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Research Article

Cancer Prevention Research

Prostatic and Dietary Omega-3 Fatty Acids and Prostate Cancer Progression during Active Surveillance

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Abstract

The association between omega-3 (ω-3) fatty acids and prostate cancer has been widely studied. However, little is known about the impact of prostate tissue fatty acid content on prostate cancer progression. We hypothesized that compared with the estimated dietary ω-3 fatty acids intake and the ω-3 fatty acids levels measured in red blood cells (RBC), the prostate tissue ω-3 fatty acid content is more strongly related to prostate cancer progression. We present the initial observations from baseline data of a phase II clinical trial conducted in a cohort of 48 untreated men affected with low-risk prostate cancer, managed under active surveillance. These men underwent a first repeat biopsy session within 6 months after the initial diagnosis of low-risk prostate cancer, at which time 29% of the men had progressed from a Gleason score of 6 to a Gleason score of 7. At the first repeat biopsy session, fatty acid levels were assessed with a food-frequency questionnaire, and determined in the RBC and in the prostate tissue biopsy. We found that eicosapentaenoic acid (EPA) was associated with a reduced risk of prostate cancer progression when measured directly in the prostate tissue. Thus, this initial interim study analysis suggests that prostate tissue ω-3 fatty acids, especially EPA, may be protective against prostate cancer progression in men with low-risk prostate cancer. *Cancer Prev Res; 7(7); 766–76. ©2014 AACR.*

Introduction

In prostate cancer, inflammation is suspected to trigger cancer progression (1). At least partly due to their anti-inflammatory properties, omega-3 (ω-3) fatty acids are proposed to have anticancer actions (2). Indeed, once incorporated into biologic membranes, ω-3 fatty acids are converted into lipid mediators, named eicosanoids, with anti-inflammatory properties via a succession of enzymes: desaturase, elongase, cyclooxygenase, and lipoxygenase (3). ω-3 and omega-6 (ω-6) fatty acids are converted by the same enzymes, but the conversion of ω-6 fatty acids leads to eicosanoids with proinflammatory properties (3). Because of the high ratio of ω-6/ω-3 fatty acids in the Western diet, these enzymes mainly convert ω-6 fatty acids leading to a proinflammatory environment that may contribute to prostate cancer progression (4). Although a recent study has shown that dietary ω-3 does not influence the expression level of cyclooxygenase-2 (5), other evidences show that an increasing tissue level of ω-3 fatty acids may lead to a shift in the ω-6/ω-3 ratio, resulting in a higher synthesis of ω-3 fatty acid–derived eicosanoids and decreased tissue inflammation. ω-3 fatty acids may also contribute to fight prostate cancer as it was shown to influence androgen metabolism (6). These findings have led to extensive clinical studies on the links between ω-3 and ω-6 fatty acids and cancer, including prostate cancer.

The clinical potential of ω-3 fatty acids in the context of prostate cancer in men is, however, not clearly demonstrated. Epidemiologic studies have generated conflicting results. Some studies show that a high dietary intake of ω-3 fatty acids may reduce the risk of prostate cancer (7–11), whereas others have seen either no association (12–14), or an increased risk of prostate cancer in people with a high intake of ω-3 fatty acids (15, 16). Moreover epidemiologic data suggest that fish or marine-derived ω-3 fatty acids may have a more pronounced effect on biologically aggressive tumors or on their progression, and a less pronounced effect on initiation of more benign or earlier-stage tumors often detected by screening (4).

All prostate cancers will grow, but many will grow at slow rates. Indeed, men with a Gleason score of 6 prostate cancer (low-risk prostate cancer) have a very modest risk of prostate cancer–related death over a period of 20 years, whereas men with Gleason score of 7 or greater prostate cancer

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(high-risk prostate cancer) face a significant risk of death from this disease over the same period (17). Thus, men affected with low-risk prostate cancer may remain untreated and be managed with active surveillance to follow the progression of their disease. Prostate cancer progression is a complex phenomenon that may be influenced by environmental factors, including fatty acid intake. Studies looking at fatty acid intake were mainly based, until now, on data gathered from food-frequency questionnaires (FFQ; refs. 18, 19), with few studies based on fatty acid profiles measured in plasma circulating lipids (13, 20) and even fewer studies in which fatty acids were measured in the membranes of red blood cells (RBC; refs. 21, 22). To the best of our knowledge, no published study examined the fatty acid profile of the prostate tissue itself in relation with prostate cancer progression during active surveillance.

The controversy about the association between ω-3 and prostate cancer may be partly explained by the use of surrogate methods to estimate the fatty acid profile of the target (prostate) tissue. Thus, we conducted a prospective cohort study of men affected by low-risk prostate cancer and managed under active surveillance in which we tested the hypothesis that, compared with the estimated *dietary* ω-3 fatty acid intake and with ω-3 fatty acid levels in RBCs, only the *prostate tissue* fatty acid content is related to cancer progression.

Materials and Methods

Study design

The CHU de Québec ethics board approved this study. All patients provided written informed consent to participate in the study and the protocol is registered at ClinicalTrials.gov (NCT01653925). The study presented herein is an observational substudy from a phase II clinical trial. An *a priori* interim analysis of the baseline data was planned after approximately 60 patients were recruited into the prospective cohort (Supplementary Fig. S1). We present these results herein. The goal of the phase II clinical trial is to understand the molecular mechanisms of drug and dietary interventions to prevent prostate cancer and reduce its progression. The study, conducted in 120 participants, focuses on men diagnosed with low-risk prostate cancer under active surveillance, to assess the impact on prostate tissue of a dietary intervention performed by a nutritionist versus use of dutasteride, a 5-alpha-reductase inhibitor (5α-RI) drug commonly prescribed for benign prostate hypertrophy (23, 24) and low-risk prostate cancer during active surveillance (25, 26). Active surveillance offers a close monitoring of prostate cancer and allows patients to avoid or delay the negative side effects of curative treatments. The dietary intervention, conceived with experts in nutritional trials on pragmatic bases, aims to increase intakes in ω-3 fatty acids while decreasing intake of ω-6, saturated and *trans* fatty acids. Men were eligible to participate if they were diagnosed with low-risk prostate cancer at their first biopsy and accepted active surveillance. Low-risk prostate cancer was defined as pathologically confirmed Gleason 6 with less than six positive biopsy cores out of the 12 cores examined: less than three positive sextants; a clinical stage $\leq$T2a; and a prostate-specific antigen (PSA) level <15 ng/mL if prostate volume was >30 mL, or a PSA <10 ng/mL otherwise. Patients were not eligible if they were already taking a 5α-RI drug, ω-3 fatty acid supplements, or if they had received radiotherapy or chemotherapy. Following the initial diagnosis of low-risk prostate cancer, patients underwent a first repeat biopsy session, performed by the same ultrasonographist, within 6 months after the initial diagnosis. At the first repeat biopsy session, patients were randomized in one of the two arms of the study: dietary intervention or 5α-RI. After 6 months of individual intervention, all the patients received both interventions for another 6 months. The total study length was 12 months. Biopsies were taken at the first repeat biopsy session, at 6 months, as well as at 12 months.

Because tumor volume estimation is rather imprecise based on the quantity of cancer in the biopsy specimens, particularly for low-risk cancers, our institutional active surveillance recommendations are mainly based on grade of tumor assessed by the Gleason score, then on the number of positive biopsy cores. Thus, our eligibility criteria are somewhere between those of other institutions, whereby we typically exclude men from surveillance when there is presence of high-grade pattern (Gleason 7 or greater) and will tolerate up to six positive biopsy cores (27).

The dietary intake of these patients was assessed by a Web-based quantitative FFQ (web-FFQ) (28). Fatty acid profiles were measured in the membrane of the circulating RBCs and in the prostate tissue. Patients were included in this substudy if fatty acid profiles from both RBCs and prostate biopsies were available. Only 3 patients did not tolerate additional prostate biopsies for research and were thus excluded. Upon central pathologic review, 5 patients were identified with a Gleason 7 prostate cancer at the initial diagnostic biopsy and were excluded from analysis. Six patients were excluded from analysis for taking ω-3 fatty acids supplements at or before the first repeat biopsy session. Two patients refused to complete the web-FFQ, but these were included in the analysis.

Biologic specimen harvesting

At the first repeat biopsy session, two additional prostate biopsy cores were taken in the normal peripheral zone of the prostate presenting without prostate nodule, without ultrasound abnormality and within a region of initial negative biopsy. Biopsy cores were immediately placed in cold Hank's Balanced Salt Solution (HBSS; Invitrogen), frozen in dry ice, and stored at −80°C. Blood samples were collected into EDTA-containing Vacutainer tubes (Becton Dickinson) the morning of the first repeat biopsy session, after overnight fast, and immediately placed on ice. Within 1 hour, plasma, buffy coat, and RBCs were separated and isolated by centrifugation (2,500 $\times g$ for 15 minutes at 4°C). The isolated fractions were placed on dry ice before final storage at −80°C.

Data collection

At the first repeat biopsy session, body weight, height, and waist circumference were measured according to

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standardized procedures by specifically trained personnel (29). Complete medical and medication history was recorded for each patient.

FFQ

Patients were asked to complete a web-FFQ within days after the first repeat biopsy session to assess food intake over the last month. The web-FFQ was developed and validated specifically for Quebecers, who constitute the totality of our study population (28). The web-FFQ is used to assess food intake over the preceding month using photographs of meals and standardized portion sizes. This web-FFQ compiles data for eight food subcategories, including the four food groups of the Canadian Food Guide, and contains 136 questions with 40 sub questions. The Nutrition Data System for Research (software version 4.03, Food and Nutrient Database 31; ref. 30) and the Canadian Nutrient File (CNF, version 2007b; 31) were used to create a food composition database for the analysis of data derived from the web-FFQ. Data showed that the web-FFQ was highly reproducible over time (28).

Determination of fatty acid profiles

Fatty acid profile of the RBC membrane reflects the past 3 months' diet (21, 32). Fatty acid profiles of RBCs and of normal prostate biopsies were determined by gas chromatography after extraction of total lipids as previously described (33, 34). Briefly, after cell disruption and addition of phosphatidylcholine C:15 (Avanti Polar Lipids) as an internal standard, lipids were extracted according to a modified Folch method (34). Fatty acid profiles were obtained after methylation in methanol/benzene 4:1 (v/v; ref. 35) and separated by capillary gas chromatography using a temperature gradient on an HP5890 gas chromatograph (Hewlett Packard) equipped with an HP-88 capillary column (100 m $\times$ 0.25 mm i.d. $\times$ 0.20 μm film thickness; Agilent Technologies) coupled with a flame ionization detector. Helium was used as carrier gas (split ratio, 1:80).

Fatty acids were identified according to their retention time using the following mixtures as standards: the FAME 37 mix (Supelco Inc.), the GLC-411 FA mix (NuChek Prep Inc.), as well as the methylated fatty acids C22:5n6 (Larodan AB) and C22:5n3 (Supelco Inc.). Fatty acid profiles were expressed as the relative proportion of total fatty acids. To assess the reproducibility of the technique, the determination of the fatty acid profile of prostate tissue was performed on two biopsy cores for the 29 first patients; coefficients of variation (CV) between duplicates were calculated (Supplementary Table S2). The mean value of the duplicates' fatty acid profile was used for statistical analysis. Prostatic fatty acids were also available in absolute concentrations (μg of fatty acid per g of prostatic biopsy). Ratios of these concentrations were calculated to assess the activity of the desaturase and elongase enzymes from the fatty acids metabolic pathway in the prostate. The fatty acid profile of RBCs was determined on a single sample, as this technique is well established (36).

Statistical analysis

Descriptive statistics of the study subjects and their diet were tabulated by prostate cancer progression status. Distributions of values were compared between the progression and the nonprogression groups using the Wilcoxon rank sum test. Because of asymmetric dispersion of values and the size of the sample, the Fligner-Policello test was used to assess significance of associations between specific fatty acids and prostate cancer progression status (37). Eicosapentaenoic acid (EPA, C20:5n3), the main factor associated with prostate cancer, was stratified in tertiles. Logistic regression was used to estimate the risk of prostate cancer progression at the first repeat biopsy session, across tertiles of dietary, RBC, and prostate tissue EPA. Covariables included in the multivariable models were age, PSA level, total energy intake, smoking status, time between the initial and the first repeat biopsies, and number of positive biopsy zones at the first repeat biopsy session. The education level was not associated with the risk of prostate cancer progression; thus, this variable was not included in this multivariable regression model. The area under the curve has been calculated for each model. All the statistical tests were two-sided using an α value of 0.05 to declare significance and were coded in SAS v.9.2 (SAS Institute Inc).

Results

Description of the study subjects

The cohort (Table 1) was composed of 48 men diagnosed with low-risk prostate cancer. At the first repeat biopsy session, the median age of the patients was 60.5 years [interquartile range (IQR), 55.0–66.0], the median body mass index (BMI) was 27.6 kg/m^2 (IQR, 24.6–29.3), and 44% had a university degree. The median PSA level was 4.3 ng/mL (IQR, 3.0–5.0). Pathologic examination of biopsy cores from the first repeat biopsy session showed that 29% (14 of 48) of the patients had a Gleason score of 7 (progression group), whereas 71% (34 of 48) of patients had a Gleason score of 6 or less (nonprogression group). The median time between the initial diagnosis and the first repeat biopsy session was 3.4 months (IQR, 2.4–4.3). The web-FFQ was completed by 46 patients; 12 in the progression group and 34 in the nonprogression group. There was no significant difference in descriptive parameters between groups. However, at the first repeat biopsy session, patients of the nonprogression group had less positive biopsy zones (median, 1.0; IQR, 0.0–1.0) than the progression group (median, 2.0; IQR, 2.0–3.0), $P < 0.0001$. This difference was not observed at the initial diagnostic biopsy session ($P = 0.39$, data not shown).

Dietary intake at the first repeat biopsy session

Table 2 gives a general overview of dietary intake for energy, macronutrients, and single fatty acids. The median caloric intake was 2,490 kcal/day (IQR, 1,871–2,970), the protein intake was 95.5 g/day (IQR, 71.2–116.8) and the fat intake was 87.5 g/day (IQR, 72.0–117.1). No significant difference was observed for nutrients between the nonprogression and the progression group. The ω-6/ω-3

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Table 1. Characteristics of the study subjects at the first repeat biopsy session

Variables	Total ($n = 48$)	Nonprogression ($n = 34$)	Progression ($n = 14$)	P^a
Age (y)				0.52
Mean ± SD	60.0 ± 7.0	59.4 ± 6.5	61.3 ± 8.1	
Median (IQR)	60.5 (55.0–66.0)	60.0 (55.0–66.0)	62.0 (55.0–66.0)	
BMI (kg/m^2)				0.75
Mean ± SD	27.2 ± 3.3	27.1 ± 3.3	27.4 ± 3.5	
Median (IQR)	27.6 (24.6–29.3)	27.5 (24.4–29.2)	27.8 (25.6–30.0)	
Waist circumference (cm)				0.94
Mean ± SD	95.9 ± 9.0	96.0 ± 8.9	95.7 ± 9.7	
Median (IQR)	95.0 (89.0–103.5)	96.0 (88.0–104.0)	94.5 (89.0–103.0)	
Education level[b] (*n*, %)				0.97
Secondary school or less	14 (30)	10 (29)	4 (33)	
Postsecondary diploma	12 (26)	9 (26)	3 (25)	
University degree	20 (44)	15 (44)	5 (42)	
Smoking status[b] (*n*, %)				0.49
Current smoker	3 (7)	3 (9)	0 (0)	
Former smoker	26 (57)	18 (53)	8 (67)	
Never	17 (36)	11 (38)	4 (33)	
PSA (ng/mL)				0.81
Mean ± SD	4.6 ± 2.5	4.6 ± 2.7	4.4 ± 2.1	
Median (IQR)	4.3 (3.0–5.0)	4.3 (3.0–4.9)	4.3 (2.8–5.0)	
Positive biopsy cores (*n*)				**<0.0001**
Mean ± SD	1.4 ± 1.4	1.0 ± 1.3	2.4 ± 1.1	
Median (IQR)	1.0 (0.0–2.0)	1.0 (0.0–1.0)	2.0 (2.0–3.0)	
Time interval[c] (mo)				0.12
Mean ± SD	3.6 ± 1.5	3.4 ± 1.5	3.9 ± 1.3	
Median (IQR)	3.4 (2.4–4.3)	3.2 (2.3–4.10)	3.9 (2.7–4.9)	

NOTE: In this study, 48 men diagnosed with low-risk prostate cancer and managed under active surveillance underwent a first repeat biopsy session. At the first repeat biopsy session, 14 men (29%) out of 48 had a progression of their cancer to Gleason 7 prostate cancer (progression group).

[a]*P* values were obtained by using the Fligner-Policello test for continuous variables and χ^2 test for categorical variables.

[b]Information was available for 46 patients out of 48.

[c]Time between the diagnostic biopsy and the first repeat biopsy session.

Bold font indicates significance at $P < 0.05$.

ratio was higher in the nonprogression group but the difference was not significant ($P = 0.08$). Supplementary Table S1 shows food groups intake, as well as total fish and fatty fish intake, assessed by the web-FFQ. Even if not significant, the intakes of vegetables, dairy products, and fish were higher in the nonprogression group, whereas red meat intake was higher in the progression group.

Fatty acid profile of the RBCs

Table 3 shows a general overview of the fatty acid profile of the RBCs. No significant difference was observed between the fatty acid profile of the nonprogression and the progression group.

Fatty acid profile of the prostate tissue

Table 4 shows a general overview of the fatty acid profile of the prostate tissue. Supplementary Table S2 shows that measurements were reproducible between the two biopsy cores. EPA was the only single polyunsaturated fatty acid to be associated with prostate cancer progression. Patients from the nonprogression group had a higher prostatic content of EPA than patients from the progression group (median, 0.13; IQR, 0.00–0.21 vs. median, 0.00; IQR, 0.00–0.09; $P = 0.004$).

Table 5 shows the results from the univariable and multivariable logistic regression models. Univariable models show that men categorized in the highest tertile of prostate tissue EPA level (mean, 0.32%; SD, 0.27) had a drastically lower risk of high-risk cancer [OR, 0.08; 95% confidence interval (CI), 0.01–0.72; $P = 0.02$) than men categorized in the lowest tertile of prostate tissue EPA (mean, 0.00%; SD, 0.00). This was also observed when associations were adjusted for age, PSA level, total energy intake, smoking status, time between the initial and the first repeat biopsies, and number of positive biopsy cores at the new repeat biopsy session; men categorized in the highest tertile of prostate tissue EPA level had a drastically lower risk of high-risk cancer (OR, 0.008; 95% CI, <0.001–0.56;

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Table 2. Daily nutrient intake assessed by the web-FFQ stratified by prostate cancer progression status

Daily intake	Total ($n = 46$)	Nonprogression ($n = 34$)	Progression ($n = 12$)	P^a
Energy (kcal)				0.28
Mean ± SD	2,585 ± 900	2,519 ± 875	2,772 ± 983	
Median (IQR)	2,490 (1,871–2,970)	2,444 (1,834–2,854)	2,533 (2,206–3,131)	
Protein (g)				0.63
Mean ± SD	102.0 ± 40.6	102.0 ± 44.4	101.8 ± 28.8	
Median (IQR)	95.5 (71.2–116.8)	94.3 (68.8–117.4)	98.3 (89.1–121.5)	
Total fat (g)				0.36
Mean ± SD	97.8 ± 38.7	95.9 ± 40.7	103.4 ± 33.3	
Median (IQR)	87.5 (72.0–117.1)	85.6 (65.4–113.8)	100.0 (78.4–122.6)	
Total ω-3 (g)				0.69
Mean ± SD	2.25 ± 0.98	2.26 ± 1.07	2.23 ± 0.73	
Median (IQR)	2.09 (1.57–2.92)	2.09 (1.53–2.92)	2.15 (1.74–2.71)	
ALA (g)				0.53
Mean ± SD	1.81 ± 0.76	1.77 ± 0.76	1.91 ± 0.77	
Median (IQR)	1.70 (1.28–2.26)	1.66 (1.27–2.12)	1.78 (1.34–2.38)	
LC ω-3 (g)				0.23
Mean ± SD	0.44 ± 0.41	0.48 ± 0.44	0.32 ± 0.26	
Median (IQR)	0.32 (0.21–0.59)	0.36 (0.21–0.67)	0.24 (0.12–0.43)	
EPA (g)				0.26
Mean ± SD	0.14 ± 0.13	0.15 ± 0.14	0.10 ± 0.08	
Median (IQR)	0.11 (0.06–0.20)	0.12 (0.07–0.21)	0.08 (0.04–0.15)	
DPA (g)				0.21
Mean ± SD	0.05 ± 0.05	0.06 ± 0.05	0.04 ± 0.03	
Median (IQR)	0.04 (0.02–0.06)	0.04 (0.02–0.07)	0.03 (0.02–0.05)	
DHA (g)				0.23
Mean ± SD	0.25 ± 0.24	0.27 ± 0.26	0.18 ± 0.15	
Median (IQR)	0.17 (0.11–0.32)	0.21 (0.12–0.39)	0.13 (0.07–0.24)	
Total ω-6 (g)				0.17
Mean ± SD	15.29 ± 5.69	14.55 ± 5.51	17.40 ± 5.91	
Median (IQR)	14.12 (10.62–19.59)	13.25 (10.18–18.64)	17.28 (11.57–22.51)	
ω-6/ω-3 ratio				0.08
Mean ± SD	7.26 ± 2.14	7.01 ± 2.25	7.95 ± 1.70	
Median (IQR)	6.93 (5.97–8.49)	6.86 (5.54–8.34)	8.07 (6.40–9.45)	

NOTE: Prostate cancer progression is defined as biopsy-detected Gleason 7 at the first repeat biopsy session.
Abbreviation: LC ω-3, long chain omega-3 fatty acids (EPA+DPA+DHA).
[a]*P* values were obtained by using the Fligner-Policello test.

$P = 0.03$) than men categorized in the lowest tertile of prostate tissue EPA. When modeled continuously, the association between EPA in prostate tissue and the risk of high-risk prostate cancer remained significant for both univariable ($P = 0.03$) and multivariable ($P = 0.01$) models. Similar modeling of associations with docosahexaenoic acid (DHA, C22:6n3), docosapentaenoic acid (DPA, C22:5n3), or total ω-3 fatty acids showed no statistically significant difference in risk of high-risk cancer between the tertiles of fatty acid levels (data not shown). The area under the curve has been calculated to estimate accuracy of the logistic regression models. Models for EPA in prostate tissue showed an accuracy of 0.73 for the univariable model and an accuracy of 0.90 for the multivariable model (data not shown).

Table 6 shows the prostate tissue activity of the key enzymes from the fatty acid metabolic pathway. The ratio of C22:5n3/C20:5n3, which measures the Elovl2 elongase activity, was the only ratio to be associated with prostate cancer progression; the nonprogression group had a higher ratio (median, 4.02; IQR, 0.00–4.98) than the progression group (median, 0.00; IQR, 0.00–3.29; $P = 0.01$).

Discussion

This is the first study to link progression of early-stage low-risk prostate cancer to the fatty acid profile of the prostate tissue during active surveillance. We found that EPA may be protective against prostate cancer progression when measured in the prostatic tissue. However, no association was observed when fatty acids were measured in the RBCs and

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Table 3. Fatty acid profile of RBCs stratified by prostate cancer progression status

Fatty acid[a]	Total ($n = 48$)	Nonprogression ($n = 34$)	Progression ($n = 14$)	P[b]
Total ω-3				0.70
Mean ± SD	7.96 ± 1.57	8.06 ± 1.69	7.71 ± 1.24	
Median (IQR)	7.46 (7.17–8.59)	7.47 (7.16–8.75)	7.37 (7.23–8.24)	
ALA				0.34
Mean ± SD	0.08 ± 0.09	0.09 ± 0.10	0.06 ± 0.09	
Median (IQR)	0.00 (0.00–0.17)	0.12 (0.00–0.17)	0.00 (0.00–0.16)	
LC ω-3				0.98
Mean ± SD	7.86 ± 1.57	7.95 ± 1.71	7.65 ± 1.20	
Median (IQR)	7.39 (7.08–8.41)	7.40 (7.04–8.58)	7.37 (7.23–8.10)	
EPA				0.33
Mean ± SD	0.83 ± 0.43	0.87 ± 0.48	0.73 ± 0.28	
Median (IQR)	0.82 (0.57–0.96)	0.82 (0.59–0.98)	0.69 (0.48–0.87)	
DPA				0.40
Mean ± SD	2.64 ± 0.33	2.66 ± 0.36	2.57 ± 0.24	
Median (IQR)	2.62 (2.45–2.77)	2.63 (2.47–2.83)	2.58 (2.43–2.72)	
DHA				0.78
Mean ± SD	4.40 ± 1.09	4.42 ± 1.19	4.35 ± 0.81	
Median (IQR)	4.15 (3.67–4.96)	4.12 (3.61–5.03)	4.34 (3.89–4.73)	
Total ω-6				0.41
Mean ± SD	28.54 ± 1.80	28.40 ± 1.99	28.88 ± 1.23	
Median (IQR)	28.82 (27.88–29.70)	28.74 (27.64–29.58)	29.13 (28.18–29.81)	
ω-6/ω-3 ratio				0.52
Mean ± SD	3.73 ± 0.79	3.69 ± 0.83	3.85 ± 0.69	
Median (IQR)	3.88 (3.34–4.07)	3.81 (3.29–4.07)	3.89 (3.57–4.07)	

NOTE: Prostate cancer progression is defined as biopsy-detected Gleason 7 at the first repeat biopsy session.
Abbreviation: LC ω-3, long-chain omega-3 fatty acids (EPA+DPA+DHA).
[a]Fatty acids are expressed as a percentage of total fatty acids in the RBC membranes.
[b]*P* values were obtained by using the Fligner-Policello test.

were at the limit of significance when assessed in the diet using the web-FFQ. The multivariable logistic regression model clearly shows that men in the highest tertile of prostatic EPA level have a drastically lower risk of progression to a high-risk cancer (7% of this group) than men in the lowest tertile of prostatic EPA (50% of this group; $P = 0.03$), with an accuracy of 0.90. We also found that anthropometric factors, such as obesity or visceral fat, clinical measures (except the number of positive biopsy zones), smoking status, educational level, and the time between the diagnosis and the first repeat biopsy session were not associated with prostate cancer progression in this cohort, as they commonly are in other cohorts (38).

The three most prevalent methods to assess fatty acid intake are the FFQ, the circulating fatty acids, and the membranes of the RBCs, but each method is exposed to error measurement. First, the FFQ is an informative tool, but is limited by its semiquantitative nature and reliance on the patient's capacity to remember his diet. Despite significant correlations between EPA assessment methods (Spearman's correlation coefficients values of 0.513 between the web-FFQ and the RBCs, 0.345 between the web-FFQ and the prostate tissue, and 0.582 between the RBC and the prostate tissue; all $P < 0.05$), the association between EPA and prostate cancer risk was only significant in prostate tissue. A recent meta-analysis suggested that long chain ω-3 fatty acids (EPA, DPA, and DHA) and ω-6 fatty acids do not affect the risk of prostate cancer, whereas a high intake of α-linolenic acid (ALA, C18:3n3) may reduce the risk of prostate cancer (16). However, this meta-analysis study did not include the Health Professionals Study, which showed that a high intake of ALA was associated with an increased risk of prostate cancer (39). This meta-analysis is also exposed to various information and selection biases as referred in details by Reese and colleagues (4). Second, the circulating plasma fatty acids are reflective of the short-term intake of fatty acids (40) and are therefore greatly influenced by the last meal's content. Moreover, circulating plasma fatty acids are, by definition, not incorporated in the tissue's membranes and are not biologically active. This measurement is hard to interpret from a mechanistic point of view. Therefore, blood should be collected after an overnight fast and timing of the blood collection and analysis reported as this affects oxidation of the samples. Results from recent studies using circulating plasma fatty acids only (13, 20) should be interpreted with caution because of exposure measurement

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Table 4. Fatty acid profile of prostate tissue stratified by prostate cancer progression status

Fatty acid[a]	Total ($n = 48$)	Nonprogression ($n = 35$)	Progression ($n = 17$)	P^b
Total ω-3				0.45
Mean ± SD	3.65 ± 0.98	3.73 ± 0.99	3.45 ± 0.97	
Median (IQR)	3.56 (2.93–4.09)	3.64 (3.01–4.06)	3.28 (2.79–4.37)	
ALA				0.67
Mean ± SD	0.31 ± 0.31	0.32 ± 0.30	0.28 ± 0.34	
Median (IQR)	0.26 (0.00–0.55)	0.33 (0.00–0.53)	0.16 (0.00–0.68)	
LC ω-3				0.90
Mean ± SD	2.99 ± 1.09	3.03 ± 1.12	2.88 ± 1.06	
Median (IQR)	2.91 (2.26–3.53)	2.89 (2.49–3.40)	3.02 (2.21–3.81)	
EPA				**0.004**
Mean ± SD	0.13 ± 0.19	0.16 ± 0.22	0.05 ± 0.08	
Median (IQR)	0.09 (0.00–0.19)	0.13 (0.00–0.21)	0.00 (0.00–0.09)	
DPA				0.79
Mean ± SD	0.84 ± 0.25	0.85 ± 0.25	0.81 ± 0.26	
Median (IQR)	0.83 (0.70–0.91)	0.83 (0.75–0.89)	0.82 (0.60–1.08)	
DHA				0.79
Mean ± SD	2.02 ± 0.77	2.02 ± 0.76	2.01 ± 0.84	
Median (IQR)	1.94 (1.52–2.51)	1.92 (1.55–2.35)	2.07 (1.50–2.62)	
Total ω-6				0.52
Mean ± SD	22.69 ± 3.05	22.59 ± 2.65	22.95 ± 3.96	
Median (IQR)	23.37 (20.17–24.53)	23.29 (20.19–24.11)	24.04 (20.15–26.48)	
ω-6/ω-3 ratio				0.13
Mean ± SD	6.53 ± 1.44	6.35 ± 1.41	6.95 ± 1.46	
Median (IQR)	6.27 (5.51–7.41)	6.21 (5.45–7.13)	7.05 (6.06–7.71)	

NOTE: Prostate cancer progression is defined as biopsy-detected Gleason 7 at the first repeat biopsy session.
Abbreviation: LC ω-3, long-chain omega-3 fatty acids (EPA+DPA+DHA).
[a]Fatty acids are expressed as a percentage of total fatty acids in the prostate tissue.
[b]*P*-values were obtained by using the Fligner-Policello test.
Bold font indicates significance at $P < 0.05$.

issues. Also, these two recent studies (13, 20) did not report information about the consumption of dietary fish or supplements of ω-3 fatty acids in particular. This raises the possibility for confounding by these variables and others, and of internal validity problem, as they were not designed specifically to determine the impact of ω-3 fatty acids on prostate cancer.

We also conducted analyses including patients consuming ω-3 fatty acid supplements ($n = 52$, data not shown). A more significant association between EPA concentration in prostate tissue and prostate cancer progression was observed ($P = 0.0006$ vs. 0.004 excluding those patients) and the regression model for the EPA in the prostate tissue was stronger ($P = 0.01$ for the univariable and 0.005 for the multivariable regression modeled continuously vs. 0.03 and 0.01). Although the analysis of patients consuming supplements has greater potential for bias (which is why we excluded them from the main analysis), it does support the protective effect of EPA in the target tissue (prostate). It has already been shown that ω-3 fatty acid supplementation increases the ω-3 fatty acid content of prostate tissue in specimens of prostatectomy (41, 42). These observations combined with our new results suggest a potential protective effect of ω-3 fatty acid supplements on prostate cancer carcinogenesis.

The membrane of the RBCs is a marker of fatty acid intake, as it is reflective of the past 3 months' diet (43). However, because of the absence of fatty acid metabolism in the RBCs, their fatty acid profile may not accurately reflect the profile found in the metabolically active tissues such as the prostate tissue. A recent meta-analysis article examined the associations between circulating fatty acids, fatty acid profile of the RBC, and prostate cancer risk (44). This study suggested that circulating DPA may be preventive against prostate cancer in contrast with EPA and DHA, which were associated with an increased risk of high-risk prostate cancer. Nevertheless, taken separately, heterogeneity was noted in the analysis of association of blood level DHA and EPA with prostate cancer and no significant association has been observed. Indeed, although DHA and EPA in humans are mostly coming from the diet, DPA is principally derived from endogenous synthesis from EPA, with partial reconversion back to EPA (45). Thus, combining single fatty acid concentrations in blood as a surrogate of their dietary intake may lead to misinterpretations as described above.

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Table 5. EPA measurements and risk of prostate cancer progression

				Univariable models			Multivariable models[a]		
Method	**EPA tertile**	***n***	**High-grade prostate cancer (%)**	**OR (95% CI)**	***P*[b]**	***P*[c]**	**OR (95% CI)**	***P*[b]**	***P*[c]**
Web-FFQ	1	16	31%	1.00		0.23	1.00		0.06
	2	16	25%	0.80 (0.17–3.80)	0.78		0.78 (0.11–5.61)	0.80	
	3	15	20%	0.55 (0.11–2.86)	0.48		0.15 (0.01–1.52)	0.11	
RBC	1	15	33%	1.00		0.33	1.00		0.64
	2	17	29%	0.83 (0.19–3.72)	0.81		0.45 (0.03–6.62)	0.56	
	3	16	25%	0.67 (0.14–3.17)	0.61		0.62 (0.07–5.80)	0.68	
Prostate tissue	1	18	50%	1.00		**0.03**	1.00		**0.01**
	2	16	25%	0.33 (0.08–1.44)	0.14		0.12 (0.01–1.22)	0.07	
	3	14	7%	0.08 (0.01–0.72)	**0.02**		0.008 (<0.001–0.56)	**0.03**	

NOTE: Logistic regression models in which outcome is the presence of high-risk prostate cancer defined as Gleason score 7 at the first repeat biopsy session versus low-risk prostate cancer or absence of prostate cancer at the first repeat biopsy session.

[a]Multivariable models were adjusted for age, PSA level, total energy intake, smoking status, time between the initial and the first repeat biopsies, and number of positive biopsy zones at the first repeat biopsy session.

[b]*P* value of category relative to referent (lowest tertile).

[c]*P* value of the nutrient variable modeled continuously.

Bold font indicates significance at $P < 0.05$.

Prostatic metabolism of fatty acids was also assessed in this study. Cancer cell metabolism, including that of prostate cancer cells, is characterized by a high *de novo* fatty acid lipogenesis (46); for example, cancerous cells have an increased Δ9-desaturase activity. In this cohort, the Δ9-desaturase activity was the same in the nonprogression group and the progression group (Table 6). Furthermore, no difference was observed for the Δ5 and Δ6-desaturase activity. However, the nonprogression group had a higher activity of the Elovl2 elongase, which converts EPA to DPA,

Table 6. Estimation of elongase and desaturase activity in the nonprogression and the progression group

Fatty acids ratio	**Enzymatic activity**		**Nonprogression ($n = 34$)**	**Progression ($n = 14$)**	***P*[a]**
20:3n6/18:3n6	Elovl5 elongase	Mean ± SD	4.33 ± 12.06	2.49 ± 6.99	
		Median (IQR)	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.89
22:4n6/20:4n6	Elovl2 elongase	Mean ± SD	0.14 ± 0.03	0.16 ± 0.04	
		Median (IQR)	0.14 (0.12–0.16)	0.15 (0.13–0.16)	0.34
20:4n3/18:4n3	Elovl5 elongase	Mean ± SD	0.12 ± 0.60	0.20 ± 0.73	
		Median (IQR)	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.88
22:5n3/20:5n3	Elovl2 elongase	Mean ± SD	3.95 ± 3.54	1.79 ± 2.87	
		Median (IQR)	4.02 (0.00–4.98)	0.00 (0.00–3.29)	**0.01**
18:1n9/18:0	Δ9-desaturase	Mean ± SD	2.14 ± 1.42	2.40 ± 2.24	
		Median (IQR)	1.73 (1.12–2.66)	1.42 (1.10–2.16)	0.70
18:3n6/18:2n6	Δ6-desaturase	Mean ± SD	0.00 ± 0.00	0.00 ± 0.00	
		Median (IQR)	0.00 (0.00–0.00)	0.00 (0.00–0.00)	1.00
18:4n3/18:3n3	Δ6-desaturase	Mean ± SD	0.15 ± 0.20	0.07 ± 0.12	
		Median (IQR)	0.07 (0.00–0.24)	0.00 (0.00–0.18)	0.15
20:4n6/20:3n6	Δ5-desaturase	Mean ± SD	4.78 ± 0.91	4.86 ± 1.42	
		Median (IQR)	4.76 (4.00–5.52)	4.52 (3.63–6.49)	0.83
20:5n3/20:4n3	Δ5-desaturase	Mean ± SD	0.29 ± 1.27	0.028 ± 0.11	
		Median (IQR)	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.80

NOTE: The progression group had a lower conversion rate of docosapentaenoic acid (22:5n3) from EPA (20:5n3) than the nonprogression group, as measured by the Elovl2 elongase activity. Prostate cancer progression is defined as biopsy-detected Gleason 7 at the first repeat biopsy session.

[a]*P* values were obtained by using the Fligner-Policello test.

Bold font indicates significance at $P < 0.05$.

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than the progression group ($P = 0.01$). As the prostatic DPA level is similar in both groups, the difference of Elovl2 elongase activity is driven by the prostatic EPA level. Nonetheless, the nonprogression group had a higher prostatic EPA level and a higher EPA dietary intake, although nonsignificant, than the progression group, suggesting that the dietary intake of ω-3 fatty acids is more important than the interindividual functional metabolic variations. This is especially true for long-chain fatty acids as their *de novo* synthesis is limited. This suggests that the microenvironment of high-risk prostate cancer (Gleason 7 or greater) is biologically different than that of low-risk prostate cancer (Gleason 6).

Previous studies have determined the link between prostatic fatty acid levels and prostate cancer, but all were based on prostatectomy specimens (42, 47). Our study is original, as it is based on prostatic biopsies from men affected with low-risk (Gleason 6) prostate cancer and managed under active surveillance. We also observed that one prostate biopsy is sufficient to determine the fatty acid profile of the prostatic tissue and that this determination is reproducible (Supplementary Table S2). The present results show association with prostate cancer progression mostly for the fatty acid profile of the prostate tissue, suggesting that an estimation of the prostate ω-3 fatty acids, more particularly of the EPA content, may be meaningful to assess the risk of prostate cancer progression. No association between the dietary fatty acid intake and the RBC measurements has been observed. Thus, determining the prostatic fatty acid profile of men under active surveillance could eventually become a predictive tool to manage men affected with low-risk prostate cancer.

The strengths of this study are its prospective design and its uniform cohort, untreated men diagnosed with Gleason 6 prostate cancer. Some limitations of this study are worth mentioning. First, the small sample size, linked to the complexity of tissue procurements, exposes this study to sampling error and instability of statistical models. Notwithstanding the need to validate these data in a larger cohort, observing strong differences in such a small sample size is promising. Second, the FFQ was administered after the initial diagnosis of prostate cancer. As approximately 29% of men diagnosed with prostate cancer change their diet toward a healthier one following the initial diagnosis (48), we do not know whether the differences observed between the nonprogression and the progression groups reflect a long-term diet or a change in the diet following the diagnosis of prostate cancer. Nonetheless, as all the men of this cohort were originally diagnosed with a similar low-risk prostate cancer and have completed the FFQ before the diagnostic information of the first repeat biopsy session was available, it is unlikely that the proportion of men who significantly changed their diet would be different between the two groups. Moreover, the dietary information we collected is not subject to differential recall across groups, which could otherwise have biased the association, as it was prospectively collected well before outcomes were available. Third, confounding from other unmeasured factors is possible. For example, we did not adjust for physical activity, which may induce variation in dietary intake and metabolism. However, we did adjust for many other potential confounding factors such as smoking habits and anthropometric measures. Also, health care coverage is universal in Canada, and thus cannot affect the observed measure. Fourth, the possibility for selection bias seems limited as we included in this study all eligible patients who tolerated the protocol (see CONSORT diagram; Supplementary Fig. S1). Fifth, as for all other studies conducted during active surveillance, it is impossible to know whether the grade progression is real or a sampling artefact. However, one important factor to consider is that in this clinical trial the same ultrasonographist performed all the first repeat ultrasound procedures, and a central review of pathologic specimens has been done for all biopsy sessions for all patients. Despite the risk of undersampling, prostate biopsy remains the most important diagnostic tool to detect prostate cancer and to decide appropriate treatments for patients (49). Finally, results presented herein are from observational data before any intervention. Thus, the present article draws inferences about the possible effect of ω-3 fatty acids on prostate cancer progression.

Marine ω-3 fatty acids, mainly EPA, seem to be protective against prostate cancer progression. Even given the small size of the cohort, this association is more precise when examined in the target prostate tissue than with surrogate methods. This likely explains part of the inconsistent associations between ω-3 fatty acids and prostate cancer previously published. Both mechanistic and specifically designed clinical trials are needed to decipher the biology and the beneficial effects of ω-3 fatty acids on prostate cancer.

Disclosure of Potential Conflicts of Interest

B. Lamarche received a commercial research grant from Atrium Innovations. No potential conflicts of interest were disclosed by the other authors.

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): X. Moreel, B. Lamarche, P. Julien, V. Fradet
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): X. Moreel, J. Allaire, C. Léger, A. Caron, P. Julien, B. Têtu, V. Fradet
Writing, review, and/or revision of the manuscript: X. Moreel, J. Allaire, M.-E. Labonté, B. Lamarche, P. Julien, V. Fradet
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): X. Moreel, J. Allaire, P. Desmeules
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Revision of pathologic issues: P. Desmeules
Central revisions of study cases: B. Têtu

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