

Research Article

Effect of a Low-Fat Fish Oil Diet on Proinflammatory Eicosanoids and Cell-Cycle Progression Score in Men Undergoing Radical ProstatectomyColette Galet¹, Kiran Gollapudi¹, Sevan Stepanian¹, Joshua B. Byrd¹, Susanne M. Henning², Tristan Grogan³, David Elashoff³, David Heber², Jonathan Said⁴, Pinchas Cohen⁵, and William J. Aronson^{1,6}**Abstract**

We previously reported that a 4- to 6-week low-fat fish oil (LFFO) diet did not affect serum insulin-like growth factor (IGF)-1 levels (primary outcome) but resulted in lower omega-6 to omega-3 fatty acid ratios in prostate tissue and lower prostate cancer proliferation (Ki67) as compared with a Western diet. In this *post hoc* analysis, the effect of the LFFO intervention on serum pro-inflammatory eicosanoids, leukotriene B4 (LTB4) and 15-S-hydroxyeicosatetraenoic acid [15(S)-HETE], and the cell-cycle progression (CCP) score were investigated. Serum fatty acids and eicosanoids were measured by gas chromatography and ELISA. CCP score was determined by quantitative real-time reverse transcriptase PCR (RT-PCR). Associations between serum eicosanoids, Ki67, and CCP score were evaluated using partial correlation analyses. BLT1 (LTB4 receptor) expression was determined in prostate cancer cell lines and prostatectomy specimens. Serum omega-6 fatty acids and 15(S)-HETE levels were significantly reduced, and serum omega-3 levels were increased in the LFFO group relative to the Western diet group, whereas there was no change in LTB4 levels. The CCP score was significantly lower in the LFFO compared with the Western diet group. The 15(S)-HETE change correlated with tissue Ki67 ($R = 0.48$; $P < 0.01$) but not with CCP score. The LTB4 change correlated with the CCP score ($r = 0.4$; $P = 0.02$) but not with Ki67. The LTB4 receptor BLT1 was detected in prostate cancer cell lines and human prostate cancer specimens. In conclusion, an LFFO diet resulted in decreased 15(S)-HETE levels and lower CCP score relative to a Western diet. Further studies are warranted to determine whether the LFFO diet antiproliferative effects are mediated through the LTB4/BLT1 and 15(S)-HETE pathways. *Cancer Prev Res*; 7(1); 97–104. ©2013 AACR.

Introduction

Prostate cancer is a leading cause of cancer death among men in the United States (1). It is estimated that 238,590 men will be diagnosed with prostate cancer and 29,720 men will die from the disease in 2013 (2). There is an ever-growing need to find new strategies to prevent the development of prostate cancer or to slow disease progression. Preclinical studies utilizing xenografts and genetically engineered mouse models demonstrated that reducing dietary fat from corn oil (omega-6 fatty acids) and increasing fish oil intake (omega-3 fatty acids) delays the development and

progression of prostate cancer (3–6). Epidemiologic studies also found that a high-fat diet and low intake of omega-3 fatty acids were associated with increased risk of developing prostate cancer and increased risk of advanced disease (7–10). However, this association is not supported by other reports (11, 12). Intake of fish and marine-derived omega-3 fatty acids has been shown to be associated with decreased prostate cancer mortality (13, 14). Epidemiologic studies have yielded conflicting results with regard to the association of circulating omega-3 fatty acid levels and prostate cancer risk (12, 15–20). In a prospective randomized trial involving men diagnosed with prostate cancer, serum from men consuming a low-fat diet reduced the proliferation of LNCaP cells in an *ex vivo* bioassay compared with men on a high-fat diet. In the same study, serum omega-6 fatty acid levels were positively associated with proliferation, whereas serum omega-3 fatty acid levels were inversely associated (21).

The proportion of omega-6 to omega-3 fatty acids in adipose tissue and blood lipids reflect the dietary intake of fatty acids (22). Through a series of steps, cyclooxygenases and lipoxygenases convert the fatty acids to metabolically active eicosanoids including prostaglandins, thromboxanes, hydroxyeicosatetraenoic acids (HETE), and leukotrienes. Eicosanoids derived from dietary omega-6 polyunsaturated

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fatty acids, including 15-S-hydroxyeicosatetraenoic acid [15 (S)-HETE] and leukotriene B4 (LTB4), have pro-inflammatory effects whereas those formed from omega-3 polyunsaturated fatty acids are less inflammatory and/or anti-inflammatory in nature (23). Inflammation is under active investigation as an important component of cancer development and progression (22, 24–26). Eicosanoids regulate inflammatory responses by selective interaction with the BLT receptors. The BLT2 receptor is expressed ubiquitously and is known to bind both LTB4 and 15(S)-HETE (27). Ligand binding to the receptor induces signaling pathways involved in cell proliferation (28). It was recently reported that BLT2 is a key regulator of androgen receptor expression in androgen-dependent cell lines and possibly a target for prostate cancer therapy (29). LTB4 also binds to the BLT1 receptor with a higher affinity than BLT2 (27). BLT1 is mainly expressed in leukocytes, where it induces signaling pathways involved in cell proliferation. BLT1 expression has been reported in ovarian, colon, and pancreatic cancer (30–32) where it has pro-proliferative effects, but has not yet been reported in prostate cancer.

We recently completed a phase II pre-prostatectomy trial in which the primary outcome (serum IGF-1 levels) was negative, but one of the positive secondary outcomes was lower proliferation (Ki67) in radical prostatectomy specimens, of men consuming a low-fat fish oil (LFFO) diet as compared with a Western diet (33). The dietary intervention also resulted in lower omega-6 and higher omega-3 fatty acids in red blood cell membranes and benign and malignant prostate tissue (33). Preclinical studies suggested that fish oil may act through the COX-2/PGE-2 pathway (3) but these factors were not affected in urine, serum, or prostate tissue in the LFFO group in our human trial (33). Given the association between dietary fat, omega-6 and omega-3 fatty acids, inflammation and prostate cancer (26, 34, 35), and the lack of information about potential mechanisms, we sought to examine the changes in serum fatty acids and pro-inflammatory eicosanoid levels in men consuming the LFFO diet compared with a Western diet. In this *post hoc* analysis, we also investigated whether the diet-induced changes in serum pro-inflammatory eicosanoids correlate with antiproliferative effects as measured by immunohistochemistry (Ki67; ref. 33) and the cell-cycle progression (CCP) score, a validated genetic risk score for predicting recurrence after radical prostatectomy and death from prostate cancer (36–38).

Materials and Methods

Patients and study design

This study was a *post hoc* analysis utilizing serum and prostate tissue obtained from a previously completed phase II randomized trial (33). Briefly, men diagnosed with prostate cancer were randomized to a low-fat diet (15% kcal fat) supplemented with 5 g of fish oil per day or to a Western diet with 40% kcal fat before radical prostatectomy (33). Baseline characteristics of the research subjects are described in Table 1. Subjects were ineligible if they were on insulin, 5- α reductase inhibitors, anti-androgens, or luteinizing hormone-releasing hormone (LHRH) agonists. Compli-

Table 1. Research subject baseline characteristics

	Western diet (n = 21)	LFFO diet (n = 27)
Ethnicity		
Caucasian (No.)	10	21
Black American (No.)	9	6
Hispanic (No.)	2	0
Age (y), mean \pm SD	60.4 \pm 6.7	60.5 \pm 6.3
Weight (kg), mean \pm SD	91 \pm 19.1	92.5 \pm 13.1
BMI (kg/m ²), mean \pm SD	29 \pm 4.2	29.8 \pm 3.8
Percent body fat, mean \pm SD	25.9 \pm 4.7	29.6 \pm 3.1
PSA (ng/mL), mean \pm SD	7.6 \pm 5.6	6.9 \pm 4.9
Gleason sum at diagnosis (No.)		
6	8	16
7	11	9
8–9	1	2

Abbreviations: BMI, body mass index; PSA, prostate-specific antigen.

ance with the research diets was high because of utilization of the NIH-funded UCLA Clinical Research Center (CRC) chefs that prepared all meals and snacks (delivered to patients), and the UCLA CRC dietitian who closely monitored dietary intake. The study was approved by the UCLA Institutional review board and registered with clinical trial.gov #NCT00836615.

Fatty acid analysis

Fatty acid analysis was performed on pre- and postintervention serum and on red blood cell membranes using gas chromatography after formation of fatty acid methyl esters (39). The method used measures phospholipids and cholesteryl esters. The intraassay coefficients were less than 6% for all fatty acids analyzed. The interassay coefficients were less than 10% for all fatty acids analyzed except for palmitoleic and linolenic acids for which the interassay coefficient was less than 12.5%.

LTB4 and 15(S)-HETE analysis

LTB4 levels were measured in duplicate by ELISA in pre- and postintervention plasma following the manufacturer protocol (Assay Designs Inc.). Prostaglandin synthetase inhibitor, indomethacin (Sigma-Aldrich), was added at a concentration of 10 μ g/mL as recommended by the manufacturer. The intra- and interassay coefficient of variation were 6% and 5%, respectively. 15(S)-HETE levels were measured by ELISA in pre- and postintervention serum following the manufacturer protocol (Enzo Life Sciences INT'L Inc.). The intra- and interassay coefficients of variation were 9.8% and 8.9%, respectively.

CCP score

The CCP score was calculated as average expression of 31 CCP genes, normalized to 15 housekeeper genes. Five paraffin-embedded unstained slides and a corresponding H&E stained slide were prepared from the dominant tumor focus from the archived paraffin-embedded radical prostatectomy specimens. The mRNA extraction, RT-PCR, and calculation of CCP score was performed by Myriad Genetics as previously described (36). Archived specimens with adequate malignant epithelium for CCP testing were not available for 12 patients (7 from the LFFO group and 5 from the Western diet group).

RNA extraction and RT-PCR

Total RNA was isolated from 22Rv1, LnCAP, PC-3, and DU145 human prostate cancer cell lines, from human peripheral blood mononuclear cells, and from frozen radical prostatectomy prostate cancer specimens from 4 patients enrolled in our study (2 patients with LFFO and 2 patients with Western diet) using trizol reagent following the manufacturer protocol (Invitrogen) followed by RNeasy clean up using the Qiagen RNeasy kit (Qiagen). RNA concentration was measured using a Nano Photometer Pearl (Implen, Inc.). One microgram of collected total RNA was treated with DNaseI and reverse transcribed using Superscript III and oligo dT following the manufacturer recommendations (Invitrogen). Human BLT1 receptor 433bp fragment and human GAPDH 420 bp fragment were amplified by standard PCR using 5 prime hotmastermix (5 prime) and specific primers. Primers for human BLT1 were 5'-CCTGAAAAGGATGCAGAAGC-3' (forward) and 5'-AAAGGACAACACCCAGATGC-3' (reverse). Primers for human GAPDH were 5'-GTCAGTGGTGGACCTGACCT-3' (forward) and 5'-AGGGGTCTACATGGCAACTG-3' (reverse). The PCR conditions were as follows: 94°C for 3 minutes; 94°C for 1 minute, 50°C (BLT1) or 55°C (GAPDH) for 1 minute, 72°C for 1 minute for 35 cycles followed by 10 minutes at 72°C for final elongation using a Peltier thermal cycler PTC 200 (MJ Research). PCR products were run on a 1.5% agarose/ethidium bromide gel.

Western blot analysis

Human peripheral blood mononuclear cells, human prostate cancer cell lines 22Rv1, LnCAP, PC-3, DU145, and the mouse prostate cancer cell line Myc CAP were lysed using 100 μ L of RIPA buffer supplemented with an EDTA-free protease inhibitor cocktail and PhosSTOP tablets as recommended by the manufacturer (Roche Applied Bioscience) and clarified by centrifugation. Equal amounts of protein were separated on SDS gels and electrophoretically transferred to PVDF membranes for Western blotting. The membrane was incubated overnight at 4°C with the BLT1 receptor antibody (#120114; Cayman Chemical) at a 1/1,000 dilution in 5% BSA/TBST blocking solution followed by a 1-hour incubation at room temperature with a 1:3,000 dilution of the anti-rabbit secondary antibody covalently coupled to horseradish peroxidase (#170-6515 Bio-Rad laboratories Inc.). All immune complexes in the Western

blots were visualized using the West Femto Supersignal (ThermoScientific) and revealed using the Bio-Rad Chemidoc XRS system (Bio-Rad laboratories Inc.). The membrane was then stripped using Restore* Western Blot Stripping Buffer (Thermo Scientific) following the manufacturer recommendations. After blocking, the membranes were incubated with a rabbit anti-GAPDH antibody (Cell Signaling Technology, Inc.) at a 1:5,000 dilution in 5% milk overnight at 4°C. The immunocomplexes were detected as described earlier.

Immunohistochemistry

The rabbit polyclonal BLT1 receptor (#120114; Cayman Chemical) was preincubated in PBS overnight at 4°C in the presence or in the absence of BLT1 receptor antibody blocking peptide (#120112; Cayman Chemical) following the manufacturer protocol. The next day, 4- μ m paraffin-embedded sections of human prostate cancer adenocarcinoma were cut, paraffin was removed with xylene, and rehydrated through graded ethanol. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in methanol for 10 minutes. Heat-induced antigen retrieval was carried out for all sections in 0.001M EDTA buffer, pH 8.0 using a vegetable steamer at 95°C for 25 minutes. The sections were then incubated in presence of the BLT1 receptor antibody \pm blocking peptide at a 1/500 dilution for 1 hour at room temperature. The signal was detected using the Novolink Polymer Detection System (Leica Microsystem Inc.). All sections were visualized with the diaminobenzidine reaction and counterstained with hematoxylin. Pictures were taken using a 20 \times objective.

Statistical analysis

Baseline subject characteristics were compared between groups using unpaired *t* tests or Fisher exact tests. Serum and red blood cell fatty acids, LTB₄, and 15(S)-HETE secondary outcomes were operationalized as the change from baseline to surgery whereas CCP score was measured only at surgery. Partial correlation coefficients were calculated between the tissue and serum biomarkers controlling for pathological Gleason score, weight loss, and race. Residual analysis was performed to check for deviations from models assumptions (normality, homoscedasticity). These outcomes were then compared between groups using unpaired *t* tests. Kolmogorov-Smirnov tests were used to assess normality assumptions within groups for these outcomes (all *P*-values > 0.05). *P*-values < 0.05 were considered significant. The data are presented as mean \pm SD or SEM where appropriate. Statistical analyses were performed using SAS Version 9.3 (SAS Institute Inc.) and IBM SPSS Version 19 (SPSS).

Results

Patients

The patient characteristics were previously described (33) and are listed in Table 1. Of the 55 patients enrolled, 48 completed the trial. Twenty-one subjects were randomized to the Western diet group and 27 to the LFFO group. The majority of patients in both groups were either overweight

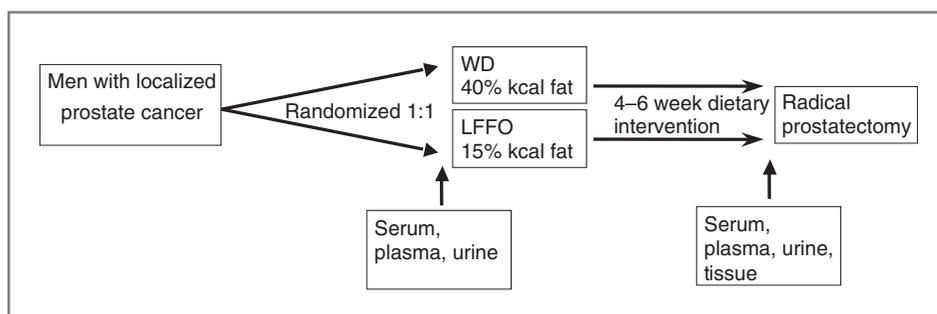


Figure 1. Schema of the previously completed prospective phase II pre-prostatectomy trial comparing an LFFO and Western diet (WD).

or obese. The average duration on the diet intervention was 28 to 30 days. Patients in both groups were compliant with the diets, and patients in the LFFO group were compliant with the fish oil capsule consumption. An overview of the clinical trial design is shown in Fig. 1.

Decreased CCP score in the LFFO prostatectomy specimens

CCP score was measured in malignant radical prostatectomy tissue from 16 Western diet patients and 20 LFFO patients. As shown in Fig. 2, CCP score was significantly lower in the LFFO group as compared with the Western diet group ($P = 0.03$).

Dietary effects on serum fatty acids and circulating eicosanoids

A significant decrease in mean levels of total omega-6 fatty acids, an increase in levels of total omega-3 fatty acids, and a decrease in the omega-6:omega-3 fatty acid ratio was observed in serum from subjects consuming the LFFO diet versus the Western diet (Table 2). Circulating levels of the pro-inflammatory eicosanoid 15(S)-HETE were significantly decreased in the LFFO group as compared with the Western diet group (Table 2). Postintervention 15(S)-HETE circulating levels were significantly reduced compared with preintervention levels in the LFFO group; whereas LTB₄ levels were not decreased in the LFFO group relative to the Western diet group (Table 2), postintervention LTB₄ levels in the LFFO group were significantly lower than preintervention levels (Fig. 3).

BLT1 expression in prostate cancer cell lines and prostatectomy specimens

Expression of the LTB₄ receptor BLT1 was assessed using RT-PCR, Western blotting, and immunohistochemistry (Fig. 4). As shown in Fig. 4A, the BLT1 gene was expressed in androgen-dependent and -independent prostate cancer cell lines 22Rv1, LnCAP, PC-3, and DU145, as well as in prostatectomy specimens. BLT1 expression was further confirmed by Western blot in all 4 cell lines as well as in a mouse prostate cancer cell line, MycCAP (Fig. 4B). 22Rv1 expressed 2 forms of the receptors, the wild type form as well as a lower molecular weight form corresponding to the deglycosylated form of BLT1 (40). BLT1 protein expression was also detected in human prostate cancer specimens by immunohistochemistry (Fig. 4C).

Correlation analyses

To determine if serum 15(S)-HETE and LTB₄ levels correlate with prostate cancer tissue markers of proliferation (Ki67 and CCP score), partial correlation analyses were performed controlling for Gleason score, weight loss, and race. Serum 15(S)-HETE change (pre- to postintervention) positively correlated with tissue Ki67 ($r = 0.472$; $P < 0.01$), but did not correlate with CCP score ($r = 0.236$; $P = 0.2$). The change in serum LTB₄ levels positively correlated with CCP score ($r = 0.41$; $P = 0.02$) but did not correlate with Ki67 ($r = -0.147$; $P = 0.3$).

Discussion

A typical Western diet is high in omega-6 polyunsaturated fatty acids from corn oil and other vegetable oils, low in omega-3 fatty acids, and has a ratio of omega-6:omega-3 fatty acids of approximately 15:1 (41); whereas in the typical Asian diet, the ratio of omega-6:omega-3 fatty acids is 4:1 (42). Although epidemiologic data are conflicting on the anticancer or cancer preventative effects of dietary omega-3 and omega-6 fatty acids (9–14), preclinical data clearly show beneficial anticancer effects of lowering omega-6 intake from corn oil and lowering the dietary ratio of

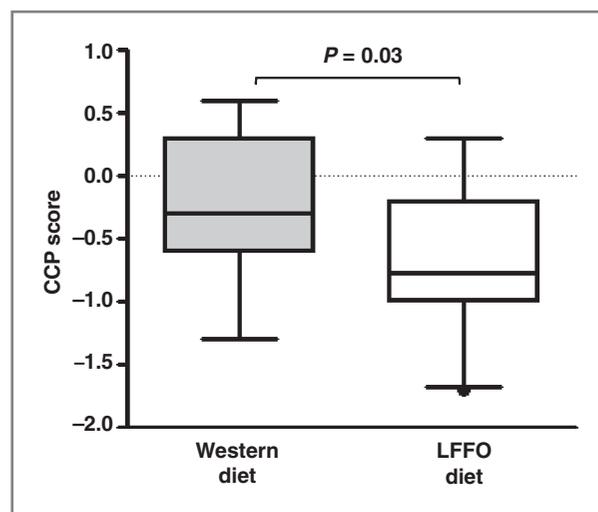


Figure 2. Effect of an LFFO diet compared with a Western diet on CCP score in malignant epithelial prostate cells in radical prostatectomy specimens (Western diet: $n = 16$, LFFO: $n = 20$). Statistical significance was assessed using unpaired t test.

Table 2. Changes in serum fatty acids and eicosanoids

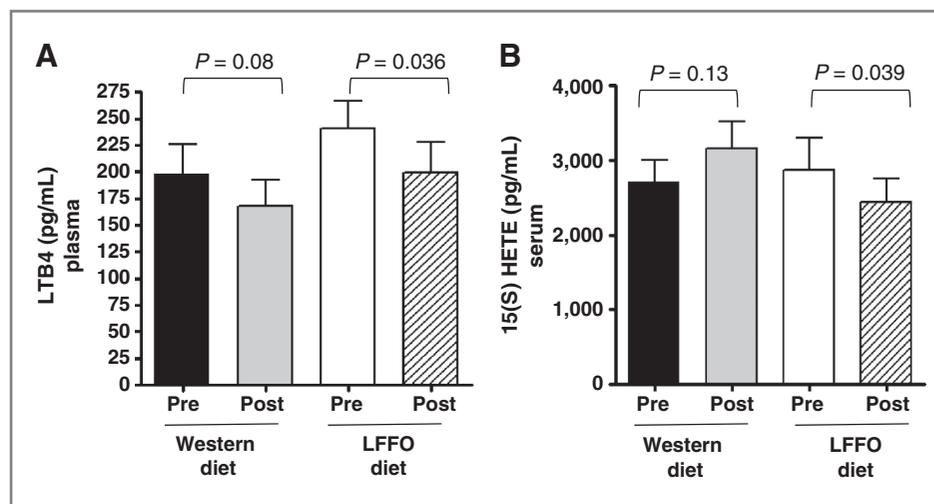
Fatty acids ^a	Western diet postintervention minus preintervention (n = 21; mean ± SEM)	LFFO postintervention minus preintervention (n = 27; mean ± SEM)	P value
Palmitic	-0.8 ± 0.46	-0.62 ± 0.32	0.7
Palmitoleic	-0.45 ± 0.22	-0.28 ± 0.1	0.9
Stearic	-0.14 ± 0.08	-0.42 ± 0.13	0.2
Oleic	-1.17 ± 0.63	-2.35 ± 0.43	0.2
Linoleic acid (n-6)	2.4 ± 0.93	-1.68 ± 0.63	<0.01
α-Linolenic (n-3)	0.03 ± 0.04	0.009 ± 0.05	0.9
Eicosadienoic (n-6)	-0.005 ± 0.009	-0.009 ± 0.009	0.6
Arachidonic acid (20:4, n-6)	0.48 ± 0.27	-0.75 ± 0.21	<0.01
EPA (20:5, n-3)	-0.23 ± 0.05	1.97 ± 0.14	<0.01
Docosapentaenoic (n-3)	0.007 ± 0.02	0.16 ± 0.03	<0.01
DHA (22:6, n-3)	-0.12 ± 0.08	3.98 ± 0.19	<0.01
Total n-6	2.88 ± 1.12	-2.44 ± 0.67	<0.01
Total n-3	-0.32 ± 0.13	6.12 ± 0.3	<0.01
n-6/n-3	1.41 ± 0.42	-5.76 ± 0.42	<0.01
	% Change from baseline (n = 21; mean ± SEM)	% Change from baseline (n = 27; mean ± SEM)	P value
Eicosanoids ^b			
15(S)-HETE	24.7 ± 11.4	-7.2 ± 6.6	0.02
LTB4	-9.7 ± 7.4	-14.9 ± 5.6	0.6

^aChanges in serum fatty acids are expressed as postintervention minus preintervention.
^bEicosanoid changes were calculated for each patient as percentage of change from baseline.

omega-6:omega-3 fatty acids (3, 5). The major omega-6 fatty acid in corn oil is linoleic acid. Linoleic acid is elongated to form arachidonic acid, which is then metabolized through cyclooxygenase (COX) and lipoxygenase (LOX) pathways. The COX-2/PGE2 pathway has been implicated in prostate cancer development and progression in preclinical and clinical studies (3, 43, 44). We initially hypothesized that an LFFO diet would result in lower serum insulin-like growth factor (IGF)-1 levels (primary outcome) as well as lower prostate tissue proliferation, lower COX-2

tissue levels, and lower tissue and serum PGE-2 levels (secondary outcomes). Although an LFFO intervention resulted in lower prostate cancer proliferation (Ki-67) in our pre-prostatectomy phase II trial (33), there was no effect on IGF-1 (primary outcome), COX-2, or PGE-2 levels suggesting that alternative mechanisms may be responsible for the observed antiproliferative effects. Similarly, Lloyd and colleagues recently published a xenograft study demonstrating that a fish oil-based diet slowed prostate cancer progression but did not affect the IGF-1 axis or the COX2/PGE2

Figure 3. Effect of LFFO and Western diet on plasma levels of LTB4 (A) and serum levels of 15(S)-HETE (B). 15(S)-HETE and LTB4 were measured by ELISA in duplicate (LFFO group, n = 27; Western diet group, n = 21). Values are expressed as mean ± SE. Statistical significance was assessed using paired t test.



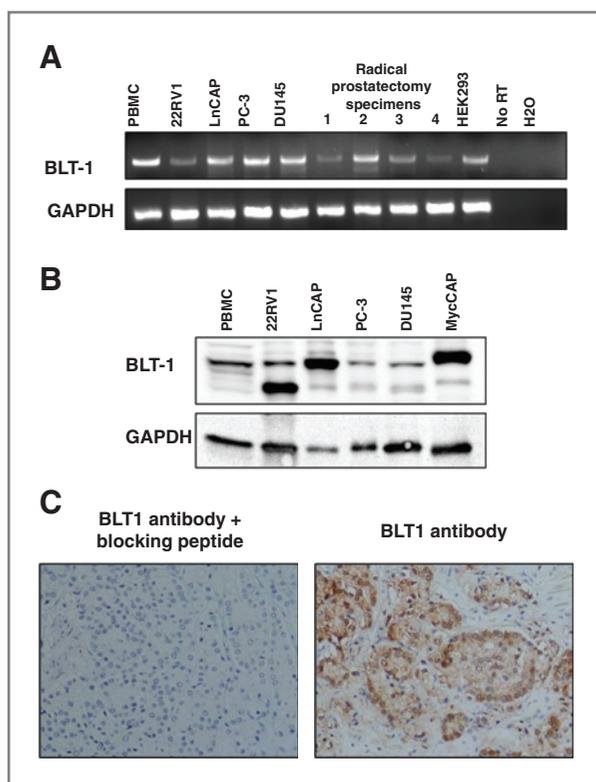


Figure 4. Expression of the BLT1 receptor in prostate cancer cell lines and radical prostate cancer specimens from 4 patients. **A**, BLT1 gene expression was assessed by RT-PCR (in triplicate) in prostate cancer cell lines and malignant prostatectomy tissue from 4 patients. Peripheral blood mononuclear cells and HEK293 cells were used as positive control. A negative control with no reverse transcriptase was performed in each RT-PCR. **B**, BLT1 protein expression in prostate cancer cell lines was assessed by Western blot. Peripheral blood mononuclear cell extract was used as a positive control. **C**, BLT1 receptor expression was assessed by immunohistochemistry in radical prostatectomy specimens from 4 patients. Preincubation of the BLT1 antibody with a blocking peptide was used to assess the BLT1 antibody specificity.

pathway compared with olive oil, corn oil, or animal fat diets (6). These results led us to hypothesize that an LFFO diet may induce a reduction in serum levels of 2 major products of arachidonic acid metabolism, 15(S)-HETE and LTB₄, through lipoxygenase pathways.

15S-HETE is the major hydroxy derivative of arachidonic acid and is the end product of the enzyme 15-lipoxygenase whereas LTB₄ is formed through the 5-lipoxygenase pathway (23). In this study we found that an LFFO diet significantly reduced serum 15S-HETE levels relative to a Western diet, and that postintervention LTB₄ levels were lower than preintervention levels, although LTB₄ levels in the LFFO group were not significantly reduced relative to the Western diet group. Although involvement of the COX2/PGE2 pathway in the progression of prostate cancer has been studied extensively, there are limited reports on the role of the lipoxygenase pathway (35). Preclinical and clinical studies demonstrated a potential role for 5-lipoxygenase and its metabolite 5-HETE in prostate cancer progression. Gupta and colleagues reported that levels of 5-lipoxygenase

and its product, 5-HETE, were significantly higher in malignant prostate tissue as compared with benign prostate tissue, and that inhibition of 5-lipoxygenase induces apoptosis in prostate cancer cell lines (45). In addition, *in vitro* studies using LNCaP and PC3 cell lines demonstrated a role for the 5-lipoxygenase pathway in proliferation and survival of prostate cancer cell lines (46, 47). The BLT2 receptor, a LTB₄ receptor expressed ubiquitously, is known to bind both LTB₄ and 15(S)-HETE (27) and to induce signaling pathways involved in cell proliferation (28). Recently, BLT2 was reported to be a key regulator of androgen receptor expression and possibly a target for prostate cancer therapy (29). LTB₄ also binds to the BLT1 receptor, which is known to be more specific for LTB₄ than BLT2 (27) and mainly expressed in leukocytes where it induces signaling pathways involved in cell proliferation (28). Interestingly, herein we demonstrated the expression of BLT1 in prostate cancer specimens and in prostate cancer cell lines. To our knowledge, the presence of BLT1 in prostate cancer cells has not been previously described. However, our findings are in line with other studies demonstrating the expression and the proliferative role of BLT1 receptor in other cancers such as ovarian cancer (30), colon cancer (32), and pancreatic cancer (31).

In the present study we also demonstrated that an LFFO intervention reduced expression of genes involved in CCP using Myriad Genetics CCP score. The CCP score has been previously shown to be an independent predictor of recurrence after radical prostatectomy, prostate cancer death (36–38) and, more recently, a predictor of biochemical recurrence for patients treated with external beam radiation therapy as their primary therapy (48). The finding that CCP score was lower in the LFFO versus the Western diet group provides strong support for longer-term prospective randomized trials evaluating an LFFO diet in men with prostate cancer. Correlation analyses demonstrated a significant positive correlation between changes in serum 15(S)-HETE and LTB₄ levels with Ki67 and CCP score, respectively. Further studies are required to evaluate if 15(S)-HETE is more directly linked with proliferation as measured by Ki67, and whether LTB₄ has a greater impact on the genes used to calculate the CCP score. Further preclinical and clinical studies are also warranted to determine if diet-induced changes in lipoxygenase-derived eicosanoids may impact on prostate cancer proliferation through mechanisms involving BLT1 and BLT2 receptors.

As detailed in the introduction section of this manuscript, preclinical, clinical, and epidemiologic studies have yielded conflicting results with regards to the role of omega-3 fatty acids for prostate cancer prevention and treatment. In a recent epidemiologic study, Brasky et al. reported that plasma omega-3 fatty acid levels were associated with low-grade and high-grade prostate cancer (20). On the other hand, preclinical studies suggest beneficial effects of omega-3 fatty acids for prevention and treatment of prostate cancer (3–6) and a short-term prospective randomized clinical trial suggested potential beneficial effects of omega-3 fatty acids for prostate cancer treatment (33). In this

trial, fish oil supplements were combined with a low-fat diet to achieve a reduced omega-6/omega-3 fatty acid ratio. The article by Brasky et al. did not report on other components of the participants' diets or supplement intake. Ultimately, prospective randomized trials will be required to establish dietary and supplement recommendations for prostate cancer prevention and treatment.

Limitations of the present report include that it was a *post hoc* analysis and is therefore only useful for generating new hypotheses. In addition, the samples size was small (48 patients completed the trial) and malignant radical prostatectomy tissue was only available in 36 patients for measurement of the CCP score. Therefore, these results will need to be validated in larger clinical trials.

In summary, a 4–6 week LFFO dietary intervention decreased serum pro-inflammatory eicosanoids and the prostate cancer tissue CCP score. We also demonstrated the expression of the LTB4 receptor BLT1 in prostate cancer cell lines and in human prostate cancer specimens. Furthermore, circulating levels of 15S-HETE and LTB4 correlated with proliferation and CCP score in prostate cancer specimens suggesting a potential role for the 5- and 15-lipoxygenase pathways in the anticancer effect of an LFFO diet. Further preclinical and clinical studies are warranted to explore whether dietary modulation of pro-inflammatory eicosanoids impacts on prostate cancer proliferation, and if serum eicosanoids levels may function as surrogate biomarkers for efficacy of an LFFO intervention.

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Disclosure of Potential Conflicts of Interest

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