

## Phase II Prospective Randomized Trial of a Low-Fat Diet with Fish Oil Supplementation in Men Undergoing Radical Prostatectomy

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### Abstract

Preclinical studies suggest lowering dietary fat and decreasing the ratio of omega-6 to omega-3 polyunsaturated fatty acids decreases the risk of prostate cancer development and progression. We conducted a phase II randomized trial to test the effect of decreasing dietary fat combined with decreasing the dietary omega-6:omega-3 ratio on biomarkers related to prostate cancer development and progression. Patients undergoing radical prostatectomy were randomly assigned to receive a low-fat diet with 5 grams of fish oil daily (dietary omega-6:omega-3 ratio of 2:1) or a control Western diet (omega-6:omega-3 ratio of 15:1) for four to six weeks prior to surgery. The primary endpoint was change in serum insulin-like growth factor I (IGF-1) between arms. Secondary endpoints were serum IGFBP-1, prostate prostaglandin E2 levels, omega-6:omega-3 fatty acid ratios, COX-2, and markers of proliferation and apoptosis. Fifty-five patients were randomized and 48 completed the trial. There was no treatment difference in the primary outcome. Positive secondary outcomes in the low-fat fish oil versus Western group were reduced benign and malignant prostate tissue omega-6:omega-3 ratios, reduced proliferation (Ki-67 index), and reduced proliferation in an *ex vivo* bioassay when patient sera was applied to prostate cancer cells *in vitro*. In summary, four to six weeks of a low-fat diet and fish oil capsules to achieve an omega-6:omega-3 fatty acid ratio of 2:1 had no effect on serum IGF-1 levels, though in secondary analyses, the intervention resulted in decreased prostate cancer proliferation and decreased prostate tissue omega-6:omega-3 ratios. These results support further studies evaluating reduction of dietary fat with fish oil supplementation on modulating prostate cancer biology. *Cancer Prev Res*; 4(12); 2062–71. ©2011 AACR.

### Introduction

Preclinical studies utilizing xenografts and genetically engineered mouse models showed that reducing dietary fat and decreasing the omega-6 to omega-3 fatty acid ratio delays the development and progression of prostate cancer

(1–5). Epidemiologic studies also found that a high-fat diet and low intake of fish and marine-derived omega-3 fatty acids were associated with increased risk of developing prostate cancer and increased risk of advanced disease (6–12), though other reports do not support this association (13–15). Other studies found increased intake of fish and marine-derived omega-3 fatty acids was associated with decreased prostate cancer mortality (16, 17). Studies have been mixed with regard to the relationship between circulating marine-derived omega-3 fatty acid levels and prostate cancer risk with one showing a negative association (18), others showing a positive association with high-grade prostate cancer (19, 20) and others showing no association (15, 21, 22). The main mechanisms underlying the purported anticancer effects of modulating dietary fat seem to be through reduced insulin-like growth factor (IGF) signaling (5, 23, 24) and alterations of membrane omega-6 to omega-3 fatty acid ratios leading to suppressed COX-2-dependent PGE2 production, though other mechanisms may also be involved (1, 4, 25, 26).

The aim of this preprostatectomy trial was to examine the effects of modulating dietary fat and the omega-6/omega-3 fatty acid ratio in men with prostate cancer on the

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

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IGF/IGFBP system and the COX-2/PGE2 pathways. To obtain a dietary omega-6 to omega-3 fatty acids ratio of 2:1, we combined dietary fat reduction with fish oil capsule supplementation. Other endpoints examined in this trial (and established in preclinical models) were fatty acid ratios in prostate tissue membranes and markers of angiogenesis, proliferation, and apoptosis (4, 5, 24). This trial was designed to establish whether modulating dietary fat and the dietary omega-6 to omega-3 fatty acid ratio alters prostate cancer biomarkers and may, therefore, support the conduct of large-scale prospective trials incorporating dietary fat modulation.

## Patients and Methods

### Patients

Participants were recruited from the urology clinics at the Veterans Administration Greater Los Angeles Healthcare System, UCLA, and Santa Monica UCLA from 2005 to 2008. Participants were required to have a diagnosis of clinically localized prostate adenocarcinoma and scheduled to undergo radical prostatectomy at least 4 weeks from study entry. The diagnostic needle biopsy was required to have more than 5% cancer in one core or to have more than 1 core with cancer to increase the likelihood of having prostate cancer tissue for experimental studies. Subjects needed to be willing to stop nutritional supplements and herbal therapies (i.e., lycopene, selenium, vitamin E, fish oil, and saw palmetto), and medications that inhibit the COX-2 pathway (i.e., aspirin, nonsteroidal anti-inflammatory agents) at least 1-week before starting the intervention. Subjects were ineligible if they were on insulin or ever took 5-alpha reductase inhibitors, antiandrogens, or LHRH agonists. The study was approved by the Institutional Review Board.

### Study design

This was a phase II, randomized trial designed to study intermediate biological endpoints. This trial is registered with ClinicalTrials.gov (# NCT00836615). All subjects signed informed consent documents prior to study entry. Randomization (1:1) was by a permuted random block design and prerandomization stratification was done by study site. Study duration was 4 to 6 weeks. The Western diet provided 40% kcal from fat, 15% kcal from protein, and 45% kcal from carbohydrates (15 grams of fiber/d) and the dietary omega-6:omega-3 fatty acid ratio was 15:1. The low-fat/fish oil diet provided 15% kcal from fat, 15% kcal from protein, 70% kcal from carbohydrates (39 grams of fiber/d), and subjects took five 1.1-gm fish oil capsules daily, bringing the dietary ratio of omega-6:omega-3 fatty acids to 2:1. Subjects were instructed to consume 3 fish oil capsules with breakfast and 2 with dinner. The fish oil capsules were provided by Pharmavite (Northridge, CA). Each 1.1-gm capsule contained 200 mg eicosapentaenoic acid and 367 mg docosahexaenoic acid. The same lot of fish oil capsules was used throughout the study. The research dietitian designed the dietary intervention for each patient in both

arms to maintain patient weight. To tightly control the dietary components in both arms, all meals were prepared by the UCLA General Clinical Research Center nutrition staff. Packaged meals were delivered to homes of patients in coolers 2 d/wk and 1 d/wk subjects picked up the packaged meals and returned uneaten food which was weighed by the dietary staff. Compliance was determined by the research dietitian on the basis of weekly meetings with subjects, daily food checklists completed by subjects, and measurement of uneaten foods. Body weight was measured weekly and caloric intake was adjusted when weight changes exceeded 1 kg/wk. Fish oil capsule compliance was measured by pill counts every 2 weeks. Participants were asked to maintain their usual physical activity level throughout the study.

At baseline, all subjects received a history and physical examination and completed a 3-day food diary. At baseline and within 5 days of the radical prostatectomy, all subjects had urine and fasting AM blood collected and bioelectrical impedance was conducted to determine body composition.

### Outcomes

The primary objective was to compare the change between the baseline and presurgery fasting serum IGF-1 levels between arms. Secondary endpoints measured in fresh frozen radical prostatectomy tissue and compared between arms were benign and malignant prostate tissue membrane omega-6:omega-3 fatty acid ratios and PGE2 levels. Other secondary endpoints compared between arms were prostate cancer tissue proliferation (Ki-67), apoptosis [terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL)], COX-2, and angiogenesis (CD31), urine prostaglandin E2 (PGE2) metabolite (PGEM) levels (a stable metabolite of PGE2; ref. 27), peripheral red blood cell membrane omega-6:omega-3 fatty acid ratios, serum-stimulated growth, and proliferation of 22RV1 cells in an *ex vivo* bioassay (24), fasting serum IGF1, IGF1BP-3, insulin, lipids, and PSA levels.

### Fatty acids analysis

Fatty acid analysis was done on membrane preparations of red blood cells and fresh frozen benign and malignant radical prostatectomy tissue using gas chromatography after formation of fatty acid methyl esters (28). Our method measures phospholipids and cholesteryl esters. The intra-assay coefficients were less than 6% for all fatty acids analyzed. The interassay coefficients were less than 10% for all fatty acids analyzed, except for palmitoleic and linolenic acids for which the interassay coefficient was less than 12.5%.

### Prostaglandin E2 and PGEM ELISAs

PGE2 levels in extracts from fresh frozen prostate tissue specimens were measured in duplicate by EIA following the manufacturer protocol (Assay Designs Inc.). The intra- and interassay coefficient of variation were 6.1% and 12.6%, respectively. The total protein concentration was assessed to standardize the results. Urinary PGEM measurements (Cayman Chemical Company) relative to

urinary creatinine (Assay Designs Inc.) levels were done in triplicates with EIA assays following manufacturer protocols. PGEM EIA intra- and interassay coefficients of variation were 5.5% and 11.2%, respectively. The creatinine EIA intra- and interassay coefficients of variation were 3.2% and 2.2%, respectively.

### Ex vivo bioassay

The *ex vivo* bioassay measuring 22RV1 proliferation in pre- and postintervention patient serum was carried out twice in duplicate. 22RV1 cells were obtained from American Type Culture Collection and grown in RPMI medium without phenol red (Invitrogen) supplemented with 10% FBS, 100 IU penicillin, 100 µg/mL streptomycin, 10 mmol/L HEPES. 22RV1 cells cultures were maintained at 37°C and supplemented with 5% CO<sub>2</sub> in a humidified incubator. The mitogenic effect of human serum on 22RV1 proliferation was studied using an in-house bioassay. The cells were plated at  $5 \times 10^3$  cells per well in 96-well plate and incubated for 24 hours before changing to fresh media containing 10% study subject serum or 10% FBS (control). The cell proliferation in media containing study subject serum or FBS was measured using the bromodeoxyuridine (BrdU) cell proliferation assay kit. BrdU was added for the last 4 hours of the 48-hour incubation and uptake measured per manufacturer's instructions (Millipore). The intra-assay and interassay coefficients of variation were 8.4% and 10%, respectively.

### Serum analysis

Serum IGF-1, IGFBP-1, and IGFBP-3 levels were measured in triplicate using an in-house ELISA assay (29). The intra-assay coefficients of variation for human IGF-1, IGFBP-1, and IGF-BP3 are <10%, <10%, and <6%, respectively. The interassay coefficients of variation for human IGF-1, IGFBP-1, and IGF-BP3 are <10%, <10%, and <8%, respectively. PSA, lipids, and insulin were measured in the UCLA clinical laboratory.

### Prostate tissue harvesting

Following surgical removal, the prostate was immediately placed in a container surrounded by crushed ice. Within 10 to 15 minutes the prostate was inked and serially sectioned (typically into 5 levels for the average prostate). Levels 2 and 4 were used for research purposes and each divided into 6 blocks, embedded in OCT (Tissue-Tek; Sakura Finetek), and stored at -80°C. The other sections were fixed in 10% neutral buffered formalin for whole-mount embedding in paraffin blocks. Cryostat sections of the OCT blocks were evaluated by the pathologist and areas of adenocarcinoma circled. Circled areas and adjacent areas of benign tissue were macrodissected from the OCT blocks for fatty acid and PGE2 measurements.

### Immunohistochemistry

Serial sections for immunohistochemical analysis were cut from paraffin embedded blocks with the largest

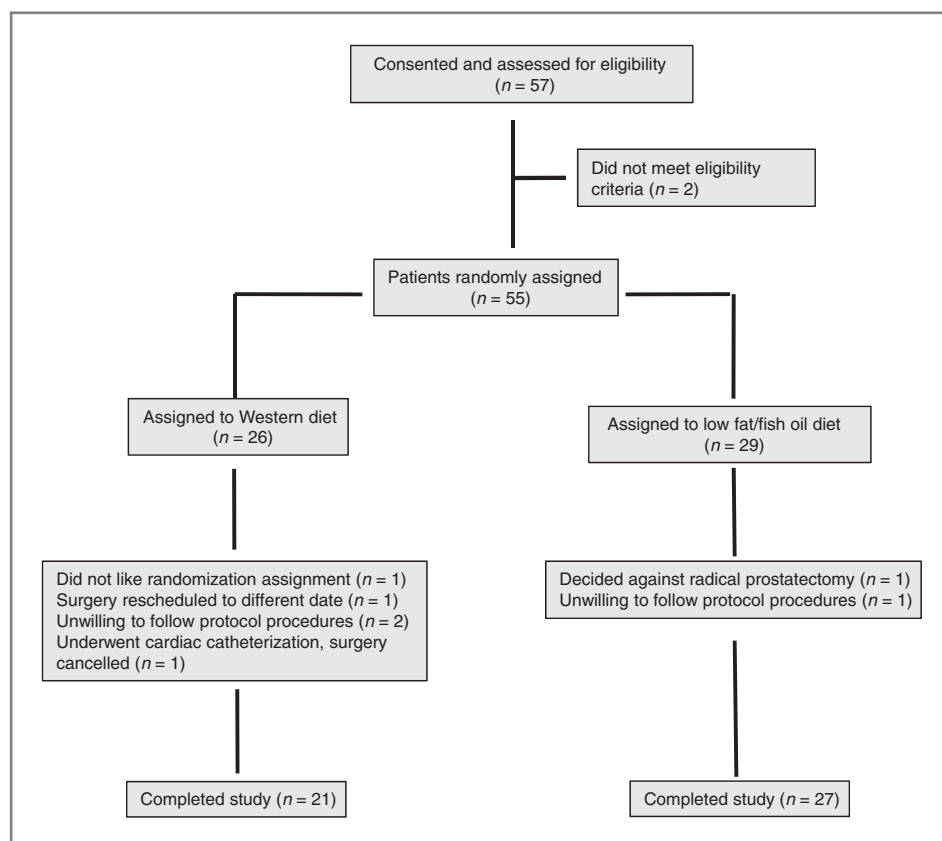


Figure 1. CONSORT diagram.

cancer volume and with the Gleason grade corresponding to the grade on the final pathology report. Slides were stained for Ki-67 and TUNEL (4), the areas of adenocarcinoma were circled by the study pathologist (J.S) and digitally scanned using the Ariol SL-50 high-throughput scanning system (Applied Imaging) in the Translational Pathology Core Laboratory (TPCL) at UCLA. The cancerous glands within the areas of adenocarcinoma were circled by the study pathologist (J.S), and the Ariol software was trained to score Ki-67 or TUNEL-positive cells within the circled glands. The number of Ki-67 or TUNEL-stained nuclei per 1,000 nuclei scored was used to calculate the percent of positive stained cells (30).

For angiogenesis, the number of CD31-stained vessels were visually counted in five 20× fields per specimen. COX-2 staining was visually scored by the same pathologist for percent of cancer epithelial cells with positive staining (1 = 5%–25%, 2 = 26%–50%, 3 = 51%–75%, and 4 = 76%–100%) and intensity of COX-2 staining was scored on a scale of 1 to 3 (1 = low, 2 = medium, and 3 = high intensity; ref. 31) The COX-2 immunoreactivity score was calculated by multiplying the percent of cells staining positive by the intensity score (32). In all cases the pathologist was blinded to treatment arm.

#### Statistical analysis

A sample size of 70 (35 per group) was estimated to provide 80% power to detect a 20% difference in between group changes in serum IGF-1 levels with a 2-sided alpha of 0.05. This power calculation was based on results from a prior intervention (24).

#### Primary analysis

After 48 subjects fully completed the trial, an interim analysis was done to evaluate the primary outcome. We conducted a conditional power analysis simulation and estimated that, with completion of enrollment to the trial, there was only a 7% chance of finding a significant difference in the mean change of IGF-1 levels between groups. Therefore, study enrollment was closed and secondary endpoint analyses were done.

Baseline subject characteristics were compared between groups using *t* tests or Fisher's exact tests. Secondary outcomes were either measured only at surgery or operationalized as the change from baseline to surgery. These outcomes were then compared between groups using *t* tests. For outcomes that had skewed distributions  $\log(x+1)$  transformation was used. *P* values less than 0.05 were considered significant. The data are presented as mean ± SD or SEM where appropriate.

Linear regression models were constructed to examine the effect of covariates on the log-transformed Ki-67 outcome. Factors included race, the change in weight during the study, and the biopsy or radical prostatectomy Gleason grade which was separately analyzed as the Gleason sum or as a categorical variable with both the primary and secondary Gleason scores.

## Results

### Baseline characteristics

Between August 2005 and November 2008, fifty-five subjects signed consent forms and were randomized. Twenty-six subjects were randomized to the Western diet group and 29 to the low-fat/fish oil group. Of these 55 subjects, 7 withdrew from the study and 48 completed the trial (Fig. 1). The baseline characteristics of these 48 subjects are shown

**Table 1.** Research subject baseline characteristics

|                                     | Western diet<br>(n = 21) | Fish oil diet<br>(n = 27) |
|-------------------------------------|--------------------------|---------------------------|
| Ethnicity                           |                          |                           |
| Caucasian (no.)                     | 10                       | 21                        |
| Black American (no.)                | 9                        | 6                         |
| Hispanic (no.)                      | 2                        | 0                         |
| Age (y), mean ± SD                  | 60.4 ± 6.7               | 60.5 ± 6.3                |
| Weight (kg), mean ± SD              | 91 ± 19.1                | 92.5 ± 13.1               |
| BMI (kg/m <sup>2</sup> ), mean ± SD | 29 ± 4.2                 | 29.8 ± 3.8                |
| Percent body fat,<br>mean ± SD      | 25.9 ± 4.7               | 29.6 ± 3.1                |
| IGF-1 (ng/mL),<br>mean ± SD         | 120.4 ± 47.9             | 141.8 ± 42.9              |
| IGFBP-1(ng/mL),<br>mean ± SD        | 15.9 ± 15.5              | 11.3 ± 13.2               |
| IGFBP- 3 (ng/mL),<br>mean ± SD      | 2,166 ± 617              | 2,181 ± 579.9             |
| PSA (ng/mL)                         |                          |                           |
| Mean                                | 7.6                      | 6.9                       |
| Range                               | 2.1–28.1                 | 1.4–22.3                  |
| SD                                  | 5.6                      | 4.9                       |
| Median                              | 6.3                      | 5.3                       |
| Gleason sum at diagnosis            |                          |                           |
| 6                                   |                          |                           |
| No.                                 | 8                        | 16                        |
| %                                   | 40                       | 59.3                      |
| 7                                   |                          |                           |
| No.                                 | 11                       | 9                         |
| %                                   | 55                       | 33.3                      |
| 3+4                                 |                          |                           |
| No.                                 | 7                        | 7                         |
| %                                   | 35                       | 25.9                      |
| 4+3                                 |                          |                           |
| No.                                 | 5                        | 2                         |
| %                                   | 25                       | 7.4                       |
| 8–9                                 |                          |                           |
| No.                                 | 1                        | 2                         |
| %                                   | 5                        | 7.4                       |
| No. of positive cores               |                          |                           |
| Mean                                | 4                        | 3.7                       |
| Range                               | 1–12                     | 1–10                      |
| SD                                  | 2.5                      | 2.6                       |
| Median                              | 4                        | 3                         |

in Table 1. The majority of patients in both groups were either overweight or obese. The median PSA in the Western diet group was 6.25 ng/mL (range: 2.1–28.1 ng/mL) and the median PSA in the low-fat/fish oil group was 5.30 ng/mL (range: 1.4–22.3 ng/mL). The mean intake of fat, carbohydrates, and protein at baseline (calculated from the 3-day food records) for the group as a whole and expressed as a percentage of total energy intake was  $35\% \pm 8\%$ ,  $44\% \pm 10\%$ , and  $19\% \pm 5\%$ , respectively, and the mean omega-6:omega-3 fatty acid ratio at baseline for the group as a whole was  $9.6 \pm 4$  to 1.

### Treatment duration and compliance

Subjects remained on the diet intervention prior to prostatectomy for a mean of  $27.7 \pm 0.5$  days in the Western diet group and  $30.2 \pm 1.9$  days in the low-fat/fish oil group. Subjects in both groups were compliant with the dietary intervention with  $94.8\% \pm 5.0\%$  of prepared food consumed in the Western diet group, and  $89.5\% \pm 8.3\%$  of prepared food consumed in the low-fat/fish oil group. Subjects were also compliant with consuming fish oil capsules with greater than 95% of capsules consumed based on pill counts.

### Biomarker results

The anthropometrics and changes in fasting serum biomarkers and urine PGEM levels are shown in Table 2. There were no statistically significant between-group changes in serum IGF-1 levels (the primary endpoint). There were also no statistically significant changes in serum IGFBP-1, IGFBP-3, insulin, and PSA and no changes in urine PGEM levels. Triglyceride and cholesterol levels were significantly reduced in the low-fat/fish oil group versus the Western diet

group. There was a trend for increased weight loss in the low-fat/fish oil group versus the Western diet group ( $P = 0.06$ ). There was a greater reduction in 22RV1 cell proliferation (measured by BrdU incorporation) in media containing postintervention versus preintervention patient serum in the low-fat/fish oil group versus the Western diet group ( $-5.0 \pm 1.8\%$  vs.  $0.6 \pm 1.9\%$ ,  $P = 0.039$ ).

There was a significant decrease in mean levels of omega-6 fatty acids, an increase in levels of total omega-3 fatty acids, and a decrease in the omega-6:omega-3 fatty acid ratio in benign and malignant prostate tissue membranes (Fig. 2 and Table 3) and in red blood cell membranes (Supplementary Table S1) from subjects consuming the low-fat/fish oil diet versus the Western diet.

### Immunohistochemistry and tissue prostaglandin E2 levels

There was a significant 32.2% decrease in malignant epithelial cell proliferation (Ki-67) in the low-fat/fish oil group versus the Western diet group (Fig. 3). After controlling for race, change in weight, and Gleason grade (prostate needle biopsy or radical prostatectomy) either as the sum or the primary and secondary scores, we found that the intervention effect on Ki-67 remained significant ( $P < 0.05$ ). There was no significant difference in mean PGE2 levels in benign or malignant prostate tissue between treatment groups and no difference in COX-2, angiogenesis (CD-31), or apoptosis (TUNEL) immunostaining (Supplementary Fig. S1).

### Safety

Overall the diet interventions were well tolerated and all adverse events were grade 1. In the low-fat/fish oil

**Table 2.** Changes in serum and urine biomarkers and anthropometrics

|                                | Western diet<br>post-intervention<br>minus pre-intervention | Fish oil diet<br>post-intervention<br>minus pre-intervention | P           |
|--------------------------------|---|--|-------------|
| Body weight (kg)               | $-1.7 \pm 0.3$  | $-2.5 \pm 0.3$   | 0.06        |
| % of body fat                  | $-0.5 \pm 0.4$  | $-0.76 \pm 0.3$  | 0.84        |
| % of lean body mass            | $-0.5 \pm 0.4$  | $-0.8 \pm 0.3$   | 0.58        |
| IGF-1 (ng/mL)                  | $-0.4 \pm 4.3$  | $8.8 \pm 6.2$  | 0.25        |
| IGF BP-1 (ng/mL)               | $3.3 \pm 1.9$   | $3.8 \pm 1.6$  | 0.84        |
| IGF BP-3 (ng/mL)               | $16.4 \pm 35.3$   | $-62.8 \pm 37.5$   | 0.14        |
| Cholesterol (mg/dL)            | $-10.4 \pm 4.4$   | $-31.7 \pm 7.0$  | <b>0.02</b> |
| LDL (mg/dL)                    | $-4.1 \pm 3.4$  | $-15.2 \pm 7.2$  | 0.22        |
| HDL (mg/dL)                    | $-2.9 \pm 1.5$  | $-5.2 \pm 1.1$   | 0.24        |
| Triglycerides (mg/dL)          | $-13.6 \pm 14.8$  | $-56.5 \pm 12.5$   | <b>0.03</b> |
| Cholesterol<br>non-HDL (mg/dL) | $3.5 \pm 5.4$   | $-18.1 \pm 10.3$   | 0.13        |
| PSA (mg/mL)                    | $-0.09 \pm 0.3$   | $0.08 \pm 0.4$   | 0.53        |
| Insulin ( $\mu$ U/mL)          | $-0.1 \pm 1.2$  | $-2.7 \pm 1.2$   | 0.17        |
| PGEM/creatinine ratio          | $0.3 \pm 1.5$   | $-2.1 \pm 2$   | 0.36        |

NOTE: The data represent the mean  $\pm$  SEM for each diet group.

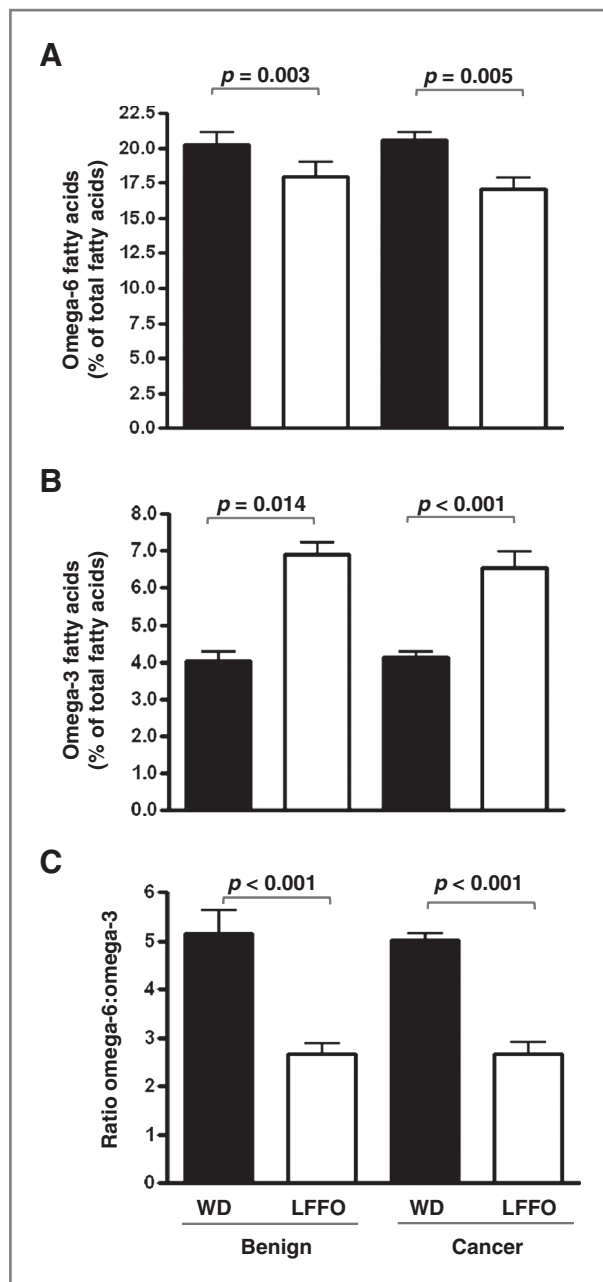


Figure 2. Effect of a low-fat/fish oil diet and Western diet on omega-3 and omega-6 fatty acid levels in benign (WD: n=18, LFFO: n=19) and malignant (WD: n=7, LFFO: n=6) prostate tissue obtained from postintervention radical prostatectomy specimens. A, omega-6 fatty acid levels in benign and malignant prostate membranes are expressed as a percent of total fatty acids. B, omega-3 fatty acids levels in benign and malignant prostate membranes are expressed as a percent of total fatty acids. C, omega-6:omega-3 fatty acid ratio in benign and malignant prostate tissue membranes. In all graphs the data are presented as the mean  $\pm$  SEM for each diet group. Statistical significance was assessed using unpaired *t* test. WD, Western diet; LFFO, low-fat fish oil diet.

group 5 subjects reported increased flatulence, 2 reported diarrhea that was self limited, and 1 reported eructation (burping). In the Western diet group, adverse events reported were increased frequency of bowel movements

in 1 subject, flatulence in 1 subject, and constipation in 1 subject.

## Discussion

On the basis of preclinical studies showing that decreasing dietary fat and decreasing the omega-6:omega-3 fatty acid ratio inhibits carcinogenesis and prostate cancer progression (1, 4, 5, 23), we conducted a prospective randomized phase II trial to evaluate biomarkers associated with prostate cancer development and progression. The rationale for the primary endpoint of this trial (between group change in serum IGF-1 levels) was based on prior preclinical studies suggesting decreased dietary fat and omega-6 fatty acid intake decreased prostate cancer development and progression through a reduction in serum IGF-1 levels (4, 24). In this trial, we found no significant change in serum IGF-1, IGFBP-1, or insulin levels. Several other dietary intervention trials incorporating dietary fat reduction also showed no change in serum IGF-1 or IGFBP-1 levels (33–37). In prior clinical trials, decreased IGF-1 and increased IGFBP-1 levels were typically found in the setting of significant weight loss or reduction in serum insulin (24, 38–40). Subjects in this study did not have significant weight loss or reduction in serum insulin, which might explain the lack of effect on IGF-1 and IGFBP-1 levels.

Although we found no effect on the primary outcome, we did find significant effects in some secondary analyses. Malignant epithelium proliferation, as measured by Ki-67 immunostaining, was significantly reduced in the low-fat/fish oil group versus the Western diet group. Ki-67 immunostaining has been shown to independently predict recurrence after radical prostatectomy and prostate cancer-specific survival (41–44). The mechanism through which the dietary intervention affected malignant epithelium proliferation is unknown. The fact that there were no changes in the serum IGF axis parameters and no change in tissue COX-2 and PGE2 levels suggests that the intervention targeted other pathways involved in proliferation. The finding that the low-fat/fish oil intervention reduced serum-stimulated proliferation of 22RV1 cells in an *ex vivo* bioassay suggests that alterations in serum growth factors may be responsible for the reduced proliferation seen in the tissue. Potential targets affected by the low-fat/fish oil intervention include eicosanoid synthesis pathways and expression of inflammatory cytokines (26, 45). Given the well-established association of Ki-67 and prostate cancer progression, and the impact of the low-fat/fish oil intervention on Ki-67, future trials are warranted for evaluating whether altering dietary fat and the dietary omega-6:omega-3 ratio favorably alters proliferation and other clinical prostate cancer endpoints.

A novel finding in this trial was that reducing dietary fat and the omega-6:omega-3 fatty acid ratio resulted in significant changes in the fatty acid levels in benign and malignant prostate tissue membranes. Subjects in the low-fat/fish oil group had lower omega-6 levels and higher omega-3 levels in the prostate tissue membranes relative to the Western diet group. The ratio of omega-6:omega-3 fatty

**Table 3.** Fatty acid levels in benign and malignant prostate tissue membranes from radical prostatectomy specimens

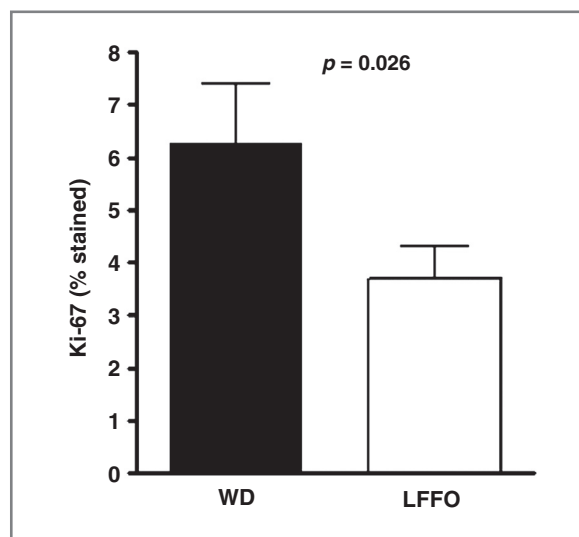
| Fatty acids            | Benign prostate tissue   |                      |                  | Malignant prostate tissue |                     |                  |
|------------------------|--------------------------|----------------------|------------------|---------------------------|---------------------|------------------|
|                        | Western diet<br>(n = 18) | Fish oil<br>(n = 19) | P                | Western diet<br>(n = 7)   | Fish oil<br>(n = 6) | P                |
|                        | mean ± SEM               | mean ± SEM           |                  | mean ± SEM                | mean ± SEM          |                  |
| Palmitic               | 30.42 ± 0.87             | 30.02 ± 0.49         | 0.69             | 30.05 ± 0.38              | 31.06 ± 0.36        | 0.08             |
| Palmitoleic            | 1.43 ± 0.38              | 1.33 ± 0.24          | 0.82             | 0.81 ± 0.11               | 0.75 ± 0.12         | 0.73             |
| Stearic                | 18.74 ± 1.09             | 18.32 ± 0.99         | 0.78             | 20.07 ± 0.77              | 21.13 ± 0.68        | 0.33             |
| Oleic                  | 25.63 ± 1.78             | 26.90 ± 1.55         | 0.59             | 23.59 ± 1.32              | 22.47 ± 1.46        | 0.58             |
| LA (18:2, n-6)         | 11.23 ± 0.75             | 10.50 ± 0.57         | 0.44             | 12.11 ± 0.46              | 9.73 ± 0.48         | <b>0.005</b>     |
| α-linolenic (n-3)      | 0.67 ± 0.13              | 0.57 ± 0.08          | 0.50             | 0.18 ± 0.02               | 0.18 ± 0.02         | 0.98             |
| Eicosadienoic (n-6)    | 0.72 ± 0.07              | 0.72 ± 0.13          | 0.98             | 0.82 ± 0.06               | 1.00 ± 0.17         | 0.33             |
| AA (20:4, n-6)         | 7.11 ± 0.58              | 5.85 ± 0.44          | 0.09             | 8.44 ± 0.60               | 7.32 ± 0.68         | 0.24             |
| EPA (20:5, n-3)        | 0.10 ± 0.02              | 0.42 ± 0.05          | <b>&lt;0.001</b> | 0.07 ± 0.01               | 0.44 ± 0.07         | <b>&lt;0.001</b> |
| Docosapentaenoic (n-3) | 0.70 ± 0.07              | 0.81 ± 0.33          | 0.33             | 0.85 ± 0.06               | 0.90 ± 0.08         | 0.58             |
| DHA (22:6, n-3)        | 3.25 ± 0.30              | 4.55 ± 0.38          | <b>0.011</b>     | 3.01 ± 0.13               | 5.03 ± 0.39         | <b>&lt;0.001</b> |
| Total n-6              | 18.34 ± 0.50             | 16.35 ± 0.41         | <b>0.003</b>     | 20.5 ± 0.57               | 17 ± 0.86           | <b>0.005</b>     |
| Total n-3              | 4.72 ± 0.38              | 6.35 ± 0.50          | <b>0.014</b>     | 4.11 ± 0.15               | 6.54 ± 0.43         | <b>&lt;0.001</b> |
| n-6/n-3                | 4.48 ± 0.52              | 3.07 ± 0.42          | <b>0.042</b>     | 5.02 ± 0.46               | 2.68 ± 0.24         | <b>&lt;0.001</b> |

NOTE: The fatty acids were measured in postintervention radical prostatectomy tissue. Fatty acid content is expressed as a percent of total fatty acids. n-3, omega-3; n-6, omega 6; LA, linoleic acid; AA, arachidonic acid; EPA, Eicosapentaenoic acid, DHA, docosahexaenoic acid.

acid levels in cell membranes is believed to play an important role in signaling pathways leading to prostate cancer development and progression (1, 4, 25, 26, 45, 46). In prior xenograft studies, decreasing the dietary omega-6:omega-3 fatty acid ratio resulted in a reduction in the omega-6:omega-3 fatty acid ratio in the xenograft membranes and decreased COX-2 and PGE2 levels (2, 4). PGE2 is known to increase prostate cancer proliferation, invasiveness, and angiogenesis (26, 45, 47). Whereas altering the dietary omega-6:omega-3 fatty acid ratio in our trial led to alteration in the fatty acid composition of benign and malignant prostate tissue membranes, there was no significant difference in COX-2 or PGE2 levels in prostate tissue and no difference in urinary PGEM levels, a stable metabolite of PGE2 (27). In a 3-month trial evaluating 3 grams of fish oil per day (without modifying dietary fat) in men on expectant management, Chan and colleagues found no change in COX-2 expression in prostate tissue (48). Berquin and colleagues previously showed that a high omega-3 diet delayed the development and progression of prostate cancer in a prostate-specific PTEN knockout mouse model (1). In their model, they saw increased apoptosis in the omega-3 fed group, possibly through effects on bad phosphorylation. In the present trial, altering the omega-6:omega-3 fatty acid ratio in humans did not impact on apoptosis in malignant tissue.

One shortcoming of this trial is the short duration of the intervention. In our experience, patients that elect to undergo radical prostatectomy generally desire their surgery within 1 to 2 months, and it is not feasible to enroll large

numbers of patients for a longer duration. A potential criticism of this trial is that dietary fat reduction alone or intake of fish oil capsules without dietary fat reduction



**Figure 3.** Effect of a low-fat/fish oil diet compared with a Western diet on Ki-67 expression in malignant epithelial prostate cells in postintervention radical prostatectomy specimens (WD: n=21, LFFO: n=22). Ki-67 immunostaining was digitally quantified using Ariol software and expressed as percent of positive cells. The data represent the mean ± SEM for each diet group. The Ki-67 results had a skewed distribution and were therefore log-transformed using log(x+1) for statistical analysis. Statistical significance was assessed using unpaired t test. WD, Western diet; LFFO, low-fat fish oil diet.

was not tested. As such, we cannot discern whether either treatment alone would have affected proliferation or whether the combination of dietary fat and fish oil supplementation is required. Demark-Wahnefried and colleagues previously found no effect of dietary fat reduction on proliferation (Ki-67) in men undergoing radical prostatectomy, suggesting that dietary fat reduction alone does not alter proliferation (34). However, the low-fat intervention in that study was targeted to 20% kcal fat, whereas in our trial the low-fat diet provided 15% kcal from fat, so it remains possible that a more stringent reduction of dietary fat (as was the case in this trial) may have led to reduced proliferation. It is unknown whether consumption of the fish oil capsules without changing the dietary fat content would have affected proliferation. We elected to combine the 2 interventions (dietary fat reduction and fish oil supplementation) based on preclinical trials that showed decreased development and progression of prostate cancer associated with reducing the ratio of omega-6:omega-3 fatty acids and reducing dietary fat intake (2, 4, 45, 49). We would not have been able to achieve an omega-6:omega-3 ratio of 2:1 without reducing fat intake because lowering dietary fat intake reduces omega-6 fatty acid intake and, when combined with the omega-3 fish oil capsules, allows for a significant reduction in the omega-6:omega-3 ratio. Another potential criticism of this trial is that there was a difference in carbohydrate intake between the groups with 45% of energy from carbohydrates (15 grams fiber/d) in the Western diet group versus 70% of energy from carbohydrates (39 grams fiber/d) in the low-fat fish oil group, and, potentially, the alteration in carbohydrate and/or fiber intake may have been responsible for the change in prostate cancer proliferation. The low-fat/fish oil group also had a reduction in serum cholesterol levels relative to the Western diet group, and cholesterol (through a number of mechanisms) may potentially affect prostate cancer growth (50). In addition, there was a trend for increased weight loss in the low-fat/fish oil group relative to the Western group and this may potentially affect proliferation. It is likely that multiple factors play a role in nutritional effects on tumor biology including nutrient-nutrient interactions, gene-nutrient interactions,

and host susceptibility factors (11, 45). The findings in this trial that modulation of dietary fat with fish oil intake modified benign and malignant prostate tissue fatty acid levels and affected prostate cancer proliferation, suggests that the dietary intervention has the potential to affect important aspects of tumor biology related to progression. These results are to be considered hypothesis generating, and further prospective randomized trials are warranted to evaluate alteration of quantity and quality of dietary fat on tumor biology and carcinogenesis.

In summary, 4 to 6 weeks of neoadjuvant dietary fat reduction with fish oil supplementation did not affect serum IGF-1 levels. In secondary analyses, we found this intervention resulted in a decrease in omega-6:omega-3 fatty acid ratios in benign and malignant prostate tissue and a decrease in malignant epithelial cell proliferation as measured by Ki-67 immunostaining. Validation of these results with proliferation as the primary outcome will support the performance of long-term dietary intervention trials with clinical progression endpoints.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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