Flaxseed Supplementation (Not Dietary Fat Restriction) Reduces Prostate Cancer Proliferation Rates in Men Presurgery

Wendy Demark-Wahnefried,[1] Thomas J. Polascik,[2] Stephen L. George,[3,4] Boyd R. Switzer,[8] John F. Madden,[5] Mack T. Ruffin IV,[9,10] Denise C. Snyder,[6] Kouros Owzar,[3,4] Vera Hars,[4] David M. Albala,[2] Philip J. Walther,[2] Cary N. Robertson,[2] Judd W. Moul,[2] Barbara K. Dunn,[13] Dean Brenner,[10,11] Lori Minasian,[13] Philip Stella,[12] and Robin T. Vollmer[5,7]

[1]Division of Cancer Prevention and Population Sciences, The University of Texas M. D. Anderson Cancer Center, Houston, Texas; [2]Division of Urologic Surgery and Duke Prostate Center, Duke University Medical Center (DUMC); [3]Department of Biostatistics and Bioinformatics, Duke University Medical Center; [4]Duke Comprehensive Cancer Center Biostatistics, DUMC; [5]Department of Pathology, DUMC; [6]Duke University School of Nursing; [7]Department of Surgical Pathology and Cytopathology, Durham VA Medical Center, Durham, North Carolina; [8]Department of Nutrition, University of North Carolina, Chapel Hill, North Carolina; [9]Department of Family Medicine University of Michigan (UM); [10]UM Community Clinical Oncology Program Research Base; [11]Department of Internal Medicine, UM; [12]Michigan Cancer Research Consortium CCOP, Ann Arbor, Michigan; and [13]Division of Cancer Prevention, National Cancer Institute, Bethesda, Maryland

Abstract

Background: **Prostate cancer affects one of six men during their lifetime. Dietary factors are postulated to influence the development and progression of prostate cancer. Low-fat diets and flaxseed supplementation may offer potentially protective strategies.**
Methods: **We undertook a multisite, randomized controlled trial to test the effects of low-fat and/or flaxseed-supplemented diets on the biology of the prostate and other biomarkers. Prostate cancer patients (n = 161) scheduled at least 21 days before prostatectomy were randomly assigned to one of the following arms: (a) control (usual diet), (b) flaxseed-supplemented diet (30 g/d), (c) low-fat diet (<20% total energy), or (d) flaxseed-supplemented, low-fat diet. Blood was drawn at baseline and before surgery and analyzed for prostate-specific antigen, sex hormone-binding globulin, testosterone, insulin-like growth factor-I and binding protein-3, C-reactive protein, and total and low-density lipoprotein cholesterol. Tumors were assessed for proliferation (Ki-67, the primary endpoint) and apoptosis.**
Results: **Men were on protocol an average of 30 days. Proliferation rates were significantly lower (P < 0.002) among men assigned to the flaxseed arms. Median Ki-67-positive cells/total nuclei ratios (×100) were 1.66 (flaxseed-supplemented diet) and 1.50 (flaxseed-supplemented, low-fat diet) versus 3.23 (control) and 2.56 (low-fat diet). No differences were observed between arms with regard to side effects, apoptosis, and most serologic endpoints; however, men on low-fat diets experienced significant decreases in serum cholesterol (P = 0.048).**
Conclusions: **Findings suggest that flaxseed is safe and associated with biological alterations that may be protective for prostate cancer. Data also further support low-fat diets to manage serum cholesterol.** (Cancer Epidemiol Biomarkers Prev 2008;17(12):3577–87)

Introduction

This year in the United States, ~186,320 men will be diagnosed with prostate cancer and 28,660 will die from it (1). Diet is presumed to play a major role in prostate cancer, yet few studies have prospectively explored the efficacy of dietary interventions in either the preventive or complementary care settings (2, 3). Although several dietary factors may be important for prostate cancer (2, 3), we undertook a randomized controlled trial to determine the effects of flaxseed supplementation and a low-fat diet on the biology of prostate cancer and associated biomarkers, because our previous studies (4-8) and the work of others suggested potential benefit (9-12).

Flaxseed, an oilseed commonly consumed in the Middle Ages as a component of breads and cereals, has largely vanished from the modern-day food supply because of its abbreviated shelf-life (13). Given its unique nutrient profile, however, flaxseed has gained recent attention as a potential functional food (14, 15). First, flaxseed is an exceptionally rich source of dietary lignan, possessing over 800-fold the amount in most other foods (13, 14). Previous research suggests that lignan shows antimitotic, antiangiogenic, antioxidant, and phytoestrogenic effects (9, 11, 15). Furthermore, lignan has been shown to reduce testosterone (total and free) and 5α-reductase, the enzyme that converts testosterone to its most active form, dihydrotestosterone (9, 11). Such effects may be important for prostate cancer, a hormonally

Received 1/4/08; revised 8/19/08; accepted 9/4/08.
Grant support: NIH grants CA85740, CA07464830, and M01-RR-30. Flaxseed was donated by ENRECO.
Requests for reprints: Wendy Demark-Wahnefried, Division of Cancer Prevention and Population Sciences, The University of Texas M. D. Anderson Cancer Center, P.O. Box 301439, Unit 1330, Houston, TX 77230-1439. Phone: 713-563-7366; Fax: 713-794-4730. E-mail: wdemarkw@mdanderson.org

doi:10.1158/1055-9965.EPI-08-0008

driven neoplasm (2, 3). Additionally, flaxseed is a rich source of plant-based ω-3 fatty acids (ω3FA), which have been shown to increase natural killer cell activity, alter tyrosine kinase cell signaling pathways, inhibit cell membrane synthesis, affect cell receptor status, and influence the eicosanoid milieu (suppressed production of prostaglandins E_2 and I_2 and 5-hydroxyeicosatetraenoic acid via cyclooxygenase and lipoxygenase pathways; ref. 16). Despite the favorable effects of ω3FA, the role of α-linolenic acid (ALA), the predominant fatty acid in flaxseed, is unclear (17). Some reports link ALA to decreased risk of prostate cancer or find no association with risk (18-20), whereas others suggest increased risk, although such findings come largely from observational studies where food sources of ALA were predominantly meat, dairy products, and salad dressings (not flaxseed; refs. 21, 22). It has been suggested that the metabolism of ALA may vary depending on the concurrent intake of ω6FA; biochemical conversion of ALA to longer-chained ω3FA, eicosapentanoic acid (EPA) and docosahexanoic acid, is enhanced if ALA is consumed simultaneously with a reduced intake of ω6FA as in low-fat diets (23). Given this rationale, our pilot studies of flaxseed supplementation have always employed concurrent dietary fat restriction (5, 6). However, low-fat diets have been independently associated with reduced risk of prostate cancer (10, 12), although results have been inconsistent (24, 25). Thus, there was a need to disentangle the potential effects of flaxseed supplementation and dietary fat restriction using a rigorous randomized controlled approach and to determine whether these effects operate independently or synergistically. Herein, we report the results of a National Cancer Institute-funded phase II randomized clinical trial (NCT00049309) that employed a presurgical model to assess the effect of flaxseed supplementation and/or dietary fat restriction on the biology of prostate cancer and associated biomarkers.

Patients and Methods

Study Overview. A detailed description of the methods used in this trial are reported elsewhere (26). In brief, the trial employed a 2×2 factorial design, with the presence or absence of the two factors, flaxseed supplementation and dietary fat restriction, defining the following four treatment arms: (*a*) control (usual diet), (*b*) flaxseed-supplemented diet, (*c*) low-fat diet, and (*d*) flaxseed-supplemented, low-fat diet. The specific aims were to (*a*) determine the differences between study arms in tumor proliferation, as assessed by Ki-67 staining of prostatectomy specimens (primary aim), as well as rates of apoptosis (secondary aim); (*b*) determine the differences between study arms with regard to change in serum prostate-specific antigen (PSA), total testosterone, sex hormone-binding globulin (SHBG), insulin-like growth factor (IGF)-I and IGF-binding protein-3 (IGFBP-3), C-reactive protein, and serum lipids (secondary aim); (*c*) determine the effects of flaxseed supplementation and/or a low-fat diet on nutritional biomarkers (levels of lignans in the urine and seminal fluid and fatty acid profiles of erythrocytes and prostatic tissue); and (*d*) explore associations between dietary change and changes in nutritional biomarkers, hormonal intermediates, and study endpoints (secondary aim).

Study Population. Patients with biopsy-confirmed prostatic carcinoma electing prostatectomy as their primary treatment and at least 21 days from scheduled surgery were enrolled from the Duke University Medical Center, the Durham Veterans Administration Medical Center, and the five sites within the University of Michigan Community Clinical Oncology Program Research Base. Only mentally competent, English-speaking, and English-writing men with telephone access were considered because evaluative surveys and intervention delivery relied on telephone counseling and written materials. Other exclusion criteria were (*a*) recent flaxseed use and/or adherence to a diet ≤30% of kilocalories from fat (patients' diets were screened using the National Cancer Institute Percent Energy from Fat Screener; ref. 27), (*b*) dietary supplements started within the past 3 months (exception: standard multivitamin and mineral preparations), (*c*) current antibiotic use (antibiotics reduce the intestinal microflora that convert dietary lignans to biologically active mammalian-based lignans; refs. 13, 28), or (*d*) history of hormonal or other neoadjuvant therapies. The study was approved by the institutional review boards at each center. All participants provided written informed consent.

Baseline Measures. Participants received instructions and supplies to collect a chilled 24-h urine and ejaculate sample. Men also were asked to complete the National Cancer Institute Diet History Food Frequency Questionnaire (29) and the Aerobics Center Longitudinal Study Physical Activity Questionnaire (30). A baseline visit after a 12-h fast was scheduled at least 14 days after biopsy and at least 3 days after digital rectal exam (31). At this visit, heights and weights were measured (26), surveys were reviewed, sociodemographic and medical history information was recorded, and biological samples were collected.

Blood was drawn via venipuncture (21.5 mL) and configured into plasma and sera, and cryovials were prepared and stored at -70°C until completion of the study whereupon samples were batch analyzed at a commercial laboratory (LabCorp) via immunochemiluminometric assay for PSA, total testosterone, SHBG, total and low-density cholesterol, C-reactive protein, IGF-I, and IGFBP-3 (26). These tests were selected because previous studies suggest that these biomarkers are associated with prostate cancer growth and/or are influenced by a low-fat diet or flaxseed supplementation (25, 26). Erythrocytes were washed repeatedly with saline and stored at −70°C until study completion whereupon erythrocyte membranes were batch analyzed via capillary gas chromatography for fatty acid composition (32, 33).

Start and stop times for 24-h urine collections were recorded, and samples were measured for volume and aliquoted. Creatinine was measured using kinetic methods (Duke University Health Systems Clinical Laboratories and LabCorp) to confirm 24-h collection and to use as a benchmark for expressing lignan excretion (marker of dietary adherence to the flaxseed-supplemented regimen). Remaining cryovials were stored at −70°C until completion of the study whereupon they were batch analyzed. Urinary lignans were hydrolyzed and

quantified via high-performance liquid chromatography using techniques described previously (34, 35).

Collection times and volumes of ejaculate samples were noted. Samples were held at room temperature until liquefaction was complete and centrifuged at 2,500 rpm for 10 min. The resulting supernatant (seminal fluid) was pipetted off, evenly aliquoted into two 2 mL Teflon-stoppered cryovials, and stored at −70°C until completion of the study whereupon it was batch analyzed. Seminal fluid lignans were assessed using the same high-performance liquid chromatography methods described above (34, 35). As in the urine, converted plant lignans are expressed in the seminal fluid and have been associated with reduced risk for prostate cancer in previous studies (9, 34).

Randomization and Interventions. After all baseline data and biospecimens were collected, the Duke Clinical Trials Office (located off-site) randomly assigned participants using stratification variables of race (Black versus non-Black) and biopsy Gleason sum (<7 versus ≥7) to one of the following arms:.

Control: Men in this arm were asked to continue their usual diet.

Flaxseed Supplementation (FS): Men assigned to this arm were provided with ample ground flaxseed to last until their date of surgery. To reduce the variability in nutrient composition that could occur between crops, the flaxseed used for this study was obtained from ENRECO in one lot (150 kg) and was analyzed for nutrient content at two time points during the study period. Given its propensity for rancidity (13), the flaxseed was stored in whole-grain form under cold storage (4°C) and ground and packaged in daily dose (30 g) sealed opaque packets as needed; the dose of 30 g (~3 rounded tablespoons) was chosen based on positive effects observed with an identical dose in our pilot studies among men with prostate cancer as well as a similar dose (25 g used successfully by Thompson et al. in a clinical trial among women with breast cancer; ref. 36). Starter kits with stepped doses of ground flaxseed were provided (10 g for days 1-3, 20 g for days 4-6, and 30 g for day 7 and beyond); a stepped dose was used to accustom the gut to the considerable fiber load imposed by the flaxseed (~9 g fiber/30 g dose). Men receiving flaxseed also were instructed to drink at least 64 ounces/d of fluids to reduce potential risk of colonic impaction or dehydration resulting from the increased fiber load (13, 37) and to keep their flaxseed packets under refrigeration (to retard spoilage). Participants in this arm also were provided with logs to record their daily intakes of flaxseed to the nearest quarter of a packet and to return any unused packets at follow-up. These procedures were adapted from pill counts, which provide a valid measure of adherence in pharmacologic trials (including fiber supplement trials; refs. 37, 38).

Low-Fat Arm (LF): Men randomized to this arm were instructed by registered dietitians on a diet with ≤20% of energy from dietary fat. Fat gram ''budgets'' were individually calculated using the following formula: ideal body weight (pounds) × 15 × 0.2 kcal from fat/9 kcal/g. Men were provided with fat gram counters (T-Factor 2000; W.W. Norton) and instructed to record all foods consumed with corresponding fat gram counts and to tally their number of fat grams daily. Participants also received written and verbal instruction on meal planning, food preparation, shopping, and dining.

Flaxseed-Supplemented, Low-Fat Diet (FS + LF) Arm: Men in this arm received instruction and supplies for both diet regimens described above.

Men in all arms were contacted weekly by study staff to maintain contact, assess and reinforce adherence, and answer any diet-related questions. Additionally, participants' wives or partners were encouraged to attend the baseline appointment.

End-of-Study Measures. Follow-up visits were conducted within 3 days of surgery. All baseline measures (except height) were repeated, and changes in health status and medication use were assessed. Potentially relevant side effects (nausea, vomiting, diarrhea, decreased libido or impotence, and allergy) were collected using the National Cancer Institute Common Toxicity Criteria (version 3.0; ref. 39). Men assigned to diet-modified arms also were asked to report the number of days per week they adhered to their assigned diet regimen and the average amount of flaxseed consumed, if appropriate. On prostatectomy, prostatic tissue was retrieved from defined central and peripheral zone regions using a 3 mm biopsy punch. Tissue samples were flash-frozen in liquid nitrogen and stored at −70°C until study completion.

Histopathologic Outcomes. The primary study pathologist (R.V.), blinded with regard to study condition, reviewed clinical pathology reports and all slides for each case; he then chose one slide and one block per case for determination of proliferation and apoptosis. Slides (and blocks) were chosen based on the presence of adequate tumor, as well as benign tissue, and the histologic grade of tumor on the slide was representative of the entire tumor in the specimen. Proliferation counts were assessed using the antibody MIB-1 for Ki-67 hybridoma clone at a dilution of 1:200 (Biocare; ref. 40). This marker has validated use in nutrition intervention trials (41) and has been endorsed by the Prostate Cancer Chemoprevention Trial Consensus Panel as an accurate and reproducible measure (41). The labeled streptoavidin/biotin/peroxidase/diaminobenzidine tetrachloride method (Biocare) was used with antigen retrieval by pressure-cooking in citrate buffer (DAKO). Slides were counterstained with hematoxylin, and tonsillar tissue with lymphoid hyperplasia served as a control. Prepared slides were independently reviewed by the primary and secondary study pathologists (R.V. and J.F.M.), both of whom were blinded to study condition. The following method was used: (*a*) at low magnification, a random starting point in the tumor was chosen, and (*b*) at high magnification, sequentially encountered tumor cell nuclei were evaluated for Ki-67 positivity. The result was reported as the ratio of positive nuclei divided by the total number evaluated × 100.

The degree of apoptosis was measured using the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling method (TdT-FragEL kit with manufacturer control; Oncogene; ref. 42). Pretreated slides were incubated in biotin-labeled/unlabeled deoxynucleotides containing TdT at 37°C for 1.5 h. The biotinylated nucleotides were detected using a streptoavidin-horseradish peroxidase conjugate and then reacted with diaminobenzidine, forming an insoluble,

brown precipitate at the site of DNA fragmentation. Using light microscopy and assessing the degree of nuclear staining against a methyl green counterstain, labeled nuclei were then evaluated. Because preliminary results with the TdT-mediated dUTP nick end labeling stain indicated a very low number of positive nuclei, formal counts were not done. Instead, many microscopic fields (>1,000 cells) were examined, and staining for TdT-mediated dUTP nick end labeling was ranked by the study pathologists as follows: score of ''0'' for no positive cells to rare cells, ''1'' for occasional positive cells, and ''2'' for frequent positive cells. Averaged values of the two pathologists' scores were then used in statistical analyses (see ref. 26 for greater detail and issues regarding histopathologic assessments and analysis).

Statistical Design and Analysis. This trial employed a 2 × 2 factorial design with the presence or absence of flaxseed supplementation and low-fat, generating the four treatment arms. Thus, there were two primary tests, one for flaxseed supplementation and one for low-fat. The primary statistical outcome variable was proliferation rate. Our preliminary studies suggested that the combination of flaxseed supplementation with a low-fat diet resulted in log proliferation rates that were on average 33% lower than the rates observed among control subjects or an effect size of ~0.56. Here, effect size was defined as the absolute value of the ratio of the differences of the two means to the (common) SD. For a sample size of 128, the asymptotic power of the two-sided, two-sample t test, at a level of 0.05, is 0.90 for detecting an effect size of 0.50, assuming that the proliferation indices in both arms are log-normal with common variance. The accrual target was set at 160 patients (40 per arm) to account for attrition and the possibility of a weak negative interaction between flaxseed supplementation and low-fat factors with respect to proliferation rate. Although power calculations were based on the primary endpoint (proliferation index), data from our pilot study suggested that there also would be comparable power to detect differences between arms with respect to secondary outcomes. No adjustment was made for multiple comparisons because these analyses were considered exploratory.

Analyses were based on the intent-to-treat principle, and all participants were included in the arm to which they were randomized. For the primary hypotheses, the analysis population was restricted to those patients from whom cell counts (numerator and denominator) were available from both readers. Kruskal-Wallis tests were done on the number of evaluated cells to ensure that denominators did not differ between study arms; they did not (P = 0.90). For each study participant, the proliferation ''score'' was defined as the log of the average of the proliferation index from each reader.

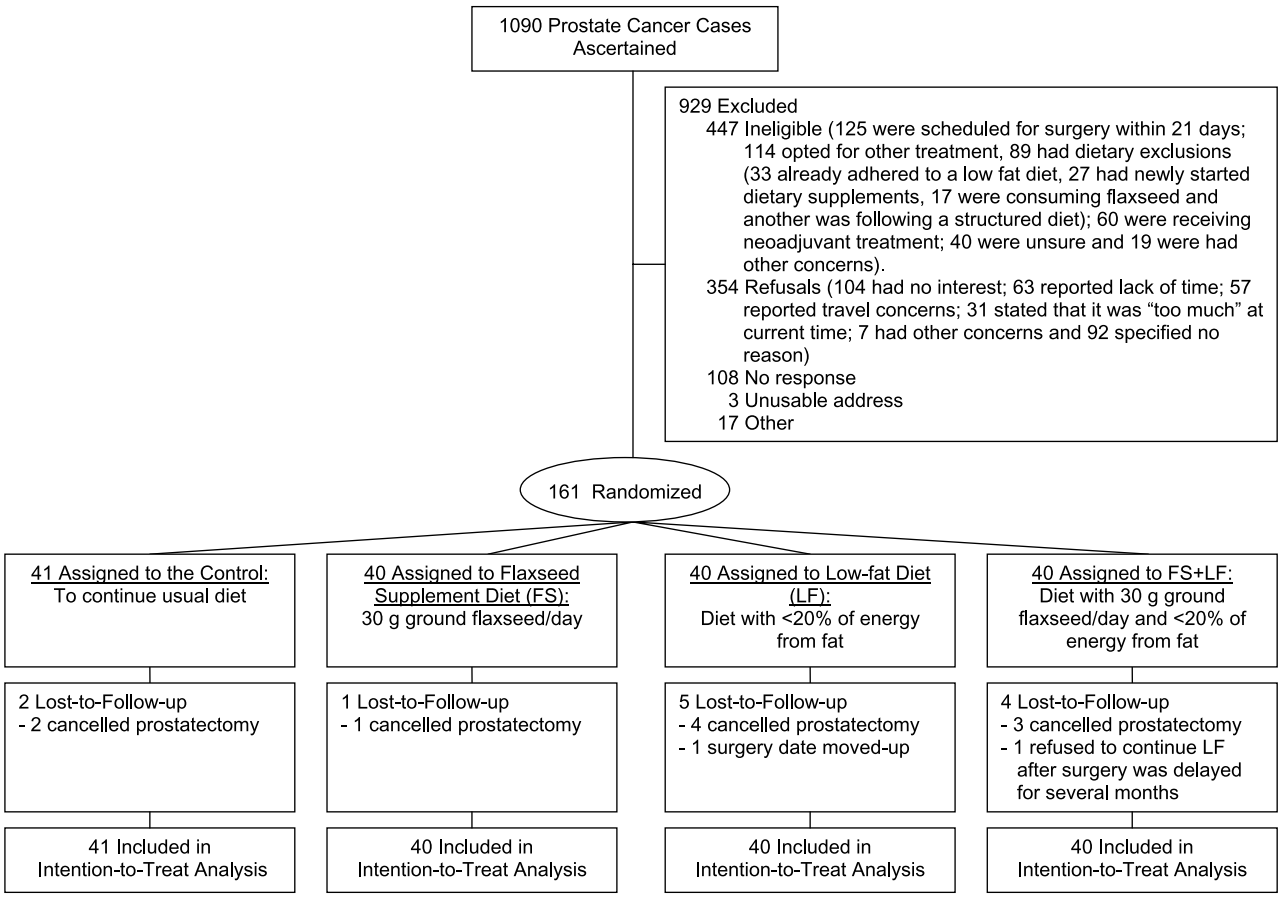

Figure 1. CONSORT trial flow diagram.

Table 1. Characteristics of the study sample

	Total (n = 161)	Controls (n = 41)	FS (n = 40)	LF (n = 40)	FS + LF (n = 40)	Significance
Age (y), range = 36-73						
Mean ± SD	59.2 ± 7.3	58.2 ± 6.8	60.2 ± 7.0	59.2 ± 8.0	59.3 ± 7.6	NS
<65, % (n)	74 (119)	78 (32)	73 (29)	70 (28)	75 (30)	NS
≥65, % (n)	26 (42)	22 (9)	27 (11)	30 (12)	25 (10)	
Race, % (n)						
White	70 (112)	68 (28)	67 (27)	70 (28)	72 (29)	NS
African American	26 (42)	27 (11)	28 (11)	25 (10)	25 (10)	
Other	4 (7)	5 (2)	5 (2)	5 (2)	3 (1)	
Education, % (n)						
High school or less/unknown	9 (15)	7 (3)	8 (3)	10 (4)	13 (5)	NS
High school graduate/GED	20 (32)	22 (9)	20 (8)	18 (7)	20 (8)	
Some college/trade	29 (46)	32 (13)	32 (13)	30 (12)	20 (8)	
College graduate/postgraduate	42 (68)	39 (16)	40 (16)	42 (17)	47 (19)	
Current smoker, % (n)	11 (18)	15 (6)	13 (5)	5 (2)	13 (5)	NS
Body mass index (kg/m^2)* % (n)						
Mean ± SD	28.8 ± 4.1	28.8 ± 4.0	28.5 ± 3.9	29.5 ± 4.3	28.5 ± 4.5	NS
18.5-24.9 (normal weight), % (n)	19 (31)	20 (8)	22 (9)	15 (6)	20 (8)	
25-29.9 (overweight), % (n)	46 (74)	46 (19)	45 (18)	47 (19)	45 (18)	
≥30 (obese), % (n)	35 (56)	34 (14)	33 (13)	38 (15)	35 (14)	
Comorbidity, % (n)						
Cardiovascular disease	35 (57)	34 (14)	28 (11)	38 (15)	43 (17)	NS
Diabetes	17 (27)	12 (5)	23 (9)	15 (6)	18 (7)	
Medication use, % (n)						
Nonsteroidal anti-inflammatory agents	38 (61)	39 (16)	33 (13)	40 (16)	40 (16)	NS
Statins	25 (41)	29 (12)	20 (8)	30 (12)	23 (9)	
Thiazolidinediones	8 (13)	10 (4)	10 (4)	3 (4)	10 (4)	
PSA at diagnosis (ng/mL), % (n)						
Mean ± SD	5.7 ± 3.8	5.2 ± 2.7	5.2 ± 2.4	5.6 ± 5.0	6.8 ± 4.3	NS
<4	23 (38)	27 (11)	23 (9)	28 (11)	18 (7)	NS
4-10	70 (112)	63 (26)	72 (29)	70 (28)	72 (29)	
10-20	6 (9)	10 (4)	5 (2)	0 (0)	8 (3)	
>20	1 (2)	0 (0)	0 (0)	2 (1)	2 (1)	
Biopsy Gleason score						
≤5 (2,2/2,3/3,2)	1 (4)	0	5 (2)	0	5 (2)	NS
6 (3,3)	63 (102)	66 (27)	60 (24)	67 (27)	60 (24)	
7 (3,4)	24 (39)	22 (9)	27 (11)	27 (11)	20 (8)	
7 (4,3)	6 (10)	7 (3)	5 (2)	3 (1)	10 (4)	
≥8 (4,4/5,3/5,4)	4 (6)	5 (2)	3 (1)	3 (1)	5 (2)	

NOTE: No participants were underweight (body mass index < 18.5).

Per protocol, the association between the log-transformed proliferation score and flaxseed supplementation and the log-transformed score and low-fat were to be tested using a two-sample t test. For cases where the score was zero, the minimum nonzero score was imputed. We also used a Generalized Estimating Equation approach (Poisson variance function) as well as a Generalized Linear Mixed Effects approach (Poisson distribution conditional on the random effects) to model the proliferation counts as a function of the experimental factors and covariables while accounting for the variability between the readers and among the patients. However, the results from these approaches added little to the simpler approaches. Analyses revealed no evidence that the low-fat diet was associated with the proliferation score; however, there was strong statistical evidence that flaxseed supplementation was associated with the score. Thus, to follow-up further on the flaxseed supplementation result, we employed a battery of sensitivity analyses. Unstratified and stratified (by low-fat) two-sample Wilcoxon tests were used to assess sensitivity with respect to the assumption of normality, the log transformation, and the imputation for ratios of zero. For two patients, the readings from one of the pathologists were not available. Both of these patients were from the flaxseed-supplemented arms. For these missing data, we imputed the maximum score for the reader to generate a worst-case scenario unfavorable to the flaxseed supplementation effect. These results supported our initial finding that flaxseed supplementation is associated with the score.

Following the protocol, standard linear regression models were used to analyze the effect of baseline variables such as race, age, body mass index, and biopsy Gleason sum in a multivariate model. For all other univariate analyses, the association between continuous outcomes and factors were tested using t tests, whereas the association between frequency outcomes and factors were tested using χ^2 tests for contingency tables.

Results

Patients. Between July 5, 2002 and April 17, 2006, a total of 1,090 men with histopathologically confirmed prostatic carcinoma were screened and 161 were randomly assigned to one of four treatment arms (Fig. 1). Leading reasons for nonaccrual were ineligibility due to selection of other treatment besides prostatectomy or because surgery was scheduled within a 21-day window

Table 2. Days on protocol, side effects, self-rated adherence, biomarkers of adherence, and nutrition-related changes in target tissues

	Controls (n = 41)	FS (n = 40)	LF (n = 40)	FS + LF (n = 40)	P*
Days on protocol					
Median (95% CI)	30 (22-29)	31 (25-32)	30 (25-29)	30 (24-30)	FS = 0.960/LF = 0.900
Side effects (grade 1/2/3 toxicity), %					
Nausea	7/0/0	5/0/0	21/3/0	8/0/0	0.467
Vomiting	0/0/0	0/0/0	0/0/0	0/0/0	NA
Diarrhea	5/3/0	18/0/0	6/0/0	14/0/0	0.308
Libido/impotence	15/5/10	8/3/8	24/0/6	11/3/8	0.633
Allergy	0/3/0	0/0/0	0/0/0	0/3/0	0.436
Flaxseed (% of 30 g dose taken)					
Median (95% CI)	NA	97.5 (97.5-100)	NA	100 (100-100)	—
Dietary lignans (μg/d)					
Median (95% CI)					
Baseline	253 (192-313)	282 (217-320)	263 (182-306)	289 (223-310)	FS < 0.0001/LF = 0.905
Follow-up	257 (219-292)	260,935 (257,229-268,591)	250 (227-329)	262,402 (258,984-264,174)	
Urinary lignans (ng/mg creatinine)					
Median (95% CI)					
Baseline	257 (113-456)	228 (147-609)	308 (130-431)	277 (197-447)	FS < 0.0001/LF = 0.493
Follow-up	239 (123-473)	10,566 (5,187-15,897)	415 (194-754)	10,358 (4,003-13,434)	
Seminal fluid lignans (μg/mL)					
Median (95% CI)†					
Baseline	182 (157-274)	180 (162-266)	202 (176-278)	195 (139-244)	FS = 0.013/LF = 0.797
Follow-up	262 (139-423)	430 (202-535)	293 (91-410)	362 (137-701)	
Energy intake					
Median (95% CI)					
Baseline	2,187 (1,999-2,503)	2,131 (1,749-2,572)	1,769 (1,530-1,955)	1,748 (1,439-2,392)	FS = 0.591/LF = 0.068
Follow-up	1,959 (1,591-2,376)	2,143 (1,715-2,654)	1,689 (1,423-1,997)	1,536 (1,370-1,929)	
% Calories from fat					
Median (95% CI)					
Baseline	34.6 (29.7-41.5)	35.3 (32.5-37.9)	36.4 (31.6-38.4)	34.5 (32.4-38.6)	FS = 0.092/LF < 0.0001
Follow-up	34.2 (29.7-39.7)	34.0 (28.8-37.2)	27.5 (25.2-31.7)	24.8 (22.2-28.7)	
Dietary ω3FA (% kcal)					
Median (95% CI)					
ALA (18.3)					
Baseline	0.074 (0.067-0.082)	0.072 (0.062-0.078)	0.077 (0.064-0.087)	0.076 (0.064-0.084)	FS < 0.0001/LF = 0.469
Follow-up	0.076 (0.067-0.090)	0.315 (0.260-0.392)	0.064 (0.059-0.074)	0.433 (0.334-0.474)	
EPA (20:5)					
Baseline	0.000 (0.000-0.001)	0.001 (0.001-0.002)	0.001 (0.001-0.002)	0.001 (0.001-0.002)	FS = 0.701/LF = 0.005
Follow-up	0.001 (0.001-0.002)	0.001 (0.001-0.002)	0.002 (0.001-0.002)	0.002 (0.001-0.005)	
Docosahexanoic acid (22:6)					
Baseline	0.000 (0.000-0.001)	0.001 (0.000-0.001)	0.001 (0.000-0.001)	0.001 (0.001-0.001)	FS = 0.163/LF = 0.006
Follow-up	0.001 (0.001-0.001)	0.001 (0.000-0.001)	0.001 (0.001-0.001)	0.001 (0.001-0.001)	
Dietary ω6FA (% kcal)					
Median (95% CI)					
Linoleic acid (18:2)					
Baseline	0.745 (0.623-0.867)	0.705 (0.632-0.772)	0.755 (0.645-0.876)	0.799 (0.704-0.870)	FS = 0.780/LF < 0.0001
Follow-up	0.782 (0.688-0.867)	0.733 (0.679-0.803)	0.582 (0.519-0.669)	0.616 (0.587-0.686)	
Arachidonic acid (20:4)					
Baseline	0.006 (0.005-0.007)	0.006 (0.005-0.007)	0.006 (0.005-0.007)	0.006 (0.005-0.007)	FS = 0.036/LF = 0.180
Follow-up	0.006 (0.005-0.007)	0.005 (0.004-0.007)	0.006 (0.005-0.007)	0.005 (0.004-0.006)	
Dietary ω3FA/ω6FA ratio					
Median (95% CI)					
Baseline	0.11 (0.10-0.12)	0.10 (0.09-0.11)	0.11 (0.10-0.12)	0.11 (0.10-0.11)	FS < 0.0001/LF = 0.0102
Follow-up	0.10 (0.10-0.12)	0.46 (0.36-0.60)	0.11 (0.11-0.12)	0.71 (0.52-0.82)	
Erythrocyte ω3FA (%)					
Median (95% CI)					
ALA (18:3)					
Baseline	0.000 (0.000-0.000)	0.000 (0.000-0.000)	0.000 (0.000-0.000)	0.000 (0.000-0.000)	FS = 0.520/LF = 0.290
Follow-up	0.000 (0.000-0.000)	0.000 (0.000-0.000)	0.000 (0.000-0.000)	0.000 (0.000-0.000)	
EPA (20:5)					
Baseline	0.633 (0.539-0.765)	0.723 (0.581-0.941)	0.651 (0.440-0.890)	0.664 (0.613-0.793)	FS = 0.005/LF = 0.705
Follow-up	0.651 (0.533-0.793)	0.784 (0.651-0.890)	0.475 (0.372-0.620)	0.825 (0.702-1.101)	
Docosahexanoic acid (22:6)					
Baseline	5.95 (5.54-6.64)	6.01 (4.73-6.80)	5.62 (4.55-6.53)	5.82 (5.00-6.32)	FS = 0.077/LF = 0.320
Follow-up	6.03 (5.01-7.09)	5.51 (4.05-6.72)	4.92 (4.36-6.38)	6.07 (5.43-7.08)	
Erythrocyte ω6FA (%)					
Median (95% CI)					
Linoleic acid (18:2)					
Baseline	11.11 (10.30-11.72)	11.04 (10.03-12.11)	11.05 (9.99-11.85)	11.09 (10.14-11.45)	FS = 0.544/LF = 0.037
Follow-up	10.46 (9.23-11.53)	10.83 (9.73-11.53)	9.71 (8.39-11.64)	9.94 (9.32-10.83)	

(*Continued on the following page*)

Table 2. Days on protocol, side effects, self-rated adherence, biomarkers of adherence, and nutrition-related changes in target tissues (Cont'd)

	Controls (n = 41)	FS (n = 40)	LF (n = 40)	FS + LF (n = 40)	P*
Arachidonic acid (20:4)					
Baseline	0.347 (0.292-0.371)	0.368 (0.293-0.428)	0.290 (0.230-0.419)	0.336 (0.279-0.391)	FS = 0.650/LF = 0.250
Follow-up	0.294 (0.218-0.337)	0.358 (0.259-0.390)	0.303 (0.217-0.357)	0.324 (0.269-0.380)	
Erythrocyte ω3FA/ω6FA ratio					
Median (95% CI)					
Baseline	0.60 (0.49-0.72)	0.62 (0.50-0.71)	0.55 (0.44-0.72)	0.56 (0.48-0.66)	FS = 0.480/LF = 0.090
Follow-up	0.61 (0.47-0.78)	0.54 (0.42-0.76)	0.50 (0.39-0.76)	0.64 (0.59-0.78)	
Prostatic ω3FA (%)					
Median (95% CI)					
ALA (18:3)					
Follow-up	0.000 (0.000-0.000)	0.000 (0.000-0.000)	0.000 (0.000-0.000)	0.000 (0.000-0.000)	FS = 0.662/LF = 0.924
EPA (20:5)					
Follow-up	0.216 (0.148-0.268)	0.300 (0.221-0.385)	0.195 (0.133-0.259)	0.298 (0.245-0.428)	FS = 0.010/LF = 0.970
Docosahexanoic acid (22:6)					
Follow-up	3.53 (3.11-4.27)	3.68 (3.17-4.53)	3.05 (3.34-4.21)	3.94 (3.37-4.37)	FS = 0.580/LF = 0.130
Prostatic ω6FA (%)					
Median (95% CI)					
Linoleic acid (18:2)					
Follow-up	9.50 (8.99-10.28)	9.48 (8.47-10.66)	9.37 (8.34-10.52)	9.47 (8.75-10.35)	FS = 0.210/LF = 0.820
Arachidonic acid (20:4)					
Follow-up	0.269 (0.235-0.307)	0.247 (0.221-0.295)	0.259 (0.235-0.274)	0.241 (0.231-0.285)	FS = 0.230/LF = 0.760
Prostatic ω3FA/ω6FA ratio					
Median (95% CI)					
Follow-up	0.40 (0.32-0.45)	0.42 (0.34-0.50)	0.42 (0.34-0.48)	0.43 (0.38-0.47)	FS = 0.175/LF = 0.974

NOTE: Average dose of flaxseed compared with amount prescribed and/or percentage of days adhered to <20% of kilocalories from fat.
*For factors that have both baseline and follow-up data, factorial testing was done under intent-to-treat analysis on change scores and P values are reported for both FS and LF conditions.
† Note that seminal fluid was unavailable on several participants; the numbers of samples for baseline and follow-up among the study arms are as follows: control, n = 19 and n = 13; FS, n = 17 and n = 12; LF, n = 22 and n = 10; and FS + LF, n = 16 and n = 10, respectively.

(see ref. 26 for details). Cancelled or rescheduled prostatectomy was the sole reason for attrition; dropout rates were 7.5%. No age or race differences were observed between participants and nonparticipants and study completers and those lost to follow-up. No differences in attrition were observed between study arms.

Table 1 provides the baseline characteristics of the study sample. The trial was successful in accruing a racially representative sample, although the proportion of college-educated participants was higher than the population at large (42% versus 31%; ref. 43). Data on PSA and biopsy Gleason sum suggested that most participants had earlier-stage disease, with roughly two-thirds having biopsy Gleason sums of ≤6. As in the general population, a majority of these middle-aged men were overweight or obese, and substantial numbers had cardiovascular disease or diabetes and regularly took medications associated with these conditions. No differences existed between study arms with regard to any of these variables.

Table 2 presents data on protocol duration, reported side effects, self-rated adherence, biological markers of adherence, and effect of dietary modification on body fluids and target tissues. The average duration on study was 30.7 days, with little variation among arms. By and large, there was a complete absence or only mild side effects reported. However, several participants reported symptoms of low libido or erectile dysfunction, although no differences were observed between study arms for this or any other side effects.

Dietary logs revealed good to excellent adherence. Adherence to flaxseed supplementation was supported by significantly higher lignan intakes and expression within urine and seminal fluid. Participants assigned to low-fat diets significantly reduced their fat intakes to 25% to 28% of calories and had higher dietary intakes of EPA because they substituted fish for red meat in efforts to reduce overall fat consumption. However, the proportion of dietary ω3FA:ω6FA was significantly higher among flaxseed-supplemented participants owing to significantly higher intakes of ALA. Both erythrocytes and prostatic tissue had significantly higher levels of EPA (not ALA) in the flaxseed-supplemented arms, suggesting that dietary ALA from flaxseed sources may be converted to longer chained ω3FA *in vivo*. The prostatic tissue of flaxseed-supplemented participants also had significantly higher proportions of ω3FA (ALA + EPA + docosahexanoic acid) to ω6FA (arachidonic acid + linoleic acid).

Tumor proliferation rate (primary endpoint) was significantly lower in the flaxseed-supplemented arms (Table 3; Fig. 2). Although the significance level varied depending on the various methods used, findings always remained significant (P values = 0.0007-0.02; ref. 25). Although the flaxseed supplementation effect appeared somewhat stronger among African American patients and also among those with Gleason sums of <7, these results were not statistically significant based on standard statistical interaction tests. In contrast, there was no statistical evidence, suggesting an effect of the low-fat diet on proliferation. Furthermore, no differences among treatment arms were noted for apoptosis or Gleason sum.

Over the presurgical study period, serum PSA, testosterone, IGF-I, and IGFBP-3 decreased in all arms, with no differences in change observed between arms. Within the control arm, these decreases were significant

Table 3. Histopathologic, serologic, and physiologic outcomes

	Controls (n = 41)	FS (n = 40)	LF (n = 40)	FS + LF (n = 40)	P*
Tumor proliferation rate (Ki-67), median (95% CI)	3.23 (2.42-3.92)	1.66 (1.13-2.64)	2.56 (2.00-3.69)	1.50 (1.05-2.65)	FS = 0.0013/LF = 0.661
Tumor apoptotic rate (TdT-mediated dUTP nick end labeling), % (n)					
0	84 (33)	74 (29)	74 (26)	89 (32)	FS = 0.880/LF = 0.730
>0 to 1	13 (5)	16 (6)	14 (5)	3 (1)	
>1 to 2	3 (1)	10 (4)	12 (4)	8 (3)	
Gleason sum, median (95% CI)					
Biopsy	6 (6-7)	6 (6-6)	6 (6-6)	6 (6-7)	FS = 0.538/LF = 0.918
Surgical	7 (6-7)	6 (6-7)	6 (6-7)	7 (6-7)	
PSA (ng/mL), median (95% CI) †					
Baseline	5.3 (3.7-5.8)	6.2 (4.8-7.7)	5.5 (4.6-6.7)	5.9 (4.9-9.4)	FS = 0.286/LF = 0.764
Follow-up	4.9 (3.5-6.2)	6.4 (5.0-7.0)	5.6 (3.9-6.7)	5.7 (4.9-8.6)	
Testosterone (ng/dL), median (95% CI) †					
Baseline	442 (386-458)	424 (358-475)	423 (387-476)	414 (346-446)	FS = 0.120/LF = 0.394
Follow-up	372 (334-429)	387 (351-430)	377 (327-399)	382 (346-448)	
SHBG (nmol/L), median (95% CI) †					
Baseline	31 (27-39)	34 (25-41)	33 (28-37)	31 (28-35)	FS = 0.597/LF = 0.066
Follow-up	28 (22-37)	31 (28-37)	31 (26-35)	32 (26-35)	
Free androgen index, median (95% CI) ‡					
Baseline	13.3 (10.9-14.3)	12.6 (11.5-14.2)	13.2 (11.8-14.3)	12.8 (11.9-14.7)	FS = 0.148/LF = 0.123
Follow-up	12.1 (11.0-13.0)	12.0 (10.9-15.0)	11.3 (10.2-13.0)	11.8 (10.6-13.2)	
IGF-I (ng/mL), median (95% CI) †					
Baseline	120 (106-133)	124 (115-148)	133 (109-150)	129 (110-148)	FS = 0.174/LF = 0.370
Follow-up	112 (98-128)	119 (107-133)	123 (100-141)	125 (113-139)	
IGFBP-3 (mg/L), median (95% CI) †					
Baseline	4.2 (3.6-4.5)	4.1 (3.9-4.5)	4.2 (4.0-4.5)	4.1 (3.9-4.9)	FS = 0.859/LF = 0.853
Follow-up	3.7 (3.3-4.5)	4.0 (3.5-4.3)	3.8 (3.3-4.3)	3.8 (3.6-4.3)	
C-reactive protein (mg/L), median (95% CI) †					
Baseline	1.5 (1.1-2.2)	1.4 (1.0-2.7)	1.6 (1.2-2.5)	1.2 (0.9-2.5)	FS = 0.668/LF = 0.982
Follow-up	1.6 (1.1-2.4)	1.8 (1.3-2.3)	2.0 (1.1-3.2)	1.1 (0.8-2.1)	
Total cholesterol (mg/dL), median (95% CI) †					
Baseline	230 (212-252)	217 (205-245)	222 (206-240)	218 (210-228)	FS = 0.174/LF = 0.048
Follow-up	196 (180-226)	211 (118-220)	182 (161-192)	183 (168-206)	
Low-density cholesterol (mg/dL), median (95% CI) †					
Baseline	130 (113-144)	124 (110-138)	134 (122-146)	123 (115-141)	FS = 0.621/LF = 0.032
Follow-up	111 (90-128)	111 (89-121)	105 (90-124)	107 (93-121)	
Body mass index (kg/m^2), median (95% CI)					
Baseline	28.9 (27.4-30.0)	28.0 (26.8-29.8)	29.0 (27.0-31.0)	27.1 (25.8-30.4)	FS = 0.496/LF = 0.003
Follow-up	29.1 (27.2-30.0)	27.6 (26.8-29.7)	28.0 (26.5-29.5)	26.9 (25.3-29.5)	

*For factors that have both baseline and follow-up data, factorial testing was done under intent-to-treat analysis on change scores and P values are reported for both FS and LF conditions.

† Intraassay and interassay coefficients of variation, with accompanying mean (SD) are as follows: PSA 1.2%, 4.39 (0.05) ng/mL and 3.7%, 4.61 (0.17) ng/mL; testosterone 4.4%, 365.4 (12.7) ng/dL and 6.6%, 365.4 (18.1) ng/dL; SHBG 2.5%, 21 (0.52) nmol/L and 5.2%, 21 (1.1) nmol/L; IGF-I 3.8%, 169 (6.9) ng/mL and 5.4%, 169 (9.1) ng/mL; IGFBP-3 4.2%, 3.59 (0.15) mg/L and 7.2%, 3.59 (0.26) mg/mL; C-reactive protein 1.8%, 6.2 (0.07) mg/L and 2.9%, 6.2 (0.1) mg/L; total cholesterol 0.8%, 231 (5.98) mg/dL and 210.1 (5.44) mg/dL; and low-density cholesterol 1.1%, 166 (3.9) mg/dL and 1.8%, 166 (4.3) mg/dL.

‡ Free androgen index derived using the formula: total testosterone/SHBG.

for testosterone and PSA (P values < 0.05) and of borderline significance for IGF-I (P = 0.0547). No between arm differences were observed in change scores for SHBG, free androgen index, and C-reactive protein.

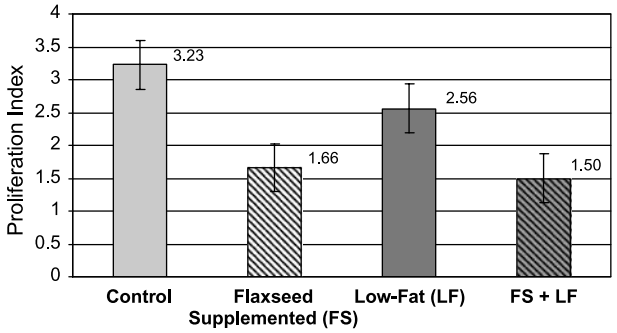

Figure 2. Median tumor proliferation rates.

Participants in the low-fat arms experienced significant reductions in body mass index and total and low-density lipoprotein cholesterol.

Discussion

Although other studies have employed presurgical models to test the effects of complementary therapies on prostate cancer (44, 45), to our knowledge, this has been the largest effort to date. Not only does it show the feasibility of implementing complex prevention trials within the community setting, it also suggests that flaxseed is well-accepted and safe to use and may affect tumor proliferation rates. These effects appear independent of dietary fat intake, although we lacked adequate power to detect interactions. Furthermore, mean decreases in fat intake to only 25%–28.4% of total calories, instead of the prescribed level of <20%, may have jeopardized our ability to observe effects that may

have accompanied better adherence. The low-fat diet has shown success in other studies, although these studies also included exercise and endpoints differed (10, 12).

Our observation of lower proliferation rates with flaxseed supplementation is consistent with our previous *in vitro* work in LNCaP, DU-145, and PC-3 prostate cancer cell lines, which also found inhibited cell growth with exposure to flaxseed-derived lignans (8). Lower cellular proliferation and reduced tumor burden and urogenital weight also were found in our preclinical study using the transgenic adenocarcinoma mouse model, which compared 5% flaxseed supplementation versus an elemental control diet (AIN-76A; ref. 7). Furthermore, the lower proliferation rates were observed with flaxseed supplementation in the current study, parallel findings from our previous clinical studies, one that also used a presurgical model and found lower proliferation indexes among 25 patients assigned to a flaxseed-supplemented, low-fat diet compared with historic controls matched on Gleason sum, PSA at diagnosis, disease laterality, race, and age (5), and another that found reduced pre-post proliferation rates in the benign epithelium of patients with abnormal biopsies scheduled for rebiopsy (6). Reduced proliferation rates with flaxseed also have been observed by Thompson et al. who employed a presurgical model in breast cancer ($n = 32$) and found a 34.2% reduction in the Ki-67 labeling index ($P = 0.001$; ref. 36); the strength of this study is that the tumor proliferation rate in biopsy specimens serves as a strong baseline measure from which to assess change in tumors in the breast, whereas in prostate cancer the ability to assess change from biopsy to surgery is limited given its multifocal and biologically diverse nature. Animal studies by Thompson et al. also support reduced proliferation rates with flaxseed supplementation (46, 47). Therefore, all published studies have observed lower or reduced proliferation rates with flaxseed supplementation, thus providing a consistent finding.

However, unlike previous reports, including one of our recent studies that found that flaxseed-derived lignans induced apoptosis in LNCaP cells via a mitochondrial-mediated case-dependent pathway (4-7, 46, 47), we did not observe differences in apoptosis between treatment arms. Indeed, we observed little variation in TdT-mediated dUTP nick end labeling scores in this study, because apoptosis was either absent or negligible in the majority of our samples. Reasons for this are unknown.

In contrast to our previous studies conducted among men with prostate cancer and those with abnormal biopsies that showed high-grade prostatic intraepithelial neoplasia or foci of atypical cells (5, 6), we did not observe differences in PSA change between the study arms. Curiously, all study arms experienced significant decreases in both PSA and testosterone during the presurgical period. Although Nakashima et al. (48) report consistent decreases in testosterone among patients from pre-anesthesia to 7 days post-prostatectomy, there are no antecedent reports of decreases in testosterone or PSA during the presurgical period. A handful of reports exist, however, describing declines in testosterone with acute stress imposed in the laboratory setting or observed in community-dwelling subjects under a variety of situational factors (49). Therefore, the decreases in testosterone observed in this study may relate to the acute stress attendant with impending surgery—a decline in testosterone that then drives PSA downward. Further study is needed to support or refute this conjecture. The decreases in PSA noted within the control arm also point to the importance of a randomized controlled design during this period and provide evidence that subjects are unable to serve as their own controls.

Our initial premise that flaxseed exerts its effects through androgen and IGF pathways was unsupported at least with respect to the biomarkers tested. Indeed, it is possible that other biomarkers assessed along these pathways might be responsible for the effects that we witnessed. For example, reductions in intracellular 5α-reductase, effects on IGFBP-1 or IGFBP-2, or other mechanisms may be at play, such as natural killer cell activity, vascular endothelial growth factor, etc. (2, 3). Eicosanoid-related pathways may hold particular promise because our data suggest that the ALA in flaxseed may be converted to EPA in both the erythrocytes and the target tissue. Therefore, membrane-mediated events that directly relate to the mechanical integrity of cell membranes or to signal transduction also warrant further exploration, as do mechanistic studies that build on recent work, suggesting that ω3FA may affect HER2 (erbB-2) oncogene expression and thus hold promise for both breast and prostate cancers (50). In addition to mechanistic studies, investigations also are needed to determine dose-response and effects among patients who manifest recurrent disease after surgery or those electing expectant management.

An unexpected finding of this study was despite the fact that ALA intakes were significantly higher among flaxseed-supplemented men, we did not find any evidence that this translated into higher levels of ALA in the erythrocytes or prostatic tissue. Instead, we found evidence that EPA levels were higher, thus suggesting that conversion of ALA to higher-chained ω3FA may occur and may not be as rate-limiting as thought previously (23). Speculation exists as to whether ALA from various sources is metabolized differently or may be influenced by energy balance or temporal changes in the hormonal milieu, thus calling for further investigation. Therefore, more research is needed regarding ALA and prostate cancer, especially studies that control for salient risk factors and that can distinguish between markers of dietary intake or of energy balance and those that are on the causal pathway (17). Although erythrocyte levels of fatty acids provide a reliable measure of intermediate intake (51, 52) in conducting further study, the use of other methods, such as radioisotope tracing to discern immediate effects on metabolism as well as fatty acid analyses of fat biopsy tissue for longer-term investigations, would be of interest.

Additionally, this trial produced findings that again support the benefits of a low-fat diet in reducing serum lipids and helping with weight management via a reduction in energy intake. Although the reduction in dietary fat to 25%–28.4% of total energy did not translate specifically into favorable outcomes in prostate cancer associated endpoints, because cardiovascular disease is a leading comorbid factor among men with prostatic carcinoma (2), this study provides favorable findings for both interventions. However, unlike a low-fat diet that has proven benefit for cardiovascular disease (53),

further studies are needed before we can definitively support flaxseed supplementation as a proven complementary therapy for prostate cancer. To date, however, the evidence suggests that flaxseed (*a*) is a good, low-cost source of select vitamins and minerals and fiber, (*b*) is well-accepted and safe to use, and (*c*) warrants further testing as a preventive or complementary therapy for prostate cancer.

Caution, however, is warranted in generalizing these findings. Limitations that are specific to the study design (lack of a placebo control and lack of power to detect the potential effect of the low-fat diet or interactions by study arm or race), the study sample (overrepresentation of more highly educated men), or inherent difficulties in conducting prostate cancer research (the multifocality of prostate cancer or small volume disease) may have influenced our findings. Although overcoming these challenges may be difficult (e.g., the creation of a food product that could successfully mask a 30 g dose of flaxseed), others such as conducting further research to determine dose-response and additional mechanisms of action, as well as further studies aimed at determining the potential synergy between low-fat and flaxseed regimens, or potentially stronger effects among African Americans are of particular interest.

In summary, this pre-prostatectomy evaluation of the chemopreventive potential of two nutritional interventions uses surrogate endpoint biomarkers as primary endpoints. In our study, the modest sample size and short duration, together with the infrequency of cancer recurrence, precluded investigation of clinical cancer endpoints. Furthermore, the development and validation of molecular markers as modifiable surrogates for preferred clinical endpoints remains a work in progress. Although the strength of conclusions drawn from our data is limited by these factors, our study makes several important contributions to clinical intervention trial implementation in cancer prevention. Indeed, the down-regulation of Ki-67, a candidate surrogate for cell proliferation, in the flaxseed-treated arms is highly suggestive of an anticarcinogenic effect on prostate cancer cells *in vivo*. Thus, this study serves to generate hypotheses for future larger trials in which flaxseed supplementation can be juxtaposed against prostate cancer recurrence, thereby testing the cancer preventive efficacy of the intervention as well as contributing to the literature documenting the validity of Ki-67 as a surrogate endpoint biomarker. The current emphasis on biomarker development is thus well-served by this study. Therefore, this study not only contributes to development of nutritional preventive interventions for prostate cancer but also exemplifies the successful implementation of a study model in which biomarker development is carried out in a cancer prevention trial that targets accrual within the community setting.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank the following individuals who helped to facilitate this trial: Mary Jo Beck; Jennifer Blevins; Martha Boggs; Virginia Bolin; Luellen Bratt; Philipp Dahm, M.D.; Diane Dowdee; Luisa Eisen; Brian Evans, M.D.; Nicholas Fitzsimons, M.D.; Linda Hofbauer; Rita Joost; Kuravilla Kurian, Ph.D.; Beth Lavasseur; Deborah Lee; Charles Marguet, M.D.; Peggy McGraw; Paige Miller; Alister Muir, Ph.D.; Kristy McKiernan-Borawski, M.D.; Brian Murphy, M.D.; Regina Norris, M.D.; David Paulson, M.D.; Mary Beth Reardon; Allana Richmond; Charles Scales, M.D.; Candice Singletary; Courtney Streeter; Gregory Taylor, Ph.D.; Wayne Terrell; Jody Thomas; Timothy Tseng, M.D.; Valeda Stull; Petra Wahnefried; and Jeremy Wiygul, M.D., as well as Norwood and David Bryan and Wesley Jones, M.D, for support.

References

1. Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2008. CA Cancer J Clin 2008;58:71–96.
2. Canby-Hagino ED, Thompson IM. Mechanisms of disease: prostate cancer—a model for cancer chemoprevention in clinical practice. Nat Clin Pract Oncol 2005;2:255–61.
3. Nelson WG, De Marzo AM, Isaacs WB. Prostate cancer. N Engl J Med 2003;349:366–81.
4. Chen L-H, Fang J, Li H, Demark-Wahnefried W, Lin X. Enterolactone induces apoptosis in human prostate carcinoma LNCaP cells via a mitochondrial-mediated, caspase-dependent pathway. Mol Cancer Ther 2007;6:2581–90.
5. Demark-Wahnefried W, Price DT, Polascik TJ, et al. A pilot study of dietary fat restriction and flaxseed supplementation in men with prostate cancer pre-surgery: exploring effects on hormonal levels, PSA and histopathology. Urology 2001;58:47–52.
6. Demark-Wahnefried W, Robertson CN, Walther PJ, Polascik TJ, Paulson DF, Vollmer RT. Pilot study to explore effects of low-fat, flaxseed-supplemented diet on proliferation of benign prostatic epithelium and prostate specific antigen. Urology 2004;63:900–4.
7. Lin X, Gingrich JR, Bao W, Li J, Haroon ZA, Demark-Wahnefried W. The effect of flaxseed supplementation on prostatic carcinoma in transgenic mice. Urology 2002;60:919–24.
8. Lin X, Switzer BR, Demark-Wahnefried W. Effect of mammalian lignans on the growth of prostate cancer cell lines. Anticancer Res 2001;21:3995–4000.
9. Denis L, Morton MS, Griffiths K. Diet and its preventive role in prostatic disease. Eur Urol 1999;35:377–87.
10. Link LB, Thompson SM, Bosland MC, Lumey LH. Adherence to a low-fat diet in men with prostate cancer. Urology 2004;64:970–5.
11. McCann MJ, Gill CI, McGlynn H, Rowland IR. Role of mammalian lignans in the prevention and treatment of prostate cancer. Nutr Cancer 2005;52:1–14.
12. Tymchuk CN, Barnard RJ, Heber D, Aronson WJ. Evidence of an inhibitory effect of diet and exercise on prostate cancer cell growth. J Urol 2001;166:185–9.
13. Thompson LU. Flaxseed, lignans, and cancer. In: Cunnane SC, Thompson LU, editors. Flaxseed in human nutrition. Chicago (IL): AOCS Press; 1995. p. 219–36.
14. Hall C III, Tulbek MC, Xu Y. Flaxseed. Adv Food Nutr Res 2006;51:1–79.
15. Webb AL, McCullough ML. Dietary lignans: potential role in cancer prevention. Nutr Cancer 2005;51:117–31.
16. Astorg P. Dietary N-6 and N-3 polyunsaturated fatty acids and prostate cancer risk: a review of epidemiological and experimental evidence. Cancer Causes Control 2004;15:367–86.
17. Demark-Wahnefried W. Flaxseed and prostate cancer: demon seed or seed of salvation? Semin Prev Altern Med 2006;2:205–7.
18. Freeman VL, Meydani M, Yong S, et al. Prostatic levels of fatty acids and the histopathology of localized prostate cancer. J Urol 2000;164:2168–72.
19. Koralek DO, Peters U, Andriole G, Reding D, Kirsh V. A prospective study of dietary α-linolenic acid and the risk of prostate cancer (United States). Cancer Causes Control 2006;17:783–91.
20. Schuurman AG, van den Brandt PA, Dorant E, Brants HA, Goldbohm RA. Association of energy and fat intake with prostate carcinoma risk: results from The Netherlands Cohort Study. Cancer 1999;86:1019–27.
21. Brouwer IA, Katan MB, Zock PL. Dietary α-linolenic acid is associated with reduced risk of fatal coronary heart disease, but increased prostate cancer risk: a meta-analysis. J Nutr 2004;134:919–22.
22. Leitzmann MF, Stampfer MJ, Michaud DS, et al. Dietary intake of n-3

and n-6 fatty acids and the risk of prostate cancer. Am J Clin Nutr 2004;80:204–16.
23. Lands WE. Biochemistry and physiology of n-3 fatty acids. FASEB J 1992;6:2530–6.
24. Kushi L, Giovannucci E. Dietary fat and cancer. Am J Med 2002;113 Suppl 9B:63–70S.
25. Shike M, Latkany L, Riedel E, et al. Lack of effect of a low-fat, high-fruit, -vegetable, and -fiber diet on serum prostate-specific antigen of men without prostate cancer: results from a randomized trial. J Clin Oncol 2002;20:3592–8.
26. Demark-Wahnefried W, George SL, Switzer BR, et al. Overcoming challenges in designing and implementing a phase II randomized controlled Trial using a presurgical model to test a dietary intervention in prostate cancer. Clin Trials 2008;5:262–72.
27. National Cancer Institute. Percent calories from fat screener. Available from: http://www.riskfactor.cancer.gov/diet/screeners/fat. Accessed November 13, 2007.
28. Lampe JW, Atkinson C, Hullar MA. Assessing exposure to lignans and their metabolites in humans. J AOAC Int 2006;89:1174–81.
29. Subar AF, Thompson FE, Kipnis V, et al. Comparative validation of the Block, Willett, and National Cancer Institute food frequency questionnaires: the Eating at America's Table Study. Am J Epidemiol 2001;154:1089–99.
30. Kohl HW, Blair SN, Jr., Paffenbarger RS, Macera CA, Kronenfeld JJ. A mail survey of physical activity habits as related to measured physical fitness. Am J Epidemiol 1988;127:1228–39.
31. Polascik TJ, Oesterling JE, Partin AW. Prostate specific antigen: a decade of discovery—what we have learned and where we are going. J Urol 1999;162:293–306.
32. Pomfret EA, daCosta KA, Schurman LL, Zeisel SH. Measurement of choline and choline metabolite concentrations using high-pressure liquid chromatography and gas chromatography-mass spectrometry. Anal Biochem 1989;180:85–90.
33. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 1959;37:911–7.
34. Morton MS, Chan PS, Cheng C, et al. Lignans and isoflavonoids in plasma and prostatic fluid in men: samples from Portugal, Hong Kong, and the United Kingdom. Prostate 1997;32:122–8.
35. Gamache PH, Acworth IN. Analysis of phytoestrogens and polyphenols in plasma. Proc Soc Exp Biol Med 1998;217:274–80.
36. Thompson LY, Chen JM, Li T, Strasser-Weippl K, Goss PE. Dietary flaxseed alters tumor biological markers in postmenopausal breast cancer. Clin Cancer Res 2005;11:3828–35.
37. Stasse-Wolthuis M, Hautvast JG, Hermus RJ, et al. The effect of a natural high-fiber diet on serum lipids, fecal lipids, and colonic function. Am J Clin Nutr 1979;32:1881–8.
38. Farmer KC. Methods for measuring and monitoring medication regimen adherence in clinical trials and clinical practice. Clin Ther 1999;21:1074.
39. National Cancer Institute. Common terminology criteria for adverse events (version 3). Available from: http://ctep.cancer.gov/reporting/ctc_v30.html. Accessed November 28, 2007.
40. Bostwick DG, Montironi R, Nogle R, et al. Current and proposed biologic markers in prostate cancer. J Cell Biochem 1992;16:65–7.
41. Kucuk O. Chemoprevention of prostate cancer. Cancer Metastasis Rev 2002;21:111–24.
42. Tateyama H, Tada T, Hattori H, Murase T, Li WX, Eimoto T. Effects of prefixation and fixation times on apoptosis detection by *in situ* end-labeling of fragmented DNA. Arch Pathol Lab Med 1998;122:252–5.
43. U.S. Bureau of Census. Educational attainment, 2000. Available from: http://www.census.gov/prod/2003pubs/c2kbr-24.pdf. Accessed June 9, 2008.
44. Kucuk O, Sarkar FH, Sakr W, et al. Phase II randomized clinical trial of lycopene supplementation before radical prostatectomy. Cancer Epidemiol Biomarkers Prev 2001;10:861–8.
45. Urban D, Myers R, Manne U, et al. Evaluation of biomarker modulation by fenretinide in prostate cancer patients. Eur Urol 1999;35:429–38.
46. Chen J, Power KA, Mann J, Cheng A, Thompson LU. Flaxseed alone or in combination with tamoxifen inhibits MCF-7 breast tumor growth in ovariectomized athymic mice with high circulating levels of estrogen. Exp Biol Med 2007;232:1071–80.
47. Chen J, Hui E, Ip T, Thompson LU. Dietary flaxseed enhances the inhibitory effect of tamoxifen on the growth of estrogen-dependent human breast cancer (MCF-7) in nude mice. Clin Cancer Res 2004;10:7703–11.
48. Nakashima A, Koshiyama K, Uozumi T, Monden Y, Hamanaka Y. Effects of general anaesthesia and severity of surgical stress on serum LH and testosterone in males. Acta Endocrinol 1975;78:258–69.
49. Zitzmann M, Nieschlag E. Testosterone levels in healthy men and the relation to behavioural and physical characteristics: facts and constructs. Eur J Endocrinol 2001;144:183–97.
50. Osman I, Mikhail M, Shuch B, et al. Serum levels of shed Her2/neu protein in men with prostate cancer correlate with disease progression. J Urol 2005;174:2174–7.
51. Poppitt SD, Kilmartin P, Butler P, Keogh G. Assessment of erythrocyte phospholipid fatty acid composition as a biomarker for dietary MUFA, PUFA or saturated fatty acid intake in a controlled cross-over intervention trial. Lipids Health Dis 2005;4:30–40.
52. Sun Q, Ma J, Campos H, Hankinson SE, Hu FB. Comparison between plasma and erythrocyte fatty acid content as biomarkers of fatty acid intake in US women. Am J Clin Nutr 2007;86:74–81.
53. Yu-Poth S, Zhao G, Etherton T, Naglak M, Jonnalagadda S, Kris-Etherton PM. Effects of the National Cholesterol Education Program's Step I and Step II dietary intervention programs on cardiovascular disease risk factors: a meta-analysis. Am J Clin Nutr 1999;69:632–46.

Flaxseed Supplementation (Not Dietary Fat Restriction) Reduces Prostate Cancer Proliferation Rates in Men Presurgery

Wendy Demark-Wahnefried, Thomas J. Polascik, Stephen L. George, et al.

Cancer Epidemiol Biomarkers Prev 2008;17:3577-3587.