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# High Omega-3, Low Omega-6 Diet With Fish Oil for Men With Prostate Cancer on Active Surveillance: The CAPFISH-3 Randomized Clinical Trial

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## ABSTRACT

**PURPOSE** Men on active surveillance (AS) for prostate cancer are extremely interested in dietary changes or supplements to prevent progression of their disease. We sought to determine whether a high omega-3, low omega-6 fatty acid diet with fish oil capsules (D + FO) decreases proliferation (Ki-67) in prostate biopsies in men with prostate cancer on AS over a 1-year time period.

**METHODS** In this phase II, prospective randomized trial, men (N = 100) with grade group 1 or 2 prostate cancer who elected AS were randomly assigned to the D + FO or a control group. Same-site prostate biopsies were obtained at baseline and 1 year. The primary end point was the change in Ki-67 index from baseline to 1 year from same-site biopsies compared between the groups.

**RESULTS** The Ki-67 index decreased in the D + FO group by approximately 15% from baseline to 1 year (1.34% at baseline, 1.14% at 1 year) and increased in the control group by approximately 24% from baseline to 1 year (1.23% at baseline, 1.52% at 1 year), resulting in a statistically significant difference in the change of Ki-67 index between the groups (95% CI, 2% to 52%, $P = .043$). There was no significant difference in the secondary outcomes grade group, tumor length, Decipher genomic score, or prostate-specific antigen between the two groups. Four patients in the D + FO group were withdrawn from the trial because of adverse events related to the FO.

**CONCLUSION** A high omega-3, low omega-6 diet with FO for 1 year resulted in a significant reduction in Ki-67 index, a biomarker for prostate cancer progression, metastasis, and death. These findings support future phase III trials incorporating this intervention in men on AS.

ACCOMPANYING CONTENT

Appendix

Protocol

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## INTRODUCTION

Active surveillance (AS) is an established option for men with low risk or favorable intermediate-risk prostate cancer. However, approximately 50% of men that elect AS ultimately undergo therapy with either surgery or radiation within 5 years of the diagnosis.[1,2] Patients with prostate cancer in all stages of the disease, including those on AS, are highly interested in dietary approaches and supplements they can take to delay the progression of their disease. So far, however, specific guidelines in this regard have not been established. The MEAL clinical trial evaluated increased vegetable intake over 2 years in men on AS and reported no effect on prostate cancer progression.[3] The CANARY prostate cancer AS study evaluated healthy diet patterns with a median follow-up of 7.8 years and reported no effect on upgrading of the cancer.[4] Thus, there remains a need for prospective randomized trials evaluating diets and supplements for men with prostate cancer to delay progression.

The Western diet is notably high in omega-6 fatty acids found in corn, soy, and safflower oil, and low in omega-3 fatty acids found in fatty coldwater fish such as salmon, mackerel, herring, and sardines.[5,6] In preclinical models of prostate cancer, lowering omega-6 and raising omega-3 dietary intake delayed prostate cancer progression; however, epidemiologic studies have shown mixed results.[7-11] In a cross-sectional study nested within a phase II clinical trial in men on AS, higher prostate tissue eicosapentaenoic acid (EPA, omega-3) levels correlated with less clinical upgrading on subsequent biopsies.[12] In a short-term preprostatectomy study, combining a low-fat diet with fish oil (FO) supplementation was found to decrease tissue biomarkers associated with prostate cancer progression (Ki-67 index and

## CONTEXT

**Key Objective**

In this phase II prospective randomized trial, we sought to determine whether a high omega-3, low omega-6 fatty acid diet with fish oil capsules decreases proliferation (Ki-67 index) in prostate biopsies in men with prostate cancer on active surveillance (AS) over a 1-year time period. Same-site prostate biopsies were obtained at baseline and 1 year using an image fusion device that uses coordinates to track the cancer sites.

**Knowledge Generated**

The 1-year intervention resulted in a significant reduction in Ki-67 index (compared with the control group), a biomarker for prostate cancer progression and metastases. These findings support future phase III trials incorporating this intervention in men on AS.

**Relevance *(A. Necchi)***

A diet intervention may have a role in modifying the biology of prostate cancer in patients undergoing AS. Larger trials should expand upon this finding using more clinically impactful outcome parameters.*

*Relevance section written by *JCO* Associate Editor Andrea Necchi, MD.

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the cell-cycle progression score).[13,14] On the basis of these previous preclinical and clinical studies, we conducted the CAPFISH-3 trial, a 1-year prospective randomized trial in men with grade group 1 or 2 prostate cancer on AS with random assignment to either no treatment (control group) or to a high omega-3, low omega-6 diet with FO supplements (D + FO). The primary end point was the change in Ki-67 index from baseline to 1 year from same-site biopsies with cancer compared between the groups. Ki-67 index is a marker of proliferation and is known to predict progression, metastasis, and death from prostate cancer.[15,16]

## METHODS

### Trial Design and Conduct

The CAPFISH-3 trial was a single-center, phase II, randomized, open-label, two-arm study in men on AS for prostate cancer and was performed at the University of California Los Angeles (UCLA). Men were randomly assigned either to a control (no dietary intervention) group or to a group receiving dietary counseling to lower dietary omega-6 fat intake and increase dietary omega-3 fat intake combined with daily FO capsule supplements. Random assignment (1:1) was by a permuted random block design stratified by Gleason score 3 + 3 or 3 + 4. The UCLA institutional review board approved the protocol and all amendments, and the trial was registered with ClinicalTrial.gov (identifier: NCT02176902). See Appendix 1 (Trial Design and Conduct, online only) for details.

### Patients and Interventions

Eligible patients had biopsy-confirmed adenocarcinoma of the prostate (minimum 5% cancer in one core); Gleason score of 3 + 4 or less; clinical stage T2c or less; serum prostate-specific antigen (PSA) of 25 or less; agree to not consume FO capsules if randomly assigned to the control group; and were participating in the UCLA AS program conducted by one of the authors (L.S.M.). Exclusion criteria were intake of finasteride or dutasteride during the previous 6 months; previous treatment for prostate cancer; and the patient has allergy to fish or is a vegetarian.

Participants in the intervention arm received dietary counseling by the study-registered dietician nutritionist (P.J.) consisting of monthly individualized counseling sessions in-person, via telehealth, or by telephone. Partners/spouses or those who shared in meal preparation in the home were included in the counseling sessions. Patients were counseled to consume <30% of calories from fat with decreased consumption of foods high in omega-6 fatty acids such as fried foods, highly processed foods, chips, and baked goods, and to increase intake of omega-3 rich foods (eg, salmon, tuna; see details in Appendix 1—Nutritional Intervention). The goal was to decrease the ratio of omega-6 to omega-3 fat intake to achieve a ratio of <4:1. FO capsules were provided by Pharmavite LLC (West Hills, CA), providing a daily dose of 2.2 g of omega-3 fatty acids (docosahexaenoic acid [DHA] and eicosapentaenoic acid [EPA]). The control group did not receive dietary counseling and refrained from taking FO.

Before enrollment, a prostate biopsy was obtained by L.S.M. or one of his associates, and the coordinates of each core were recorded and tracked using an image-fusion device (Artemis, Eigen Health, Grass Valley, CA).[17] Twelve months from the initial biopsy, all participants underwent same-site biopsies at the coordinates where cancer was previously located (magnetic resonance imaging [MRI] and/or non-

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MRI targets, minimum of 3 cores per site). In addition, systematic 12-core biopsies were performed at the discretion of L.S.M. Using the tracking technology, repeat sampling of a specific site is spatially accurate within several millimeters and has been used successfully in previous studies.[17,18]

### Assessments

Dietary intake was assessed at baseline and every 3 months (intervention arm) and every 6 months (control arm) by 3-day food records (Appendix 1).

Fasting blood was collected at baseline, 6 months, and 1 year. Height was measured at baseline and anthropometrics (weight, and waist and hip circumference) were measured at baseline, 6 months, and 1 year in both groups, and at 3 and 9 months in the intervention group.

Plasma lipids (total cholesterol, HDL-cholesterol, and triglyceride), testosterone, and PSA were measured in the UCLA central clinical laboratory. RBC fatty acid analyses were measured as previously described.[19] Serum cytokines were measured as previously described.[20]

Slides from archived formalin-fixed, paraffin-embedded biopsy tissue were cut and either used for hematoxylin and eosin staining, multiplex immunofluorescence analysis, or determination of Decipher genomic classifier score (Veracyte Inc, San Diego, CA), a 22-gene panel.[21] Ki-67 in prostate biopsy tissue was analyzed using multiplex immunofluorescence analysis as described in Appendix Table A1 and Figure A1.[22] Ki-67 index was defined as the percentage of malignant cells staining for nuclear Ki-67.

### End Points and Statistical Analyses

Patient characteristics and study variables were summarized using means/standard deviations (SDs) or frequencies/percentages. The analyses were conducted with an intention-to-treat framework (as randomized) for patients who completed the study. The primary end point was the change in Ki-67 index from baseline to 1 year from same-site biopsies with cancer compared between the groups. The primary analysis of this end point was a negative binomial mixed-effects model, which allowed us to use all available data from patients who completed the trial, even if a patient was missing follow-up or baseline data (because of nonevaluable biopsies). The outcome variable in the model was the number of malignant Ki-67–positive stained nuclei. To account for variation in the total number of cells evaluated in each specimen, we included an offset term to control for the number of malignant cells evaluated. The terms in the model included fixed effects for treatment (D + FO/control), time (baseline/12 months), and the treatment by time interaction with a patient random effect (to account for multiple biopsies per patient). Secondary outcome measures included pathologic features (grade group, maximum tumor length), Decipher 22-gene classifier score, serum PSA, testosterone, lipids, and cytokines. For our primary PSA assessment, we used PSA values collected during the study (baseline and 12 months). Additionally, as a sensitivity analysis, we used PSA at screening. Other secondary outcomes included RBC omega-3 and omega-6 fatty acid levels, compliance with the diet and FO capsules, and adverse events. Longitudinal secondary outcomes were assessed using linear mixed-effects models with a similar structure to the primary outcome analysis. We presented these analyses using means/SDs at each time point as well as the *P* value from the interaction term to assess for differential changes between groups. Before the analysis, we assessed normality through visual inspections and drew on our clinical understanding of the variables. For cytokine data (Appendix Table A2) and PSA, we ran these analyses after performing a logarithmic transformation to better meet statistical assumptions. For grade group progression, progression rate was compared between groups using the Cochran-Armitage trend test. The statistical analyses were run using the Glimmix procedure in SAS V9.4 (SAS Institute, Cary, NC) and *P* values <.05 were considered statistically significant.

On the basis of the treatment effect from a previous study incorporating a low-fat FO diet, we estimated a sample size of 35 evaluable patients per treatment group would have 80% power for finding a 37% difference in log Ki-67 assuming a two-sample two-tailed *t* test with a .05 level of significance (means on log scale of 1.80 *v* 1.43, SD = 0.54).[14] We planned to randomly assign 100 patients anticipating a 10% dropout rate and anticipating 20% of patients would have nonevaluable biopsy samples, thus leaving 35 evaluable patients per treatment group.

## RESULTS

### Demographics

The CAPFISH-3 trial was conducted from December 2014 to September 2022. One hundred seven patients signed the consent form, of whom seven failed screening, leaving 100 patients who were randomly assigned (50 to the control group and 50 to the intervention group; Fig 1). The majority of patients had grade group 1 (Gleason grade 3 + 3) prostate cancer and approximately one in four patients who entered the trial had grade group 2 (Gleason grade 3 + 4) prostate cancer (Table 1). The majority of patients in both groups had cT1C prostate cancer (elevated PSA with normal digital rectal examination—data not shown). The reasons for dropout are listed in Figure 1 and the reasons for not entering the trial are listed in Appendix 1.

### Dietary and FO Capsule Intake

On the basis of analyses of the 3-day food records, patients in the D + FO group had a significant reduction in dietary omega-6 fatty acid intake and omega-6 to omega-3 dietary ratio compared with the control group, whereas there was

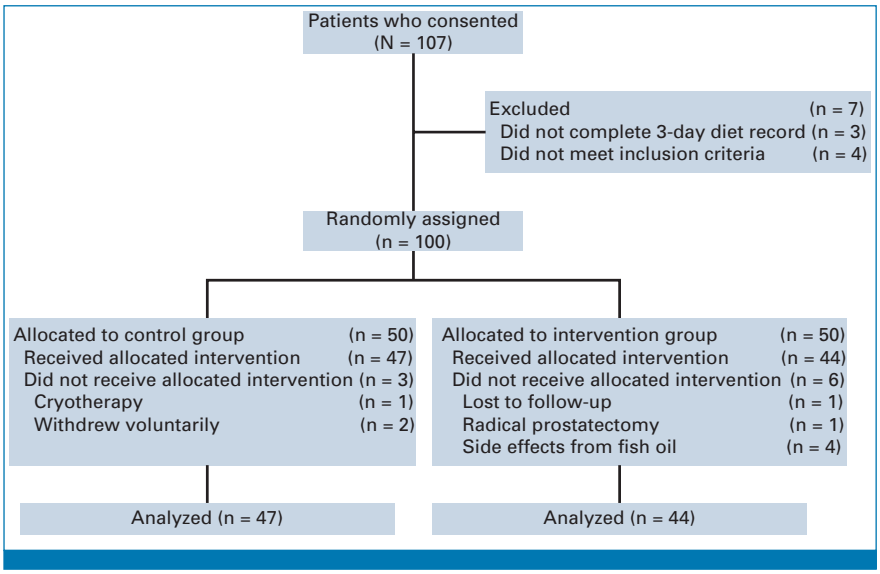


**FIG 1.** CONSORT flow diagram.

no significant difference in dietary intake (excluding FO supplements) of omega-3 fatty acids between the groups (Table 2). There was no difference in weight change between the groups and there was no significant within group weight loss (Table 3). FO capsule compliance was computed for each patient as the number of pills consumed over the number of

**TABLE 1.** Demographic and Clinical Characteristics of the Participants Who Entered the Trial

| Characteristic | Control (n = 50) | D + FO (n = 50) | Total (N = 100) |
|---|---|---|---|
| Age, years | | | |
| Mean (SD) | 64.5 (7.1) | 64.0 (6.6) | 64.2 (6.8) |
| Range | 46.0-83.0 | 50.0-79.0 | 46.0-83.0 |
| Race, No. (%) | | | |
| American Indian or Alaska | 1 (2.0) | 0 (0.0) | 1 (1.0) |
| Native, No. (%) | | | |
| Asian | 3 (6.0) | 4 (8.0) | 7 (7.0) |
| Black or African American | 2 (4.0) | 5 (10.0) | 7 (7.0) |
| White | 44 (88.0) | 41 (82.0) | 85 (85.0) |
| Ethnicity, No. (%) | | | |
| Hispanic or Latino | 5 (10.0) | 3 (6.0) | 8 (8.0) |
| Not Hispanic or Latino | 44 (88.0) | 47 (94.0) | 91 (91.0) |
| Unknown | 1 (2.0) | 0 (0.0) | 1 (1.0) |
| BMI | | | |
| Mean (SD) | 26.9 (3.7) | 27.6 (4.4) | 27.3 (4.0) |
| Range | 18.9-36.2 | 20.9-40.4 | 18.9-40.4 |
| Weight, kg | | | |
| Mean (SD) | 84.0 (13.3) | 85.6 (13.2) | 84.8 (13.2) |
| Range | 61.2-117.5 | 57.6-122.9 | 57.6-122.9 |
| Maximum Gleason score, No. (%) | | | |
| 3 + 3 | 35 (70.0) | 35 (70.0) | 70 (70.0) |
| 3 + 4 | 15 (30.0) | 15 (30.0) | 30 (30.0) |
| PSA, ng/mL | | | |
| Median (IQR) | 5.6 (4.1-7.0) | 5.9 (4.9-7.7) | 5.8 (4.2-7.6) |

NOTE. BMI is calculated as weight in kilograms divided by height in meters squared. See Appendix Table A4 for characteristics of patients who completed the trial and had evaluable Ki-67 index values.
Abbreviations: D, diet; FO, fish oil; PSA, prostate-specific antigen; SD, standard deviation.

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**TABLE 2.** Three-Day Diet Records at Baseline, 6 Months, and 12 Months

| | Control[a,b] | | | D + FO[a,b] | | | |
|---|---|---|---|---|---|---|---|
| Time (months) | 0 | 6 | 12 | 0 | 6 | 12 | Group × Time[c] |
| Calories, kcal | 1,872 (421) | 1,832 (512) | 1,798 (518) | 1,767 (418) | 1,669 (363) | 1,434 (287) | .012 |
| Protein, g | 84.5 (30.6) | 84.2 (23.7) | 79.7 (26.8) | 83.3 (26.7) | 81.4 (22.1) | 73.0 (20.0) | .57 |
| Carbohydrate, g | 204.0 (50.3) | 195.9 (72.6) | 192.8 (74.1) | 176.5 (54.4) | 178.2 (65.5) | 157.4 (38.4) | .71 |
| Fiber, g | 20.4 (8.9) | 20.7 (9.7) | 19.1 (8.0) | 17.3 (7.2) | 19.1 (9.5) | 17.7 (8.3) | .86 |
| Sugar, g | 75.4 (30.8) | 68.5 (34.7) | 64.4 (35.7) | 61.2 (27.4) | 63.4 (30.5) | 54.6 (27.4) | .33 |
| Fat, g | 75.8 (25.7) | 75.2 (28.8) | 75.0 (27.7) | 73.6 (22.3) | 64.1 (16.9) | 53.2 (16.3) | <.001 |
| SatFat, g | 23.3 (9.5) | 21.3 (8.9) | 22.1 (8.9) | 22.9 (9.0) | 19.4 (5.5) | 16.0 (6.5) | .008 |
| MonoFat, g | 28.6 (12.0) | 27.7 (11.4) | 28.5 (11.5) | 27.6 (9.7) | 24.2 (8.0) | 20.2 (6.8) | .001 |
| PolyFat, g | 17.3 (6.6) | 19.7 (11.9) | 17.9 (10.1) | 16.2 (5.8) | 14.3 (6.1) | 14.0 (14.9) | .07 |
| Cholesterol, mg | 278.6 (166.9) | 270.8 (152.1) | 281.8 (134.5) | 307.1 (169.3) | 263.9 (151.1) | 231.1 (132.3) | .08 |
| Omega-3, g | 2.1 (1.5) | 3.4 (6.1) | 2.4 (3.6) | 2.0 (1.3) | 2.5 (2.6) | 2.3 (2.0) | .59 |
| O-3 + FO[d] | — | — | — | 2.0 (1.3) | 4.7 (2.6) | 4.5 (2.0) | — |
| Omega-6, g | 15.0 (5.5) | 16.0 (8.4) | 15.2 (7.5) | 14.1 (4.9) | 11.8 (5.0) | 9.2 (4.3) | <.001 |
| Alcohol, g | 9.7 (14.0) | 9.3 (14.6) | 8.7 (13.4) | 13.0 (16.2) | 11.7 (15.5) | 8.2 (12.5) | .25 |
| % fat kcal | 35.8 (7.4) | 36.2 (7.7) | 37.4 (8.5) | 37.5 (7.3) | 34.5 (5.9) | 32.9 (6.4) | <.001 |
| Ratio n6:n3 | 8.5 (3.5) | 8.1 (3.3) | 8.5 (3.6) | 8.7 (3.3) | 6.8 (3.6) | 5.7 (3.2) | .017 |
| Ratio n6:n3 + FO[d] | — | — | — | 8.7 (3.3) | 2.8 (1.1) | 2.2 (0.7) | — |

Abbreviations: D, diet; FO, fish oil; MonoFat, monounsaturated fat; O-3, Omega-3; PolyFat, polyunsaturated fat; SatFat, saturated fat.
[a]Values in parenthesis are standard deviations.
[b]Control group baseline n = 48, 6 months n = 43, and 12 months n = 41. D + FO group baseline n = 49, 6 months n = 41, and 12 months n = 33.
[c]*P* values were assessed from the interaction term for differential changes between groups using linear mixed-effects models.
[d]Control group did not consume FO and therefore the fields in the table were left blank. The FO capsules provided 2.2 g of omega-3 fatty acids provided by docosahexaenoic acid and eicosapentaenoic acid.

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pills prescribed, resulting in an average overall compliance rate of 90.5% (SD, 12.5%) for prescribed capsules consumed in the D + FO group.

Reduction in dietary omega-6 intake was confirmed by RBC fatty acid measurements, which are a measure of long-term dietary intake (Fig 2). DHA (omega-3), EPA (omega-3), and total omega-3 RBC levels were all significantly increased in the D + FO group but not in the control group (Fig 2, Appendix Table A3).

## Primary End Point

There was a statistically significant differential reduction in Ki-67 index in the D + FO versus control group when comparing change from baseline to 1 year in same-site prostate biopsy cancer tissue (31% differential reduction [95% CI, 2 to 52], $P = .043$; Fig 3). The Ki-67 index decreased in the D + FO group by approximately 15% (from 1.34% at baseline to 1.14% at 1 year) and increased in the control group by approximately 24% (from 1.23% at baseline to 1.52% at 1 year).

## Secondary End Points

Among the patients who completed the 1-year biopsy, there was no significant trend in grade group upgrading or downgrading between the groups ($P = .499$, Table 3). For maximal tumor length (surrogate for tumor volume), there was no significant difference in change from baseline (control group: mean, 3.12, SD, 2.28; D + FO group: mean, 3.06, SD, 1.94) to 1 year (control group: mean, 3.96, SD, 3.12; FO group: mean, 3.43, SD, 2.81) between groups ($P = .40$) for same-site biopsies. For the Decipher genomic classifier score, there was no significant difference in change from baseline (Control group: mean, 0.23, SD, 0.19; D + FO group: mean, 0.18, SD, 0.10) to 1 year (Control group: mean, 0.22, SD, 0.11; D + FO group: mean, 0.24, SD, 0.18) between groups ($P = .09$) for same-site biopsies.

## Testosterone, PSA, Lipids, and Cytokines

Comparing baseline with 1 year, there was no significant difference between the groups in testosterone or PSA levels (Table 3). There was a significant decrease in triglyceride levels between the groups (Table 3). Comparing baseline with 1-year serum concentration of cytokines, macrophage colony-stimulating factor-1, also known as colony-stimulating factor-1, was significantly decreased in the D + FO group compared with the control group ($P = .017$, Appendix Table A2). Since PSA levels drawn during the trial were collected at patient entry (after the baseline prostate biopsy), and could potentially be affected by proximity to the

**TABLE 3.** Weight, BMI, Serum Lipids, Testosterone, PSA, and Grade Group Progression/Regression

| | Control[a,b] | | | D + FO[a,b] | | | |
|---|---|---|---|---|---|---|---|
| Time (months) | 0 | 6 | 12 | 0 | 6 | 12 | Group × Time[c] |
| Weight, kg | 84.0 (13.3) | 84.9 (19.7) | 85.4 (13.3) | 85.6 (13.2) | 86.2 (13.4) | 85.6 (13.0) | .689 |
| BMI, kg/m$^2$ | 26.9 (3.7) | 27.2 (5.4) | 27.3 (3.7) | 27.6 (4.4) | 27.9 (4.5) | 27.6 (4.5) | .681 |
| Triglycerides, mg/dL | 105.2 (46.0) | 117.0 (45.4) | 115.9 (54.5) | 121.0 (53.0) | 107.4 (47.4) | 110.2 (43.5) | .016 |
| Total cholesterol, mg/dL | 185.8 (34.4) | 190.3 (37.2) | 184.8 (46.6) | 191.9 (43.5) | 186.4 (38.0) | 193.6 (46.3) | .948 |
| HDL-cholesterol, mg/dL | 56.8 (12.2) | 58.0 (13.1) | 57.0 (14.2) | 57.9 (15.2) | 58.8 (16.0) | 60.4 (17.6) | .09 |
| LDL-cholesterol, mg/dL | 108.0 (34.4) | 108.9 (33.3) | 104.6 (41.7) | 109.8 (37.7) | 106.1 (33.3) | 111.1 (40.7) | .704 |
| Testosterone, ng/dL | 464.6 (153.2) | 442.8 (165.4) | 435.7 (153.9) | 423.9 (162.2) | 407.0 (149.8) | 395.7 (150.1) | .824 |
| PSA, ng/mL[d] | 6.6 (4.5-8.8) | - | 6.3 (3.7-8.4) | 7.2 (5.6-11.0) | - | 7.7 (5.0-11) | .854 |
| Grade group[e,f,g] | | | | | | | .499 |
| Progression | | | 17 (36.2) | | | 14 (31.8) | |
| No change | | | 23 (48.9) | | | 21 (47.7) | |
| Regression | | | 7 (14.9) | | | 9 (20.5) | |

NOTE. BMI is calculated as weight in kilograms divided by height in meters squared.
Abbreviations: D, diet; FO, fish oil; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PSA, prostate-specific antigen.
[a]Values in parenthesis are standard deviations or IQR.
[b]Control group baseline n = 50, 6 months n = 46, and 12 months n = 46; D + FO group baseline n = 50, 6 months n = 37, and 12 months n = 43.
[c]*P* values were assessed from the interaction term for differential changes between groups using linear mixed-effects models.
[d]PSA not collected at 6-month time point and therefore the fields in the table were left blank.
[e]Values in parenthesis are percent of total patients.
[f]Control group n = 47, D + FO group n = 44.
[g]Grade group progression rate was compared between groups using the Cochran-Armitage trend test.

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biopsy, we performed a post hoc, sensitivity analysis of PSA values collected before the biopsy that were used for screening. For this analysis, when comparing baseline with 1 year, there was also no significant difference between the groups ($P = .203$), with PSA values at eligibility measured as 5.6 (4.1-7.0) and 5.9 (4.9-7.7) for control and D + FO, respectively (median Q1-Q3).

### Safety

Four of the 50 patients randomly assigned to the D + FO group were withdrawn by the principal investigator because of adverse events attributed to the FO. One patient had grade 2 flatulence, constipation, and bloating. Another was withdrawn because of grade 2 diarrhea, GI discomfort, and eructation. The two other patients were withdrawn because of grade 1 adverse events (loose stools in one patient, and loose stools, eructation, and nausea in the other).

## DISCUSSION

CAPFISH-3, a prospective randomized trial, demonstrated that intake of a high omega-3, low omega-6 diet with FO supplements compared with a control group significantly decreased the change in Ki-67 index (the primary end point) in same-site tumor biopsy tissue from baseline to 1 year. In a meta-analysis by Berlin et al[15] of patients with prostate cancer treated with curative intent, Ki-67 index was a predictor of biochemical failure, distant metastasis, and prostate cancer survival. Moreover, a recent study by Kammerer-Jacquet et al[16] reported that Ki-67 in prostate needle biopsy tissue was an independent predictor of prostate cancer survival in conservatively managed patients with prostate cancer (patients who had no therapy 6 months after their biopsy). These authors concluded that Ki-67 in prostate biopsy tissue should be used as a viable biomarker for prognostication in patients on AS.[16] There are no previous trials in the literature evaluating serial Ki-67 index values over time in men on AS and there are no reports on Ki-67 index values in patients with grade group 1 and 2 prostate cancer. Kammerer-Jacquet et al[16] reported the mean Ki-67 index was 5% in conservatively managed patients with grade group 1 through 5 disease; however, they did not report the Ki-67 index values for the patients with grade group 1 and 2 disease.[16] The Ki-67 index cutpoint in their study predicting prostate cancer–specific death was 5%. In a meta-analysis of 21 studies, Berlin et al[15] reported a mean Ki-67 index of 6.13% in patients with prostate cancer treated with curative intent. In our trial, the baseline mean Ki-67 index values were lower (1.34% D + FO group and 1.23% control group) than previously reported, likely since we only enrolled patients with grade group 1 and 2 disease. In a similarly designed and analyzed study in men at risk for lung cancer, Mao et al observed mean Ki-67 index values in bronchial biopsies in the range of 2%-3% and observed a differential change in Ki-67 index in the celecoxib versus placebo arm (34% decrease in celecoxib and 3.8% increase in placebo).[23] These changes in Ki-67 index are similar to our trial in which

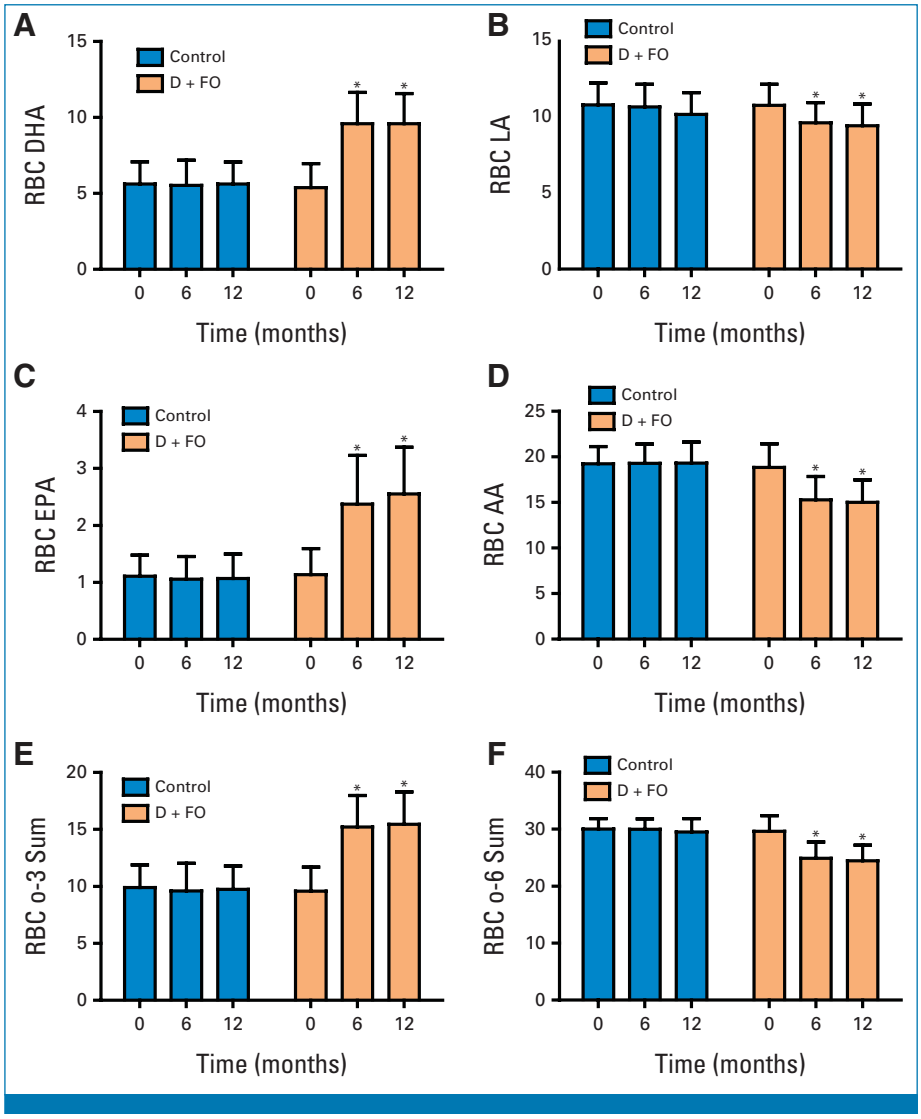


**FIG 2.** RBC fatty acid composition at baseline, 6 months, and 12 months as percent of total fat: (A) RBC DHA, (B) RBC LA, (C) RBC EPA, (D) RBC AA, (E) RBC o-3 Sum, and (F) RBC o-6 Sum. Data in the control group were collected from n = 46 (baseline), n = 42 (6 months), and n = 44 (12 months); and in the D + FO group from n = 45 (baseline), n = 39 (6 months), and n = 44 (12 months). *Compared with baseline; $P \leq .001$. *P* values were assessed from the interaction term for differential changes between groups using linear mixed-effects models. AA, arachidonic acid; D, diet; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FO, fish oil; LA, linoleic acid.

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the Ki-67 index decreased by 15% in the D + FO group and increased by 24% in the control group. The Ki-67 index values from our trial will potentially provide useful data for planning future prospective trials evaluating diet and lifestyle intervention trials in men on AS.

The intervention used in the present trial is based on previous preclinical studies and a prospective clinical trial.[14] Omega-6 polyunsaturated fat found in corn oil, soy oil, and safflower oil present in fried foods, highly processed foods, chips, and baked goods is consumed in high quantities in the typical Western diet and is known to stimulate prostate cancer progression in preclinical models.[6,11] Likewise, omega-3 fatty acids found in coldwater fish such as salmon and sardines are low in the typical Western diet and are known to delay the progression of prostate cancer in preclinical models.[6,7,10] Moreover, a short-term randomized preprostatectomy trial combining a low-fat diet low in omega-6 fatty acids with FO supplements reported a significant reduction in radical prostatectomy cancer Ki-67 and reduction in a cell-cycle progression genetic risk score in the intervention versus the control Western group.[13] Noteworthy is the cell-cycle progression score incorporates gene expression of Ki-67 in the 31-gene panel and, when combined with clinical data, is a predictor of prostate cancer progression.[24] To achieve a favorable ratio of omega-6 to

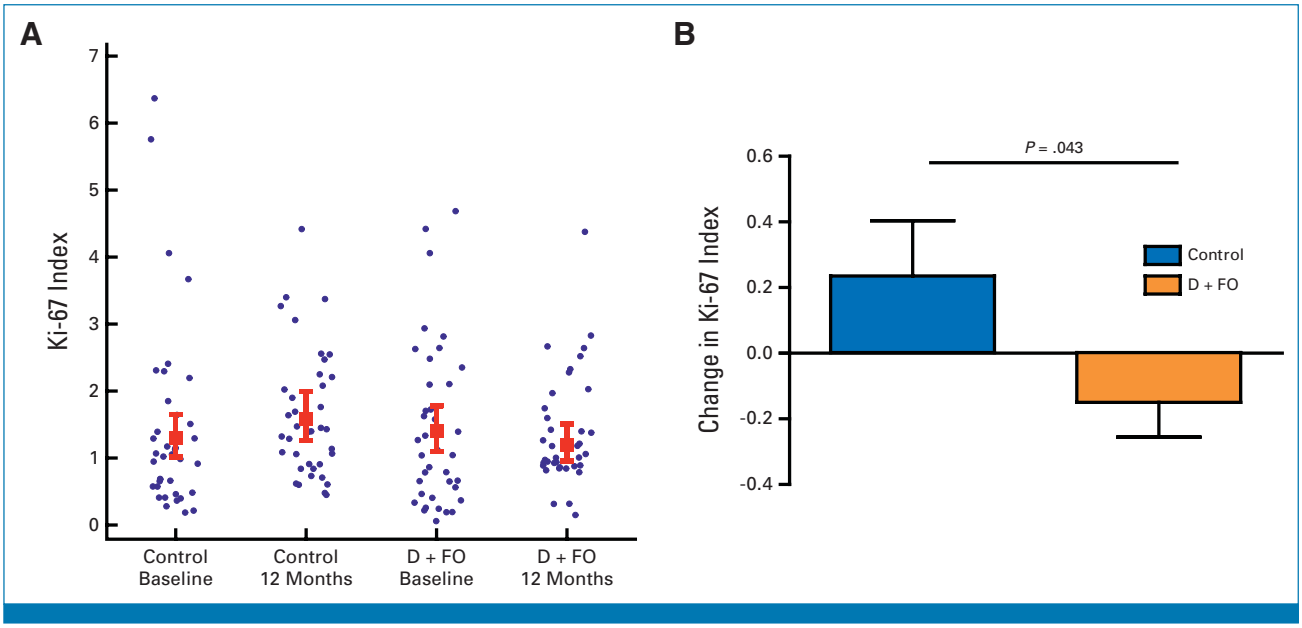


**FIG 3.** Prostate Ki-67 index in same-site biopsies at baseline and 12 months by multiplex fluorescence staining. (A) Dot plot of Ki-67 index values at baseline and 12 months for the control and D + FO groups. (B) Mean change in Ki-67 from baseline to 12 months comparing control and D + FO groups. Among the 44 patients in the D + FO group and 47 patients in the control group, 40 patients in each group had evaluable cancer tissue for Ki-67 index analysis. Prostate Ki-67 index was assessed using a negative binomial mixed-effects model. Error bars represent 95% CIs estimated from the model. D, diet; FO, fish oil.

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omega-3 fatty acids in the present trial, we combined the dietary intervention with FO supplements. The outpatient diet intervention achieved the goal of lowering omega-6 intake on the basis of analyses of the 3-day food records and RBC fatty acid levels. However, FO supplements were required to raise the omega-3 levels as this was not achieved by the diet alone as measured by the 3-day food records.

There are numerous underlying mechanisms supporting the intervention used in the present trial. The omega-6 fatty acid arachidonic acid (significantly reduced in this trial) is known to stimulate prostate cancer growth through a number of mechanisms including increasing procarcinogenic arachidonic acid metabolite levels and activation of phosphatidylinositol 3-kinase signaling.[25-27] In addition, dietary omega-3 fatty acids have been shown to decrease the number of M2-like macrophages through binding to G-protein–coupled receptors.[28] M2-like macrophages are immunosuppressive and promote angiogenesis, tumor progression, and metastases,[28] and are associated with poor outcome and increased risk of nodal and distant metastasis.[29,30] Consistent with this mechanism, in the present trial, serum levels of the cytokine macrophage colony-stimulating factor, which is known to play a role in stimulating macrophages and prostate cancer progression, was significantly reduced in the intervention group (relative to the control group).[31]

Potential shortcomings of the present trial are that there were no significant changes between the groups in other tissue markers of prostate cancer aggressiveness such as the grade group, tumor length (a surrogate of tumor volume), and serum PSA level. There was also no change between the groups in the Decipher genomic classifier score, although data are limited on use of the Decipher in patients on AS.[21] Our trial was not powered to detect differences in these secondary end points. Longer-term and phase III trials will be required to determine effects of the intervention on clinical end points. Another shortcoming is that our trial was 1 year in duration. Further studies are also required to determine the optimal dosing of omega-3 fatty acids. We chose the dosing for this trial on the basis of dosing used in our previous prospective preprostatectomy trial that demonstrated inhibitory effects on Ki-67 and a cell-cycle progression score.[14] A further shortcoming of the intervention is that four patients in the D + FO arm were withdrawn from the trial because of side effects from FO. Patients will need to be counseled about potential side effects before entering future trials incorporating FO. A strength of the trial is that it established the feasibility of performing same-site prostate biopsies for future dietary intervention trials, thus allowing longitudinal evaluation of tissue biomarkers. Ultimately, longer-term trials with larger sample sizes powered for clinical end points will be needed to provide clinical recommendations for our patients.

In summary, CAPFISH-3, a 1-year, high omega-3, low omega-6 diet + FO intervention trial, resulted in a significant reduction in prostate cancer tissue Ki-67 index, a biomarker for prostate cancer progression, metastasis, and

death. Patients in the trial were compliant with the intervention, which was well tolerated. Moreover, obtaining serial same-site prostate biopsy tissue is feasible for future biomarker intervention trials. On the basis of the underlying antiprostate cancer mechanisms of a high omega-3, low omega-6 diet with FO supplements, future trials are warranted evaluating this intervention in varying stages of prostate cancer.


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## DISCLAIMER

The funding sources had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.


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## CLINICAL TRIAL INFORMATION

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## AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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## DATA SHARING STATEMENT

**Data available:** Yes.
**Data types:** Deidentified participant data that underlie the results reported in this article.
**How to access data:** Researchers who provided a methodologically sound proposal can request data from corresponding author.
**When available:** After publication of this article.
**Mechanisms of data availability:** Data will be made available with a signed data access agreement.

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## REFERENCES


1. Timilshina N, Alibhai SMH, Tomlinson G, et al: Long-term outcomes following active surveillance of low-grade prostate cancer: A population-based study using a landmark approach. J Urol 209: 540-548, 2023
2. Simpkin AJ, Tilling K, Martin RM, et al: Systematic review and meta-analysis of factors determining change to radical treatment in active surveillance for localized prostate cancer. Eur Urol 67: 993-1005, 2015
3. Parsons JK, Zahrieh D, Mohler JL, et al: Effect of a behavioral intervention to increase vegetable consumption on cancer progression among men with early-stage prostate cancer: The MEAL randomized clinical trial. JAMA 323:140-148, 2020
4. Schenk JM, Liu M, Neuhouser ML, et al: Dietary patterns and risk of Gleason grade progression among men on active surveillance for prostate cancer: Results from the Canary Prostate Active Surveillance Study. Nutr Cancer 75:618-626, 2023
5. Simopoulos AP: An increase in the omega-6/omega-3 fatty acid ratio increases the risk for obesity. Nutrients 8:128, 2016
6. Saini RK, Keum YS: Omega-3 and omega-6 polyunsaturated fatty acids: Dietary sources, metabolism, and significance—A review. Life Sci 203:255-267, 2018
7. Liang P, Henning SM, Guan J, et al: Role of host GPR120 in mediating dietary omega-3 fatty acid inhibition of prostate cancer. J Natl Cancer Inst 111:52-59, 2019
8. Lovegrove C, Ahmed K, Challacombe B, et al: Systematic review of prostate cancer risk and association with consumption of fish and fish-oils: Analysis of 495,321 participants. Int J Clin Pract 69: 87-105, 2015
9. Lee KH, Seong HJ, Kim G, et al: Consumption of fish and omega-3 fatty acids and cancer risk: An umbrella review of meta-analyses of observational studies. Adv Nutr 11:1134-1149, 2020
10. Bilodeau JF, Gevariya N, Larose J, et al: Long chain omega-3 fatty acids and their oxidized metabolites are associated with reduced prostate tumor growth. Prostaglandins Leukot Essent Fatty Acids 164:102215, 2021
11. Ngo TH, Barnard RJ, Anton T, et al: Effect of isocaloric low-fat diet on prostate cancer xenograft progression to androgen independence. Cancer Res 64:1252-1254, 2004
12. Moussa H, Nguile-Makao M, Robitaille K, et al: Omega-3 fatty acids survey in men under active surveillance for prostate cancer: From intake to prostate tissue level. Nutrients 11:1616, 2019
13. Galet C, Gollapudi K, Stepanian S, et al: Effect of a low-fat fish oil diet on proinflammatory eicosanoids and cell-cycle progression score in men undergoing radical prostatectomy. Cancer Prev Res (Phila) 7:97-104, 2014

14. Aronson WJ, Kobayashi N, Barnard RJ, et al: Phase II prospective randomized trial of a low-fat diet with fish oil supplementation in men undergoing radical prostatectomy. Cancer Prev Res (Phila) 4:2062-2071, 2011
15. Berlin A, Castro-Mesta JF, Rodriguez-Romo L, et al: Prognostic role of Ki-67 score in localized prostate cancer: A systematic review and meta-analysis. Urol Oncol 35:499-506, 2017
16. Kammerer-Jacquet SF, Ahmad A, Moller H, et al: Ki-67 is an independent predictor of prostate cancer death in routine needle biopsy samples: Proving utility for routine assessments. Mod Pathol 32:1303-1309, 2019
17. Natarajan S, Marks LS, Margolis DJ, et al: Clinical application of a 3D ultrasound-guided prostate biopsy system. Urol Oncol 29:334-342, 2011
18. Salami SS, Tosoian JJ, Nallandhighal S, et al: Serial molecular profiling of low-grade prostate cancer to assess tumor upgrading: A longitudinal cohort study. Eur Urol 79:456-465, 2021
19. Bagga D, Capone S, Wang HJ, et al: Dietary modulation of omega-3/omega-6 polyunsaturated fatty acid ratios in patients with breast cancer. J Natl Cancer Inst 89:1123-1131, 1997
20. Kim SJ, Mehta HH, Wan J, et al: Mitochondrial peptides modulate mitochondrial function during cellular senescence. Aging (Albany NY) 10:1239-1256, 2018
21. Press BH, Jones T, Olawoyin O, et al: Association between a 22-feature genomic classifier and biopsy Gleason upgrade during active surveillance for prostate cancer. Eur Urol Open Sci 37:113-119, 2022
22. Mori H, Bolen J, Schuetter L, et al: Characterizing the tumor immune microenvironment with tyramide-based multiplex immunofluorescence. J Mammary Gland Biol Neoplasia 25:417-432, 2020
23. Mao JT, Roth MD, Fishbein MC, et al: Lung cancer chemoprevention with celecoxib in former smokers. Cancer Prev Res (Phila) 4:984-993, 2011
24. Sommariva S, Tarricone R, Lazzeri M, et al: Prognostic value of the cell cycle progression score in patients with prostate cancer: A systematic review and meta-analysis. Eur Urol 69:107-115, 2016
25. Hughes-Fulford M, Li CF, Boonyaratanakornkit J, et al: Arachidonic acid activates phosphatidylinositol 3-kinase signaling and induces gene expression in prostate cancer. Cancer Res 66: 1427-1433, 2006
26. Yang P, Cartwright CA, Li J, et al: Arachidonic acid metabolism in human prostate cancer. Int J Oncol 41:1495-1503, 2012
27. Oktem EK, Aydin B, Gulfidan G, et al: A transcriptomic and reverse-engineering strategy reveals molecular signatures of arachidonic acid metabolism in 12 cancers. OMICS 27:127-138, 2023
28. Liang P, Henning SM, Grogan T, et al: Effect of omega-3 fatty acid diet on prostate cancer progression and cholesterol efflux in tumor-associated macrophages-dependence on GPR120. Prostate Cancer Prostatic Dis 27:700-708, 2024
29. Hadimani SM, Das S, Harish KG: An immunohistochemical evaluation of tumor-associated macrophages (M1 and M2) in carcinoma prostate—An institutional study. J Cancer Res Ther 19: S300-S305, 2023
30. Han C, Deng Y, Xu W, et al: The roles of tumor-associated macrophages in prostate cancer. J Oncol 2022:8580043, 2022
31. Mougel A, Adriaenssens E, Guyot B, et al: Macrophage-colony-stimulating factor receptor enhances prostate cancer cell growth and aggressiveness in vitro and in vivo and increases osteopontin expression. Int J Mol Sci 23:16028, 2022

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## AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

**High Omega-3, Low Omega-6 Diet With Fish Oil for Men With Prostate Cancer on Active Surveillance: The CAPFISH-3 Randomized Clinical Trial**

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# APPENDIX 1. METHOD DETAILS

## Trial Design and Conduct

CAPFISH-3 trial was designed by eight of the authors and was funded by the National Institutes of Health, The Seafood Industry Research Fund, and a private donor (listed in acknowledgments). The trial was conducted in accordance with the principles of the Declaration of Helsinki and the International Council for Harmonisation guidelines for Good Clinical Practice. Patients were enrolled into the trial by the principal investigator (W.J.A.—author) and the research coordinator. All patients provided written informed consent. The trial was performed under the supervision of the Office of Regulatory Compliance of the UCLA Jonsson Comprehensive Cancer Center and Internal Data Safety Monitoring Board. There were seven screen failures (patients who signed the consent form) and the reasons for failed screening are listed in Figure 1. The design of the study, data analysis, and manuscript preparation were done by the authors independent from the Seafood Industry Research Fund, the private donor, and Pharmavite LLC (West Hills, CA), the fish oil (FO) capsule manufacturer.

## Reasons for Not Entering the Trial

1. Patients wanted to take fish oil and were not willing to be randomly assigned to the no fish oil group.
2. Entry into focal therapy trials (cryo/HIFU) was ramped up here during the later years of the study, and patients chose focal therapy over active surveillance (AS) and study entry.
3. A large majority of Dr Mark's patients have 1-2+ hour commutes and beyond, and preferred not to come back for study visits. Note that all patients in the study were recruited from Dr Mark's UCLA practice (this is listed in the Methods).
4. Some patients who elected AS were scheduled for 6-month or 18- to 24-month follow-up biopsies and therefore did not meet the inclusion criteria for a planned 12 months of biopsy.
5. No interest in participating in a study. This was rare as Dr Mark's AS patients, in general, were extremely interested in participating in a diet study.

## Nutritional Intervention

There were 12 nutrition counseling sessions for participants in the D + FO group. Initial sessions were between 45 and 60 minutes; follow-ups were 15-30 minutes on average. Before each counseling session, participants were asked about their usual diets and to record all food and beverage consumed over a 3-day period. Food diaries were used as self-monitoring tools. The study dietician reviewed the completed diaries with each participant during individual sessions to reinforce the diet and promote behavioral change.

Participants were guided on healthier, lower-fat alternatives for high-fat/high-calorie foods most often and on reducing portion size when participants did not want to totally give up on a particular food they really enjoyed. Menu samples and meal plans were offered to participants as needed. Motivational interviewing techniques were used to promote behavior change. In essence, motivational interviewing is a patient-focused, directive approach to counseling that emphasizes individual autonomy and collaborative relationship, allowing individuals to control what and how to change the behavior. The participant's spouse or partner was often involved in sessions. Whether there was someone else involved in food shopping/food preparation was one of the first questions asked at the initial session. Some printed material such as omega-3, omega-6 and total fat content of foods, how to estimate portion size, and example menus were provided.

## Assessments

Dietary data were coded and entered into a nutrition analysis software program (Food Processor 11.11.0, ESHA Research), which is linked to the Food and Nutrient Database for Dietary Studies and the latest USDA Standard Reference Database. A detailed dietary report analysis was generated providing average energy (kcal/day), select macronutrients, and nutrient profile.

Slides from archived formalin-fixed, paraffin-embedded biopsy tissue were cut and either used for hematoxylin and eosin staining, multiplex immunofluorescence analysis, or determination of Decipher genomic classifier score (Veracyte Inc, San Diego, CA), a 22-gene panel.[22] Ki-67 levels in prostate biopsy tissue were analyzed using multiplex immunofluorescence analysis (Appendix Table A1). The Tyramide signal amplification (TSA)–based Opal method was used in this study for immunofluorescence (IF) staining (Opal Polaris 7-Color Automation IHC Kit; Akoya Biosciences, Marlborough, MA; Catalog No. NEL871001KT). The Opal fluorophores were used at a 1 in 100 dilution, as recommended by Akoya (Akoya Biosciences, Marlborough, MA) when using the Leica BOND RX.

Stained slides were scanned on the Vectra Polaris (Akoya Biosciences, Marlborough, MA) at 40× magnification using appropriate exposure times. Cancer areas were marked by the study pathologist (coauthor J.S.). The data from the multispectral camera were analyzed by using the imaging software InForm (Akoya Biosciences, Marlborough, MA) and imported into RStudio 1.4.1103 add-in. Total nuclei (DAPI) number was assessed in the ductal area (not stromal area) in the cancer tissue. The number of Ki-67–positive nuclei in the ductal area was counted manually by visual inspection of personnel blinded to random assignment, but not to time point. Depending on the size of the cancer area, the software (InForm) divided the area into squares of equal size and reported a number of nuclei per square. The number of squares varied between biopsies. In total, 599 squares were included in the analysis (313 control and 286 treatment).

The analyses were performed in six batches of 10-20 biopsies each. The first three staining batches were performed in July 2021 and additional three staining batches in January 2023. Each batch included a quality control sample from a prostatectomy tissue sample. The staining was visually inspected and compared with previous batches to confirm staining quality. Pre/post samples from each individual were included in the same batch.

**TABLE A1.** Multiplex Immunofluorescent Staining Conditions

| Antibody | Manufacturer | Cat No. | BOND Tested Dilution | Antigen Retrival Time | Incubation Time RT | Opal Dye |
|---|---|---|---|---|---|---|
| Ki-67 | DAKO/Agilent | M7240 | 1-100 | ER1 20 minutes | 20 minutes | Opal 690 |

Abbreviation: RT, room temperature.

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**TABLE A2.** Serum Cytokine Concentration (log scale) at Baseline, 6 Months, and 12 Months

| | Control | | | D + FO | | | |
|---|---|---|---|---|---|---|---|
| Time (months) | 0 | 6 | 12 | 0 | 6 | 12 | Group × Time |
| IFN-γ, pg/mL | 3.63 (0.56) | 3.73 (0.76) | 3.69 (0.68) | 3.54 (0.73) | 3.62 (0.88) | 3.51 (0.67) | .511 |
| IL-10, pg/mL | 0.40 (0.29) | 0.46 (0.40) | 0.41 (0.31) | 0.37 (0.18) | 0.37 (0.16) | 0.40 (0.22) | .752 |
| IL-6, pg/mL | 0.82 (0.54) | 0.80 (0.37) | 0.84 (0.59) | 0.81 (0.40) | 0.74 (0.34) | 0.78 (0.36) | .539 |
| IL-8, pg/mL | 2.62 (0.56) | 2.65 (0.46) | 2.67 (0.44) | 2.47 (0.48) | 2.67 (0.50) | 2.64 (0.55) | .214 |
| MCP-1, pg/mL | 6.02 (0.44) | 6.04 (0.52) | 6.01 (0.43) | 5.92 (0.53) | 6.07 (0.67) | 5.94 (0.55) | .954 |
| M-CSF, pg/mL | 3.90 (0.40) | 3.89 (0.40) | 3.95 (0.41) | 3.90 (0.52) | 3.89 (0.53) | 3.86 (0.57) | .017 |
| TNF-α, pg/mL | 1.82 (0.53) | 1.79 (0.53) | 1.75 (0.48) | 1.67 (0.33) | 1.70 (0.36) | 1.64 (0.35) | .34 |

NOTE. Values in parenthesis present SD. D + FO group n = 46 (baseline), n = 37 (6 months), and n = 45 (12 months); control group n = 48 (baseline), n = 43 (6 months), and n = 46 (12 months). Statistical analysis used log transformation.
Abbreviations: D, diet; FO, fish oil; IFN-γ, interferon gamma; IL-10, interferon 10; IL-6, interferon 6; IL-8, interferon 8; MCP-1, monocyte chemoattractant protein-1; M-CSF, macrophage colony-stimulating factor; SD, standard deviation; TNF-α, tumor necrosis factor alpha.

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**TABLE A3.** RBC Fatty Acid Composition

| | Control | | | D + FO | | | |
|---|---|---|---|---|---|---|---|
| Time (months) | 0 | 6 | 12 | 0 | 6 | 12 | Group × Time |
| MA | 0.4 (0.1) | 0.4 (0.1) | 0.3 (0.1) | 0.4 (0.1) | 0.4 (0.3) | 0.4 (0.2) | .987 |
| PA | 24.3 (1.6) | 24.5 (1.9) | 24.5 (1.7) | 24.5 (2.5) | 24.7 (2.7) | 24.9 (2.8) | .75 |
| POA | 0.5 (0.2) | 0.5 (0.2) | 0.5 (0.2) | 0.5 (0.2) | 0.5 (0.2) | 0.5 (0.1) | .867 |
| SA | 20.4 (1.5) | 20.5 (1.5) | 20.6 (1.4) | 20.8 (1.8) | 20.2 (2.0) | 20.3 (2.1) | .014 |
| OA | 13.9 (1.5) | 14.0 (1.3) | 14.1 (1.1) | 13.9 (1.3) | 13.5 (1.4) | 13.4 (1.2) | <.001 |
| LA | 10.8 (1.5) | 10.7 (1.5) | 10.3 (1.2) | 10.8 (1.4) | 9.6 (1.3) | 9.4 (1.4) | <.001 |
| LNA | 0.2 (0.1) | 0.2 (0.3) | 0.2 (0.1) | 0.2 (0.1) | 0.2 (0.1) | 0.2 (0.1) | .627 |
| EDA | 0.4 (0.1) | 0.4 (0.2) | 0.4 (0.2) | 0.5 (0.2) | 0.4 (0.1) | 0.5 (0.1) | .986 |
| AA | 19.2 (1.9) | 19.3 (2.1) | 19.3 (2.4) | 18.9 (2.6) | 15.3 (2.6) | 15.0 (2.5) | <.001 |
| EPA | 1.0 (0.4) | 1.0 (0.4) | 1.0 (0.5) | 1.1 (0.5) | 2.3 (0.9) | 2.6 (0.8) | <.001 |
| DPA | 3.2 (0.7) | 3.1 (0.7) | 3.1 (0.6) | 3.1 (0.6) | 3.3 (0.6) | 3.3 (0.6) | <.001 |
| DHA | 5.6 (1.5) | 5.5 (1.6) | 5.6 (1.4) | 5.4 (1.6) | 9.6 (2.1) | 9.6 (2.0) | <.001 |
| O6 | 30.1 (1.8) | 30.0 (1.8) | 29.6 (2.2) | 29.6 (2.8) | 24.9 (2.9) | 24.4 (2.8) | <.001 |
| O3 | 9.9 (2.0) | 9.6 (2.4) | 9.7 (2.0) | 9.6 (2.1) | 15.2 (2.8) | 15.5 (2.8) | <.001 |
| Ratio O6/3 | 3.2 (0.7) | 3.3 (0.9) | 3.2 (0.7) | 3.3 (0.8) | 1.7 (0.5) | 1.7 (0.6) | <.001 |

NOTE. Values in parenthesis present SD. Values are expressed as percent of total fat. D-FO group n = 45 (baseline), n = 39 (6 months), and n = 43 (12 months); control group n = 46 (baseline), n = 42 (6 months), and n = 43 (12 months).
Abbreviations: AA, arachidonic acid; D, diet; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EDA, eicosadienoic acid; EPA, eicosapentaenoic acid; FO, fish oil; LA, linoleic acid; LNA, linolenic acid; MA, myristic acid; o-3, omega-3; o-6, omega-6; OL, oleic acid; PA, palmitic acid; POA, palmitoic acid; SA, stearic acid.

**TABLE A4.** Demographic and Clinical Characteristics of Participants Who Completed the Trial and Had Evaluable Ki-67 Index Values

| Characteristic | Control (n = 40) | D + FO (n = 40) | Total (N = 80) |
|---|---|---|---|
| Age, years | | | |
| Mean (SD) | 64.4 (7.4) | 63.4 (6.2) | 63.9 (6.8) |
| Range | 46.0-83.0 | 50.0-74.0 | 46.0-83.0 |
| Race, No. (%) | | | |
| Asian | 1 (2.5) | 3 (7.5) | 4 (5.0) |
| Black or African American | 1 (2.5) | 3 (7.5) | 4 (5.0) |
| White | 38 (95.0) | 34 (85.0) | 72 (90.0) |
| Ethnicity, No. (%) | | | |
| Hispanic or Latino | 2 (5.0) | 3 (7.5) | 5 (6.3) |
| Not Hispanic or Latino | 37 (92.5) | 37 (92.5) | 74 (92.5) |
| Unknown | 1 (2.5) | 0 (0.0) | 1 (1.3) |
| BMI | | | |
| Mean (SD) | 27.5 (3.7) | 27.7 (4.6) | 27.6 (4.1) |
| Range | 18.9-36.2 | 20.9-40.4 | 18.9-40.4 |
| Weight, kg | | | |
| Mean (SD) | 86.3 (13.5) | 86.1 (13.4) | 86.2 (13.4) |
| Range | 61.2-117.5 | 57.6-122.9 | 57.6-122.9 |
| Maximum Gleason score, No. (%) | | | |
| 3 + 3 (%) | 28 (70.0) | 26 (65.0) | 54 (67.5) |
| 3 + 4 | 12 (30.0) | 14 (35.0) | 26 (32.5) |
| PSA | | | |
| Median (IQR), ng/mL | 5.8 (4.2-7.3) | 5.8 (4.3-7.6) | 5.8 (4.2-7.6) |

NOTE. BMI is calculated as weight in kilograms divided by height in meters squared.
Abbreviations: D, diet; FO, fish oil; SD, standard deviation.

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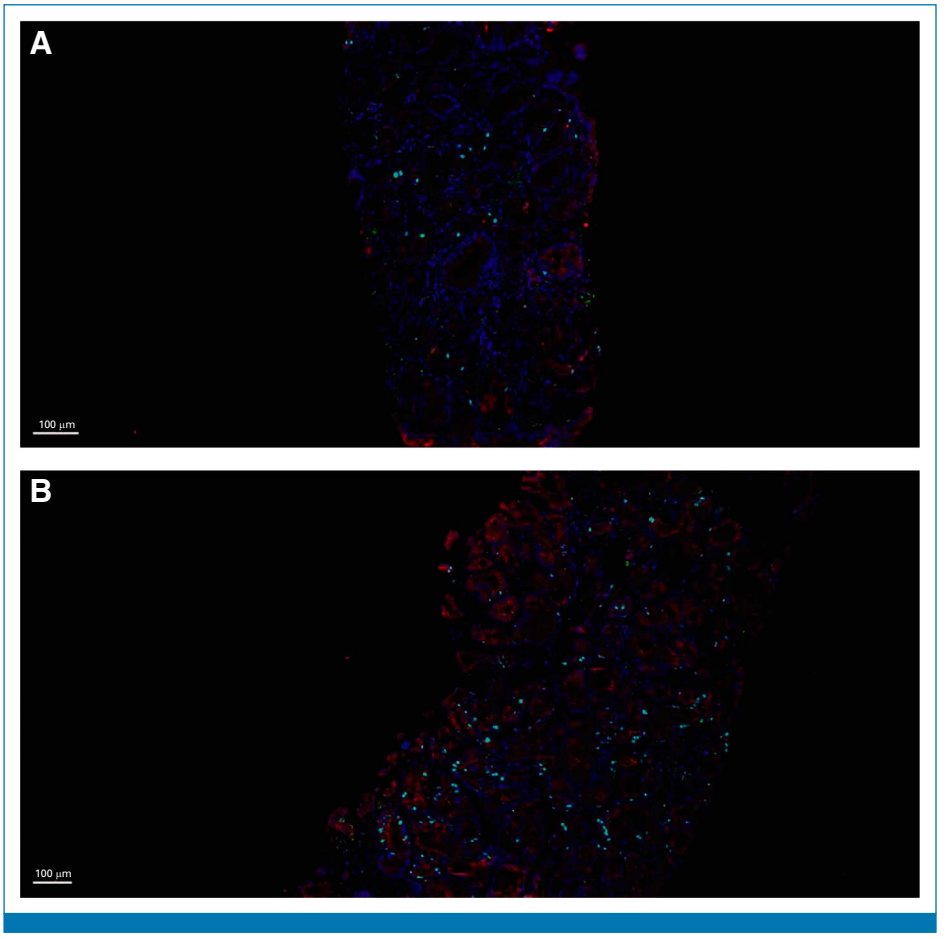


**FIG A1.** Example of multiplex immunofluorescent images. Multiplex immunofluorescence image (A) low Ki-67 expression and (B) high Ki-67 expression. Ki-67–positive nuclei are colored light blue. Total nuclei (DAPI) are colored dark blue.

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