

Table continues

## MATERIALS AND METHODS

### Study design

The Prostate Cancer Prevention Trial was a randomized, placebo-controlled trial that tested whether the 5α-reductase inhibitor, finasteride, reduces prostate cancer risk (19). In 221 study centers within the United States, men ≥55 years of age, who had no history of cancer (except nonmelanoma skin) or severe benign prostatic hyperplasia and who had prostate-specific antigen concentrations of ≤3.0 ng/mL with a normal digital rectal examination, were eligible to participate. Between January 1994 and May 1997, 18,882 men were randomized to receive finasteride (5 mg/day) or placebo. Over the course of the 7-year study, men underwent annual prostate-specific antigen and digital rectal examination testing. Men who had an abnormal digital rectal examination or finasteride-adjusted prostate-specific antigen ≥4.0 ng/mL were recommended for a prostate biopsy (20). At the final study visit, all men who had not been diagnosed with prostate cancer were requested to undergo a prostate biopsy.

### Prostate biopsies

All biopsies consisted of ≥6 cores collected under transrectal ultrasonographic guidance, which were reviewed for adenocarcinoma by both the pathologist at the local study site and a central pathology laboratory with concordance achieved in all cases. Prostate cancer cases were classified as "for-cause" if there was a prompt for biopsy on the basis of the digital rectal examination or prostate-specific antigen and "not-for-cause" if there was no prompt preceding the end-of-study biopsy. Clinical stage was assigned locally, and grade was assigned by a single pathologist at the central laboratory by using the Gleason scoring system (21). All

**Table 1.** Continued

| Characteristic | Low-Grade Cases ($n$ = 1,533) | | | High-Grade Cases ($n$ = 125) | | | Controls ($n$ = 1,803) | | |
|---|---|---|---|---|---|---|---|---|---|
| | No. | % | Mean (SD) | No. | % | Mean (SD) | No. | % | Mean (SD) |
| History of diabetes | | | | | | | | | |
| No | 1,471 | 96.0*** | | 114 | 91.2 | | 1,670 | 92.7 | |
| Yes | 62 | 4.0 | | 11 | 8.7 | | 132 | 7.3 | |
| Family history of prostate cancer[a] | | | | | | | | | |
| No | 1,193 | 77.8 | | 106 | 84.7 | | 1,421 | 78.8 | |
| Yes | 340 | 22.2 | | 19 | 15.2 | | 382 | 21.2 | |
| Treatment arm[a] | | | | | | | | | |
| Placebo | 917 | 59.8 | | 45 | 36.0*** | | 1,040 | 57.7 | |
| Finasteride | 616 | 40.2 | | 80 | 64.0 | | 763 | 42.3 | |
| For cause biopsy | | | | | | | | | |
| No | 883 | 57.6 | | 28 | 22.4 | | | | |
| Yes | 650 | 42.4 | | 97 | 77.6 | | | | |
| Clinical stage[d] | | | | | | | | | |
| T1 | 1,156 | 77.5 | | 62 | 49.6 | | | | |
| T2 | 323 | 21.7 | | 52 | 41.6 | | | | |
| T3 | 12 | 0.8 | | 11 | 8.8 | | | | |

Abbreviations: SD, standard deviation; TNM, Tumor, Node, Metastasis.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.0001$ (all vs. controls).
[a] Frequency match variable.
[b] Nonwhite controls were oversampled from the Prostate Cancer Prevention Trial population.
[c] Among drinkers.
[d] Clinical stage according to the TNM staging system.

men gave informed consent, and study procedures were approved by institutional review boards at each study center, the Southwest Oncology Group in San Antonio, Texas, and the Southwest Oncology Group Data and Statistical Center (Fred Hutchinson Cancer Research Center, Seattle, Washington).

### Case and control selection

This is a case-control study nested within the Prostate Cancer Prevention Trial. Excluding men without baseline serum available for analysis, cases ($n$ = 1,809) were all men with biopsy-confirmed invasive prostate cancer identified before the study was unblinded, and controls ($n$ = 1,809) were selected from men who were disease free at the end-of-study biopsy. Controls were frequency matched to cases on distributions of age (±5 years), treatment group (finasteride/placebo), and a first-degree relative with prostate cancer, and they were oversampled for nonwhites.

### Data collection and laboratory methods

Details regarding age, race, alcohol consumption, diabetes status, family history of prostate cancer, and history of smoking were collected at baseline by self-administered questionnaires. Participants' height and weight were measured at baseline, and body mass index was calculated (weight (kg)/height $(m)^2$).

Nonfasting blood was collected approximately 3 months prior to randomization and annually thereafter until diagnosis or the end of the study. Venous blood was drawn into collection tubes without anticoagulant, refrigerated, and shipped to the specimen repository where the samples were centrifuged, aliquoted, and stored at −70°C until analysis (22); 0.5-mL serum samples were collected at years 1 (postrandomization) and 4 and pooled before analysis to reduce intraindividual variability. Alternate years were selected if men were missing a year 1 or year 4 sample or were diagnosed before year 4 ($n$ = 320 cases, $n$ = 130 controls), and a single, prediagnostic sample was used if 2 prediagnostic blood samples were unavailable ($n$ = 78). We excluded cases diagnosed before year 1 ($n$ = 10) or missing Gleason grade ($n$ = 66), men with insufficient serum ($n$ = 57 cases, $n$ = 4 controls), or men missing ≥1 covariates ($n$ = 18 cases, $n$ = 2 controls), leaving 1,658 cases and 1,803 controls.

Detailed methods for the phospholipid fatty acid assay have been published elsewhere (23). Briefly, total lipids were extracted from serum, and phospholipids were separated from other lipids by one-dimensional thin-layer chromatography (24). Fatty acid methyl ester samples were prepared by direct transesterification and separated by using gas chromatography (25). Fatty acid composition is expressed as the weight percentage of total phospholipid fatty acids. Quality control samples were embedded randomly in each box of study samples. Samples from cases and controls were analyzed simultaneously, and all laboratory personnel

**Table 2.** Distribution of Serum Phospholipid Fatty Acids by Percent of Total Among Prostate Cancer Cases and Controls, Stratified by Prostate Cancer Grade, in the Prostate Cancer Prevention Trial, 1994–2003

| Fatty Acids | Low-Grade Cases ($n$ = 1,533) | | | | High-Grade Cases ($n$ = 125) | | | | Controls ($n$ = 1,803) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Median | Geometric Mean[a] | 95% CI | 5th–95th Percentiles | Median | Geometric Mean[a] | 95% CI | 5th–95th Percentiles | Median | Geometric Mean[a] | 95% CI | 5th–95th Percentiles |
| ω-3 Fatty acids | | | | | | | | | | | | |
| α-Linolenic acid (18:3ω3) | 0.15 | 0.14 | 0.14, 0.14 | 0.09–0.24 | 0.14 | 0.13 | 0.13, 0.14 | 0.09–0.24 | 0.14 | 0.14 | 0.14, 0.14 | 0.09–0.23 |
| EPA (20:5ω3) | 0.59 | 0.58 | 0.57, 0.60 | 0.30–1.30 | 0.56 | 0.58 | 0.53, 0.63 | 0.31–1.35 | 0.57 | 0.57 | 0.55, 0.58 | 0.30–1.22 |
| DHA (22:6ω3) | 2.73 | 2.89 | 2.82, 2.96 | 1.73–4.56 | 2.80* | 2.99* | 2.84, 3.15 | 2.00–4.35 | 2.73 | 2.84 | 2.78, 2.89 | 1.72–4.47 |
| EPA + DHA | 3.29 | 3.50* | 3.42, 3.59 | 2.18–5.75 | 3.43* | 3.61 | 3.42, 3.81 | 2.55–6.01 | 3.30 | 3.43 | 3.37, 3.50 | 2.15–5.52 |
| ω-6 Fatty acids | | | | | | | | | | | | |
| Linoleic acid (18:2ω6) | 20.13 | 19.59 | 19.40, 19.79 | 16.39–24.28 | 19.82 | 19.35 | 18.92, 19.78 | 16.26–23.48 | 20.10 | 19.56 | 19.39, 19.73 | 16.03–24.04 |
| Arachidonic acid (20:4ω6) | 10.96 | 11.10 | 10.94, 11.25 | 7.88–13.95 | 11.12 | 11.39 | 11.03, 11.75 | 8.63–14.51 | 11.08 | 11.18 | 11.05, 11.31 | 7.97–14.16 |
| Linoleic + arachidonic acid | 31.24 | 31.02 | 30.86, 31.17 | 27.77–34.02 | 31.10 | 31.08 | 30.72, 31.44 | 28.44–34.05 | 31.24 | 31.06 | 30.92, 31.20 | 28.04–34.06 |
| *trans*-Fatty acids | | | | | | | | | | | | |
| TFA 18:1 | 1.69 | 1.54 | 1.49, 1.59 | 0.84–2.85 | 1.59* | 1.43* | 1.33, 1.53 | 0.77–2.50 | 1.67 | 1.55 | 1.51, 1.59 | 0.88–2.80 |
| TFA 18:2 | 0.22 | 0.21 | 0.20, 0.21 | 0.15–0.32 | 0.21* | 0.20* | 0.19, 0.21 | 0.13–0.29 | 0.22 | 0.21 | 0.21, 0.22 | 0.15–0.33 |
| TFA 16 | 0.24** | 0.22 | 0.21, 0.22 | 0.14–0.32 | 0.22 | 0.21 | 0.20, 0.22 | 0.13–0.33 | 0.23 | 0.21 | 0.21, 0.22 | 0.14–0.31 |

Abbreviations: CI, confidence interval; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; TFA, *trans*-fatty acid.

* $P < 0.05$; ** $P < 0.01$ (both vs. controls).

[a] Adjusted for age, race, family history of prostate cancer, and treatment arm.

**Table 3.** Pearson's Correlations of Log-transformed Serum Phospholipid Fatty Acids Among Participants in the Prostate Cancer Prevention Trial, 1994–2003

| | α-Linolenic Acid | EPA | DHA | EPA + DHA | Linoleic Acid | Arachidonic Acid | Linoleic + Arachidonic Acid | TFA 18:1 | TFA 18:2 | TFA 16 |
|---|---|---|---|---|---|---|---|---|---|---|
| α-Linolenic acid | 1.00 | | | | | | | | | |
| EPA | 0.28 | 1.00 | | | | | | | | |
| DHA | 0.04 | 0.58 | 1.00 | | | | | | | |
| EPA + DHA | 0.12 | 0.77 | 0.97 | 1.00 | | | | | | |
| Linoleic acid | 0.24 | −0.42 | −0.36 | −0.41 | 1.00 | | | | | |
| Arachidonic acid | −0.41 | 0.02 | 0.02 | 0.02 | −0.63 | 1.00 | | | | |
| Linoleic + arachidonic acid | −0.10 | −0.51 | −0.44 | −0.51 | 0.64 | 0.18 | 1.00 | | | |
| TFA 18:1 | −0.12 | −0.36 | −0.26 | −0.32 | 0.34 | −0.11 | 0.32 | 1.00 | | |
| TFA 18:2 | 0.01 | −0.22 | −0.26 | −0.27 | 0.19 | −0.24 | −0.003 | 0.64 | 1.00 | |
| TFA 16 | −0.14 | −0.22 | −0.11 | −0.16 | 0.27 | −0.11 | 0.24 | 0.54 | 0.28 | 1.00 |

Abbreviations: DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; TFA, *trans*-fatty acid.

were blinded to the status of the samples. Coefficients of variation for fatty acids were as follows: 18:3ω3, 5.1%; 20:4ω6, 1.0%; 22:6ω3, 2.4%; 20:5ω3, 3.0%; 18:2ω6, 1.5%; TFA 16, 10.1%; TFA 18:1, 7.3%; and TFA 18:2, 10.3%. There was no evidence of laboratory drift.

### Statistical analysis

High-grade prostate cancer was defined as Gleason scores 8–10 ($n = 125$). Low-grade disease was defined as Gleason scores 2–7 ($n = 1{,}533$). Proportions of fatty acids were categorized into quartiles on the basis of the distribution in the controls. The following variables were calculated: eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA) as a measure of total long-chain ω-3 fatty acids; linoleic + arachidonic acids as a measure of total ω-6 fatty acids; *trans*-fats 18:1ω6t, 18:1ω7t, 18:1ω8t, 18:1ω9t, and 18:1ω10–12t as a measure of total TFA 18:1; *trans*-fats 16:1ω7t and 16:1ω9t as a measure of total TFA 16; and *trans*-fats 18:2ω6tt, 18:2ω6ct, and 18:2ω6tc as a measure of total TFA 18:2. *trans*-Fat 18:2ω6tt, from hydrogenated oils, was examined separately; however, results did not differ from those for TFA 18:2.

Differences in the characteristics of study participants between control and cancer groups were tested by using $\chi^2$ tests and *t* tests for categorical and continuous variables, respectively. Differences in age- and race-adjusted geometric mean concentrations of fatty acids were compared with controls by using an *F* test; median values were compared by use of the Wilcoxon rank-sum test.

Multivariable-adjusted polytomous logistic regression models were used to estimate odds ratios and 95% confidence intervals for the associations of fatty acids with risk of prostate cancer stratified by grade. Tests for linear trend ($P_{trend}$) across categories were based on an ordinal variable corresponding to rank from lowest to highest category (26).

All models were adjusted for the matching variables age, family history of prostate cancer, and race, and additionally adjusted for prostate cancer risk factors including history of diabetes, alcohol consumption (g/day), and body mass index (kg/m$^2$). Because finasteride reduced the risk of total prostate cancer (19), we hypothesized a priori that finasteride exposure would modify the association of fatty acids with prostate cancer risk and therefore examined all associations separately by treatment arm; analyses in combined treatment arms were further adjusted for treatment (finasteride/placebo). We also examined whether results differed by reason for biopsy (for-cause vs. not for-cause), age (<65, ≥65 years), and family history of prostate cancer. $P_{interaction}$ values were calculated by including a multiplicative term in regression models. Statistical analyses were performed by using SAS, version 9.2, software (SAS Institute, Inc., Cary, North Carolina). All statistical tests were 2 sided, and $P < 0.05$ was considered statistically significant.

## RESULTS

We restrict our presentation of results to prostate cancers stratified by grade for 2 reasons. First, many studies find that risk factors differ for low-grade compared with high-grade prostate cancer, which would not be evident in a combined analysis; Second, given the low number of high-grade cancers, the findings for total differ little from those of low-grade cancer alone. Associations of fatty acids with cancer risk did not differ by treatment arm and, thus, only combined analyses are presented.

Table 1 gives baseline demographic and lifestyle characteristics of study cases and controls. Compared with controls, high-grade cases were significantly older and were more likely to have been randomized to the finasteride treatment arm. Low-grade cases were less likely than controls to have a history of diabetes. Among cases, 45% were diagnosed by a for-cause biopsy; the majority of tumors were diagnosed as stage T1.

**Table 4.** Multivariable-adjusted Associations of Serum Phospholipid Fatty Acids in Relation to Prostate Cancer Risk, Stratified by Prostate Cancer Grade, Among Participants in the Prostate Cancer Prevention Trial, 1994–2003

| Fatty Acids, % of total | Low-Grade Cases, no. | High-Grade Cases, no. | Controls, no. | Low Grade | | High Grade | |
|---|---|---|---|---|---|---|---|
| | | | | OR[a] | 95% CI | OR[a] | 95% CI |
| ω-3 Fatty acids | | | | | | | |
| α-Linolenic acid (18:3ω3) | | | | | | | |
| <0.12 | 373 | 36 | 451 | 1.00 | Referent | 1.00 | Referent |
| 0.12–0.14 | 360 | 30 | 451 | 0.94 | 0.77, 1.15 | 0.79 | 0.47, 1.31 |
| 0.15–0.18 | 421 | 34 | 450 | 1.06 | 0.87, 1.29 | 0.87 | 0.53, 1.43 |
| >0.18 | 379 | 25 | 451 | 0.92 | 0.75, 1.13 | 0.64 | 0.38, 1.11 |
| $P_{trend}$ | | | | | 0.71 | | 0.17 |
| EPA (20:5ω3) | | | | | | | |
| <0.44 | 378 | 26 | 451 | 1.00 | Referent | 1.00 | Referent |
| 0.44–0.57 | 349 | 37 | 451 | 0.87 | 0.71, 1.07 | 1.27 | 0.75, 2.15 |
| 0.58–0.74 | 383 | 28 | 450 | 0.91 | 0.75, 1.11 | 0.90 | 0.51, 1.57 |
| >0.74 | 423 | 34 | 451 | 1.01 | 0.83, 1.24 | 1.09 | 0.63, 1.86 |
| $P_{trend}$ | | | | | 0.79 | | 0.86 |
| DHA (22:6ω3) | | | | | | | |
| <2.26 | 368 | 15 | 450 | 1.00 | Referent | 1.00 | Referent |
| 2.26–2.73 | 400 | 42 | 451 | 1.06 | 0.87, 1.29 | 2.65 | 1.44, 4.87 |
| 2.74–3.30 | 354 | 30 | 452 | 0.96 | 0.79, 1.18 | 1.84 | 0.97, 1.29 |
| >3.30 | 411 | 38 | 450 | 1.18 | 0.97, 1.44 | 2.50 | 1.34, 4.65 |
| $P_{trend}$ | | | | | 0.21 | | 0.04 |
| EPA + DHA | | | | | | | |
| <2.77 | 367 | 16 | 451 | 1.00 | Referent | 1.00 | Referent |
| 2.77–3.30 | 403 | 38 | 451 | 1.08 | 0.88, 1.31 | 2.15 | 1.18, 3.94 |
| 3.31–4.02 | 358 | 36 | 451 | 0.98 | 0.80, 1.20 | 2.00 | 1.09, 3.67 |
| >4.02 | 405 | 35 | 450 | 1.13 | 0.92, 1.38 | 1.99 | 1.08, 3.68 |
| $P_{trend}$ | | | | | 0.41 | | 0.08 |
| ω-6 Fatty acids | | | | | | | |
| Linoleic acid (18:2ω6) | | | | | | | |
| <18.49 | 363 | 35 | 450 | 1.00 | Referent | 1.00 | Referent |
| 18.49–20.10 | 390 | 31 | 451 | 1.04 | 0.85, 1.27 | 0.95 | 0.57, 1.57 |
| 20.11–21.65 | 379 | 30 | 451 | 0.94 | 0.77, 1.15 | 0.90 | 0.53, 1.51 |
| >21.65 | 401 | 29 | 451 | 1.02 | 0.83, 1.24 | 0.92 | 0.55, 1.57 |
| $P_{trend}$ | | | | | 0.88 | | 0.73 |

**Table continues**

Medians and adjusted geometric means of the percent serum phospholipid fatty acid distributions are given in Table 2. Levels of DHA were higher among high-grade cases compared with controls. Levels of TFA 18:1 and 18:2 were significantly lower among high-grade cases compared with controls. There were no other significant differences of the remaining phospholipids between control and cancer groups.

Correlations among serum fatty acids are given in Table 3. There were modest inverse correlations of ω-3 fatty acids with TFA and, as expected, moderate inverse correlations between ω-6 and ω-3 fatty acids.

Table 4 gives the multivariable-adjusted associations of percent serum fatty acids with prostate cancer risk. There were no associations of low-grade prostate cancer with any fatty acid measure. DHA was positively, but not linearly, associated with risk of high-grade prostate cancer. Compared with the lowest quartile, each quartile of percent serum DHA was associated with an approximate doubling of high-grade disease. In a post-hoc analysis contrasting quartiles 2–4 with quartile 1, the odds ratio for high-grade prostate cancer was 2.32 (95% confidence interval (CI): 1.33, 4.05). EPA was not associated with risk of high-grade prostate cancer, and associations were similar for EPA + DHA to that of DHA

**Table 4.** Continued

| Fatty Acids, % of total | Low-Grade Cases, no. | High-Grade Cases, no. | Controls, no. | Low Grade | | High Grade | |
|---|---|---|---|---|---|---|---|
| | | | | OR[a] | 95% CI | OR[a] | 95% CI |
| Arachidonic acid (20:4ω6) | | | | | | | |
| <9.79 | 399 | 33 | 451 | 1.00 | Referent | 1.00 | Referent |
| 9.79–11.08 | 413 | 28 | 451 | 1.02 | 0.84, 1.24 | 0.83 | 0.49, 1.40 |
| 11.09–12.35 | 374 | 26 | 450 | 0.93 | 0.76, 1.13 | 0.76 | 0.45, 1.31 |
| >12.35 | 347 | 38 | 451 | 0.95 | 0.78, 1.17 | 1.13 | 0.68, 1.87 |
| $P_{trend}$ | | | | | 0.71 | | 0.47 |
| Linoleic + arachidonic acid | | | | | | | |
| <30.10 | 429 | 33 | 451 | 1.00 | Referent | 1.00 | Referent |
| 30.10–31.24 | 339 | 28 | 451 | 0.81 | 0.66, 0.98 | 0.78 | 0.47, 1.30 |
| 31.25–32.35 | 343 | 26 | 450 | 0.80 | 0.66, 0.98 | 0.74 | 0.44, 1.22 |
| >32.35 | 422 | 38 | 451 | 0.97 | 0.80, 1.18 | 0.84 | 0.51, 1.40 |
| $P_{trend}$ | | | | | 0.77 | | 0.46 |
| *trans*-Fatty acids | | | | | | | |
| TFA 18:1 | | | | | | | |
| <1.29 | 386 | 38 | 450 | 1.00 | Referent | 1.00 | Referent |
| 1.29–1.67 | 349 | 37 | 452 | 0.91 | 0.74, 1.12 | 0.95 | 0.58, 1.55 |
| 1.68–2.08 | 396 | 29 | 450 | 1.02 | 0.83, 1.25 | 0.76 | 0.45, 1.28 |
| >2.08 | 402 | 21 | 451 | 1.00 | 0.81, 1.24 | 0.55 | 0.30, 0.98 |
| $P_{trend}$ | | | | | 0.71 | | 0.03 |
| TFA 18:2 | | | | | | | |
| <0.18 | 393 | 41 | 450 | 1.00 | Referent | 1.00 | Referent |
| 0.18–0.22 | 370 | 26 | 451 | 0.90 | 0.74, 1.10 | 0.63 | 0.37, 1.05 |
| 0.23–0.26 | 410 | 39 | 452 | 1.02 | 0.84, 1.25 | 0.95 | 0.59, 1.52 |
| >0.26 | 360 | 19 | 450 | 0.87 | 0.71, 1.07 | 0.48 | 0.27, 0.84 |
| $P_{trend}$ | | | | | 0.38 | | 0.06 |
| TFA 16 | | | | | | | |
| <0.19 | 348 | 34 | 451 | 1.00 | Referent | 1.00 | Referent |
| 0.19–0.23 | 340 | 39 | 450 | 0.94 | 0.76, 1.15 | 1.18 | 0.72, 1.93 |
| 0.24–0.26 | 405 | 24 | 452 | 1.09 | 0.89, 1.34 | 0.75 | 0.43, 1.31 |
| >0.26 | 440 | 28 | 450 | 1.16 | 0.94, 1.43 | 0.90 | 0.52, 1.56 |
| $P_{trend}$ | | | | | 0.08 | | 0.38 |

Abbreviations: CI, confidence interval; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; OR, odds ratio; TFA, *trans*-fatty acid.
[a] Adjusted for age, race, family history of prostate cancer, diabetes, body mass index, alcohol, and treatment arm.

alone. There was a significant inverse association of the percent serum TFA 18:1 and 18:2 with risk of high-grade prostate cancer. For TFA 18:1, comparing the highest with the lowest quartiles, there was a 45% reduction in risk of high-grade disease (95% CI: 2, 70) ($P_{trend} = 0.03$). For TFA 18:2, the highest quartile was associated with a 52% reduction in risk of high-grade prostate cancer (95% CI: 16, 73) ($P_{trend} = 0.04$). The remaining fatty acids were not associated with cancer risk. Findings did not differ when models included all 8 fatty acids.

Findings were similar across subgroups of age (<65 vs. ≥65 years) or indication for biopsy (for-cause vs. not for-cause). There were, however, statistically significant interactions between family history of prostate cancer and both linoleic acid and TFA 18:1 (Table 5). For linoleic acid, there was a positive association with risk of low-grade disease in men with a family history, and there was no association among men without a family history ($P_{interaction} = 0.02$). There were no associations with high-grade disease in men either with or without a family history. For TFA 18:1, there was a significant positive association for low-grade cancer (quartile 4 vs. 1: odds ratio (OR) = 1.70, 95% CI: 1.10, 2.65) ($P_{trend} < 0.01$) among men with a family history of prostate cancer, and there was no association for men without a family history ($P_{interaction} < 0.01$). A high percent serum TFA 18:1 was associated with a significant decreased

**Table 5.** Multivariable-adjusted Associations of Serum Phospholipid Fatty Acids in Relation to Prostate Cancer Risk, Stratified by Grade and Family History, Among Participants in the Prostate Cancer Prevention Trial, 1994–2003

| Fatty Acids, % of total | Family History of Prostate Cancer | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Yes | | | | No | | | |
| | Cases, no. | Controls, no. | OR[a] | 95% CI | Cases, no. | Controls, no. | OR[a] | 95% CI |
| Low-grade cases vs. controls | | | | | | | | |
| Linoleic acid (18:2ω6) | | | | | | | | |
| <18.49 | 66 | 102 | 1.00 | Referent | 297 | 348 | 1.00 | Referent |
| 18.49–20.10 | 87 | 100 | 1.34 | 0.86, 2.07 | 303 | 351 | 0.97 | 0.77, 1.22 |
| 20.11–21.65 | 91 | 94 | 1.40 | 0.91, 2.17 | 288 | 357 | 0.85 | 0.68, 1.07 |
| >21.65 | 96 | 86 | 1.54 | 0.99, 2.40 | 305 | 365 | 0.91 | 0.72, 1.14 |
| $P_{trend}$ | | | | 0.06 | | | | 0.25 |
| $P_{interaction}$ | | | | 0.02 | | | | |
| High-grade cases vs. controls | | | | | | | | |
| Linoleic acid (18:2ω6) | | | | | | | | |
| <18.49 | 3 | 102 | 1.00 | Referent | 32 | 348 | 1.00 | Referent |
| 18.49–20.10 | 8 | 100 | 2.79 | 0.71, 11.04 | 23 | 351 | 0.77 | 0.44, 1.36 |
| 20.11–21.65 | 3 | 94 | 0.98 | 0.19, 5.06 | 27 | 357 | 0.88 | 0.51, 1.53 |
| >21.65 | 5 | 86 | 1.86 | 0.42, 8.23 | 24 | 365 | 0.82 | 0.46, 1.45 |
| $P_{trend}$ | | | | 0.84 | | | | 0.60 |
| $P_{interaction}$ | | | | 0.43 | | | | |
| Low-grade cases vs. controls | | | | | | | | |
| TFA 18:1 | | | | | | | | |
| <1.29 | 84 | 108 | 1.00 | Referent | 302 | 342 | 1.00 | Referent |
| 1.29–1.67 | 74 | 109 | 1.00 | 0.65, 1.55 | 275 | 343 | 0.89 | 0.70, 1.12 |
| 1.68–2.08 | 81 | 83 | 1.40 | 0.89, 2.19 | 315 | 367 | 0.94 | 0.75, 1.19 |
| >2.08 | 101 | 82 | 1.70 | 1.10, 2.65 | 301 | 369 | 0.86 | 0.68, 1.10 |
| $P_{trend}$ | | | | <0.01 | | | | 0.33 |
| $P_{interaction}$ | | | | <0.01 | | | | |
| High-grade cases vs. controls | | | | | | | | |
| TFA 18:1 | | | | | | | | |
| <1.29 | 3 | 108 | 1.00 | Referent | 35 | 342 | 1.00 | Referent |
| 1.29–1.67 | 8 | 109 | 3.70 | 0.88, 15.52 | 29 | 343 | 0.80 | 0.46, 1.35 |
| 1.68–2.08 | 4 | 83 | 2.07 | 0.40, 10.64 | 25 | 367 | 0.67 | 0.38, 1.19 |
| >2.08 | 4 | 82 | 2.19 | 0.42, 11.47 | 17 | 369 | 0.44 | 0.23, 0.84 |
| $P_{trend}$ | | | | 0.61 | | | | 0.01 |
| $P_{interaction}$ | | | | 0.16 | | | | |

Abbreviations: CI, confidence interval; OR, odds ratio; TFA, *trans*-fatty acid.
[a] Adjusted for age, race, diabetes, body mass index, alcohol, and treatment arm.

risk of high-grade cancer in men without a family history (quartile 4 vs. 1: OR = 0.44, 95% CI: 0.23, 0.84) ($P_{trend}$ = 0.01). There was no association among men with a family history of prostate cancer. Nevertheless, these associations were not statistically different. There were no interactions of family history with the remaining phospholipids.

## DISCUSSION

This study found significant associations of inflammation-related phospholipid fatty acids measured in serum with prostate cancer risk, albeit in the directions opposite to those hypothesized. Percent serum DHA above the first quartile was associated with an increased risk of high-grade prostate cancer, and increasing quartiles of TFA 18:1 and 18:2 were inversely associated with risk of high-grade cancer. The remaining fatty acids were not associated with prostate cancer risk. There were some differences in associations stratified by family history of prostate cancer, which are discussed below.

We restrict our review of the previous publications on blood concentrations of fatty acids to those in which blood samples were collected before diagnosis. Several studies

examined long-chain ω-3 fatty acids (11–14, 16–18), and 4 have reported results by grade (11, 12, 14, 18). No associations were reported for total prostate cancer (13, 16, 17). Among studies that analyzed by grade, 2 small studies found no associations (14, 18). The most recent report from the Physicians' Health Study (PHS) ($n_{cases} = 476$) (11) found nonsignificant inverse associations of the percent EPA and DHA, together and separately, with risk of both aggressive and nonaggressive prostate cancers. In the European Prospective Investigation into Cancer and Nutrition (EPIC, $n_{cases} = 962$) (12), the highest quintile of percent DHA was associated with elevated risks of both low-grade (relative risk (RR) = 1.53, 95% CI: 0.96, 2.44) and high-grade (RR = 1.41, 95% CI: 0.76, 2.62) prostate cancer. They also reported significant positive associations of the percent EPA with high-grade prostate cancer (RR = 2.00, 95% CI: 1.07, 3.76). Given that the Prostate Cancer Prevention Trial and the European Prospective Investigation into Cancer and Nutrition, the 2 largest studies of blood levels of phospholipid fatty acids, reported increased risks of high-grade prostate cancer with high levels of ω-3 fatty acids, it remains a possibility that these fatty acids promote tumorigenesis.

Studies of diet, which we and others judge as less informative than studies based on blood because of their reliance on self-report, have not reported inverse associations between ω-3 fatty acid intake and total prostate cancer risk (27, 28), nor has our recent study of fish oil supplement use (29). However, in a recent meta-analysis of fish consumption and prostate cancer, Szymanski et al. (28) reported a large reduction in late stage (RR = 0.56, 95% CI: 0.37, 0.86; $n_{studies} = 1$) or fatal prostate cancer (RR = 0.37, 95% CI: 0.18, 0.74; $n_{studies} = 4$) among cohort studies. No reduction was reported for incidence of high-grade prostate cancer (RR = 1.01, 95% CI: 0.82, 1.23; $n_{studies} = 1$). These results are not necessarily inconsistent with our findings, which are based on cancers that have not yet metastasized, and the possibility remains that there may be an inverse association of fish consumption with late stage or fatal prostate cancer.

Several studies have examined α-linolenic acid in association with prostate cancer risk; however, in this and most other studies (11, 12, 16–18), there were no significant associations. There is one positive finding from a study of 141 cases that found that high levels of α-linolenic acid were associated with a doubling of prostate cancer risk (OR = 2.0, 95% CI: 1.1, 3.6) (14). A positive finding from the Physicians' Health Study (RR = 2.14, 95% CI: 0.93, 4.93) (13) has been superseded by a more recent analysis with more cases, which found no association with prostate cancer risk (RR = 1.31, 95% CI: 0.89, 1.95) (11). No previous study reported differences in the association by grade. Taken together and in support of our findings, α-linolenic acid does not appear to be associated with prostate cancer risk.

Two studies, the β-Carotene and Retinol Efficacy Trial (CARET), a randomized trial of β-carotene and retinol supplements for lung cancer prevention, and the Physicians' Health Study, examined the association of TFA and prostate cancer risk (10, 15). In the β-Carotene and Retinol Efficacy Trial, high levels of TFAs 18:1 and 18:2 were associated with increased risks of both low-grade and high-grade prostate cancer (15); in the Physicians' Health Study, the highest quintiles of TFAs 18:1 (RR = 1.96, 95% CI: 1.01, 3.80) and 18:2 (RR = 1.97, 95% CI: 1.03, 3.75) were associated with increased risks of nonaggressive prostate cancer but not with the risk of aggressive cancer (10). In contrast, we found an inverse association of TFAs 18:1 and 18:2 with high-grade and no association with low-grade prostate cancer. Similar to our study findings, no study found an association of TFA 16 with prostate cancer (10, 15).

Several smaller studies have investigated the association of the proinflammatory ω-6 fatty acids with prostate cancer risk; similar to our finding, none found an association with arachidonic acid (11–14, 16–18). Two (11, 16) of 7 studies (12–14, 17, 18) reported associations between linoleic acid and prostate cancer risk. One study reported an inverse association that did not differ by grade (tertile 3 vs. tertile 1: RR = 0.28, 95% CI: 0.12, 0.68); howeve, the number of cases was small ($n_{cases} = 46$) (16). The Physicians' Health Study found that high levels of linoleic acid were associated with significant reductions of aggressive prostate cancer risk (RR = 0.38, 95% CI: 0.17, 0.86) (11). In agreement with the remaining studies (12–14, 17, 18), our study found no association between linoleic acid and prostate cancer risk.

To our knowledge, no previous studies have examined effect modification of fatty acids stratified by family history of prostate cancer. The differences in associations that we observed for linoleic acid and TFA 18:1 by family history are nevertheless intriguing. It is possible that genetic characteristics associated with a family history of prostate cancer modify the associations of these fatty acids with prostate cancer risk; however, we had no strong a priori hypothesis when completing this analysis, and the finding may be due to chance. As with any exploratory results, replication in other studies is needed.

The most striking aspect of our findings is that they were not in the directions hypothesized. We hypothesized that the ω-3 fatty acids would be associated with reductions in prostate cancer risk, while the ω-6 fatty acids would increase risk. EPA and DHA, found in fatty fish and in fish oil supplements, are hypothesized to reduce cancer risk through their antiinflammatory and immunomodulatory properties (8, 30). They have also been shown to affect cell permeability, gene expression, and signal transduction (31). The effects of these pathways on prostate carcinogenesis are not fully understood. Although we are unaware of a proposed mechanism by which EPA or DHA would be procarcinogenic, in a previous analysis of dietary ω-3 fatty acids in the Prostate Cancer Prevention Trial, we also observed elevated risks of high-grade prostate cancer (quartile 4 vs. quartile 1: OR = 1.52, 95% CI: 0.89, 2.58) (32). *trans*-Fats, found in food products which contain hydrogenated vegetable oils and in ruminant animals (33), have been associated with increased risk of cardiovascular disease (34). There is some evidence that TFAs exhibit proinflammatory effects and therefore may promote carcinogenesis (9). With the exception of results in men with a family history of prostate cancer, high levels of TFA 18:1 were associated with reductions in high-grade prostate cancer and were not associated

with low-grade cancer. We know of no evidence suggesting anticancer properties of *trans*-fats.

We considered the possibility that the unexpected directions of our findings reflected the unique nature of the Prostate Cancer Prevention Trial design and cancer endpoints. The Prostate Cancer Prevention Trial did not use biopsy-determined absence of prostate cancer as an eligibility criterion, and thus cancers may have been prevalent at baseline. If DHA decreased the development of metastases, then men with high DHA levels would have more prevalent disease. However, all participants had a prostate-specific antigen of <3.0 ng/mL at baseline, among whom prevalent high-grade cancer is rare (35). Further, there were no associations of baseline prostate-specific antigen with DHA ($r = 0.04$), TFA 18:1 ($r = -0.00$), or TFA 18:2 ($r = -0.03$). It seems unlikely that a higher prevalence of high-grade disease at baseline among men with high levels of DHA, or a lower prevalence among men with high levels of TFAs, could explain our findings.

This study has several strengths. It is the largest prospective study to examine the association of circulating fatty acids and prostate cancer risk. The absence or presence of cancer was determined by prostate biopsy, which reduced the probability of disease misclassification. Measurement error due to intraindividual variability in fatty acid concentration was further reduced by pooling 2 blood draws.

The primary limitation of the Prostate Cancer Prevention Trial is that almost all cases were local stage, and many would likely have never been diagnosed by standard clinical practice. It is important to note that most significant associations were for risk of clinically relevant, high-grade cancer only, which was defined very conservatively as a Gleason score of 8–10. In a sensitivity analysis, we examined associations by using other definitions for prostate cancer grade. Despite smaller sample sizes, associations with high-grade tumors were stronger when they were defined as Gleason scores 8–10, compared with Gleason scores 7–10 or Gleason (4 + 3) plus 8–10. Thus, our findings for high-grade cancer are specific to the most clinically relevant, localized disease. An additional limitation is that fatty acids were parameterized as a proportion rather than a concentration. When expressed as a proportion, a positive association with one fatty acid could lead to a falsely inverse association with another (36). However, when all the fatty acids examined were included in a single model, the results did not change.

In conclusion, this large prospective investigation of inflammation-associated phospholipid fatty acids and prostate cancer risk found no support that ω-3 fatty acids reduce or *trans*-fatty acids increase prostate cancer risk. Indeed, our findings are disconcerting as they suggest that ω-3 fatty acids, considered beneficial for coronary artery disease prevention, may increase high-grade prostate cancer risk, whereas *trans*-fatty acids, considered harmful, may reduce high-grade prostate cancer risk. These findings illustrate the complexity of research on nutrition and chronic disease risk, in which the effects of nutrients may differ across multiple diseases. A comprehensive understanding of the effects of nutrients on a broad range of diseases will be necessary before making recommendations for dietary changes or use of individual dietary supplements for disease prevention.

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