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Blood fatty acid patterns are associated with prostate cancer risk in a prospective nested case-control study

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Abstract

Background—Circulating fatty acids are highly correlated with each other and analyzing fatty acid patterns could better capture their interactions and their relation to prostate cancer. We aimed to assess the associations between data-derived blood fatty acid patterns and prostate cancer risk.

Methods—We conducted a nested case-control study in the Physicians' Health Study. Fatty acids levels were measured in whole blood samples of 476 cases and their matched controls by age and smoking status. Fatty acid patterns were identified using principal component analysis. Conditional logistic regression was used to estimate odds ratio (OR) and 95% confidence interval (CI).

Results—Two patterns explaining 40.9% of total variation in blood fatty acid levels were identified. Pattern 1, which mainly reflects polyunsaturated fatty acid metabolism, was suggestively positively related to prostate cancer risk (OR_{quintile 5 vs. quintile 1}=1.37, 95%CI=0.91–2.05, P_{trend} =0.07). Pattern 2, which largely reflects *de novo* lipogenesis, was significantly associated with higher prostate cancer risk (OR_{quintile 5 vs. quintile 1}=1.63, 95%CI=1.04–2.55, P_{trend} =0.02). This association was similar across tumor stage, grade, clinical aggressiveness categories and follow-up time.

Conclusion—The two patterns of fatty acids we identified were consistent with known interactions between fatty acid intake and metabolism. A pattern suggestive of higher activity in the *de novo* lipogenesis pathway was related to higher risk of prostate cancer.

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Keywords

blood fatty acids; principal component analysis; prostate cancer; case-control study

INTRODUCTION

Many prospective cohort studies have examined associations of blood levels of individual or classes of fatty acids – reflecting both intake (1) and metabolism (2) - with risk of prostate cancer (3–15), but results are inconsistent across studies. For example, circulating levels of α -linolenic acid have been associated to a higher risk of prostate cancer in some (10, 11, 14) but not all studies (7, 9, 12, 13, 15). Moreover, a recent pooled analysis did not find compelling evidence for strong associations between circulating fatty acid levels and prostate cancer risk (16). However, significant heterogeneity across studies was identified for several of the associations evaluated warranting a closer look into the relation between circulating fatty acids and prostate cancer.

A potentially problematic methodological issue is that most studies have considered individual fatty acids as separate exposures. However, individual blood fatty acids tend to be correlated with each other, partially due to shared food sources and metabolic pathways. Additionally, fatty acid levels are often measured simultaneously in a single lab procedure, and are commonly expressed as relative amounts (e.g. percentage of total fatty acids, grams per grams of lipids) thus opening the possibility that levels of one fatty acid could influence relative amounts of other fatty acids. Hence, in addition to examining individual fatty acids, analyzing fatty acids as sets of patterns could capture these complexities and account for these technical issues, while shedding light on the biological interactions between different fatty acids and their relation with disease risk.

Principal components analysis (PCA) is a widely used statistical technique that has been used to identify food intake patterns from correlated food intakes (17) and has identified associations between food consumption patterns and chronic disease risk (18, 19). This technique could be also applied to biomarker panels such as blood fatty acid levels, but utilization so far has been limited (20–23). Therefore, in this study, we evaluated whether PCA-derived patterns of blood fatty acid levels were related to prostate cancer risk in men participating in the Physicians' Health Study.

SUBJECTS AND METHODS**Study Population**

The Physicians' Health Study (PHS) was a randomized trial of 22,071 male US physicians aged 40–84 years to assess the effect of aspirin and beta-carotene in the prevention of cardiovascular disease and cancer, initiated in 1982 (24, 25). We excluded men with history of myocardial infarction, stroke, transient ischemic attack, unstable angina, cancer (except non-melanoma skin cancer), renal or liver disease, peptic ulcer, gout, contraindication to aspirin or men who were current users of aspirin, platelet-active medications or vitamin A supplements. The aspirin arm of the trial ended in January 1988 because of the benefits of

aspirin in reducing myocardial infarction (24), while the beta-carotene arm ended as scheduled in 1995 (25). We have continued to follow the cohort for disease outcomes. The study was approved by the Human Research Committee at Brigham and Women's Hospital and written informed consent was acquired from each subject.

We obtained pre-randomization blood specimens from 14,916 (68%) participants that were processed after overnight delivery and stored at -82°C (10). This report was restricted to subjects diagnosed with prostate cancer after providing their baseline blood sample, and their matched controls. Follow-up was more than 98% complete for morbidity and 99% for mortality.

Selection of Case and Control

Whenever a participant reported a prostate cancer diagnosis, we sought hospital and pathology records for review by the PHS Endpoints Committee to confirm the diagnosis and determine the tumor stage and grade at diagnosis. We used the Gleason scoring system for histologic grade, and the Tumor Node Metastasis (TNM) staging system or conversion of the modified Whitmore-Jewett classification scheme (for prostate cancer cases diagnosed during the early years of PHS follow-up) for tumor stage. We used risk-set sampling to select one control subject for each confirmed cases among those who provided a blood sample, excluding men who had a partial or total prostatectomy or prostate cancer at the time of the case's diagnosis. Controls were individually matched to cases by age (within 1 year for men aged 55 years or younger, and within 5 years for men older than 55 years) and smoking status at baseline (current, former or never). Among the 758 cases diagnosed through 1995, 505 (67%) provided a baseline blood sample for the determination of fatty acid levels. After excluding cases and controls with blood samples received six or more days after it was drawn, we were left with 476 cases and their matched controls eligible for analysis.

Laboratory Analysis

To reduce any effect of inter-assay variability, blinded samples from cases and their matched controls were processed and analyzed together. Fatty acids were extracted from whole blood into isopropanol and hexane containing 50 mg of 2,6-di-tert-butyl-*p*-cresol as an antioxidant. Fatty acids were transmethyated with methanol and sulfuric acid, as previously described (2, 26). After esterification, the samples were evaporated and the fatty acids were redissolved in iso-octane and quantified by gas-liquid chromatography on a fused silica capillary *cis/trans* column (SP2560, Supelco, Belafonte, PA). Peak retention times were identified by injecting known standards (NuCheck Prep, Elysium, MN) and analyzed with the ChemStation A.08.03 software (Agilent Technologies). We expressed fatty acid levels in each sample as the percentage of total fatty acids. Coefficients of variation (CV) for all fatty acid peaks were measured by analyzing quality control samples (indistinguishable from other study samples) randomly distributed throughout the study samples. Fatty acids with a within-person variation exceeding the between-person variation were excluded from analysis ([12:0], [15:1n-5c], and [14:1n-5t]). The remaining 28 fatty acids had CVs ranging from 0.3% for oleic acid to 15.7% for myristoleic acid.

Identification of Fatty Acids Patterns

We conducted PCA to derive fatty acids patterns based on 28 blood levels of individual fatty acids. The factors were rotated using an orthogonal transformation that results in uncorrelated patterns to achieve a simpler structure with greater interpretability. In determining the number of factors to retain, we considered components with an eigenvalue greater than 2, the Scree plot, and the interpretability of the patterns. Cumulative percentages of variance explained by each component were not used as a criterion because it depends largely on the total number of variables included in the PCA (17). The factor score for each pattern was constructed by summing fatty acid levels weighted by the factor loadings for each individual fatty acid. The analyses were conducted by using the PROC FACTOR procedure in SAS (SAS Institute, Cary, NC).

Statistical Analysis

We calculated median values and proportions of the baseline characteristics of case and control subjects. To evaluate whether these characteristics differed between cases and controls, categorical variables were tested using the chi-square test and continuous variables were tested using the Wilcoxon rank-sum test. To estimate the association between blood fatty acid patterns and prostate cancer, we divided men into quintiles of fatty acid patterns based on the pattern distribution among the controls. Conditional logistic regression estimated the relative risk of prostate cancer in a given quintile of fatty acid pattern using men in the lowest quintile as the reference. We considered the potential confounding effects of baseline characteristics (age at baseline, smoking status) by adding terms for variables associated with prostate cancer and fatty acid patterns at $p < 0.20$. BMI met these conditions and was added to the final multivariable models. Lastly, we fitted regression models in subgroups defined by tumor stage and grade at diagnosis, clinical aggressiveness (defined by stage T3/T4/M1 at diagnosis, Gleason ≥ 8 at diagnosis, or development of distant metastases or death due to prostate cancer following diagnosis) and follow-up time (< 10 years vs ≥ 10 years). The significance of differences in stratum-specific estimates was assessed using polytomous logistic regression. Tests for linear trend were conducted by Wald statistics in all models using the median fatty acid pattern levels in each quintile as a continuous variable. Effect modification by BMI was evaluated by including cross-product terms to the multivariable model. All statistical analyses were performed using SAS, version 9.3 (SAS Institute, Cary, NC). We considered results to be statistically significant when $P < 0.05$, two tailed.

RESULTS

The correlation matrix of 28 fatty acids is in Figure 1. We identified two major fatty acid patterns in whole blood samples (Table 1). Pattern 1, which explained 25.8% of the variance, was characterized by higher blood levels of *trans* fatty acids [18:1n-12t, 18:1n-9t, 18:1n-7t, 18:2n-6t, 18:2n-6ct, 18:2n-6c] and α -linolenic acid [18:3n-3c], along with lower long chain polyunsaturated fatty acids (PUFAs) of the n-6 series (arachidonic acid [20:4n-6c]) and the n-3 series (eicosapentaenoic [20:5n-3c]; docosapentaenoic [22:5n-3c], and docosahexaenoic acid [22:6n-3c]). Pattern 2, which explained 15.1% of the variance, was characterized by higher levels of 14 and 16 carbon saturated and monounsaturated fatty acids (MUFA;

myristic [14:0], palmitic acid [16:0], myristoleic [14:1n-5c] and palmitoleic acid [16:1n-7c]) and of γ -linolenic acid [18:3n-6c], along with lower levels of linoleic acid [18:2n-6cc], aolenic acid [22:4n-6c], *trans* vaccenic acid [18:1n-7t], stearic acid [18:0], and docosanoic acid [22:0]. Oleic acid [18:1n-9] levels were high in both patterns.

There were no major differences in baseline characteristics between men who were later diagnosed with prostate cancer and their matched controls (Table 2). Most men were diagnosed with prostate cancer at a median age of 67. More than 80% of the tumors were localized at diagnosis and nearly two thirds of the tumors had a Gleason score below 7.

There was a suggestion of a positive association between fatty acid pattern 1 and prostate cancer risk that was not statistically significant ($OR_{\text{quintile 5 vs. quintile 1}}=1.37$, 95%CI=0.91–2.05, $P_{\text{trend}}=0.07$) (Table 3). Pattern 2, on the other hand, was associated with a significantly higher risk of prostate cancer. Compared to men in the lowest quintile of pattern 2, men in the highest quintile were 55% more likely to develop prostate cancer during follow-up ($P_{\text{trend}}=0.03$). Further mutual adjustment of patterns did not change the estimates appreciably ($RR=1.63$, 95%CI=1.04–2.55, $P_{\text{trend}}=0.02$) (Table 3).

Pattern 1 was positively related to tumors of Gleason <7 but not to those with Gleason ≥ 7 (Table 4). This apparent difference, however, was not statistically significant ($P_{\text{heterogeneity}}=0.10$). The associations between fatty-acid pattern 2 and prostate cancer risk were similar in strata defined by tumor stage, grade, clinical aggressiveness and follow-up time (Table 4). We found no evidence for effect modification by BMI.

DISCUSSION

We identified two patterns of fatty acids in whole blood. The first pattern, characterized by high levels of trans-fats and α -linolenic acid along with low levels of long-chain n-3 PUFAs, had a positive but not statistically significant association with prostate cancer risk. The second pattern, characterized by higher levels of MUFAs, myristic and palmitic acids, and γ -linolenic acid along with low levels of α -linoleic acid and saturated fats of 18 or more carbons, was associated with a significantly higher risk of developing prostate cancer. The relations of patterns with prostate cancer risk were similar across levels of stage, grade, clinical aggressiveness, and follow-up time, and were not modified by BMI.

The two patterns identified in this study are consistent with the current understanding of shared pathways and regulations between metabolism of dietary fatty acids and *de novo* lipid synthesis. The fatty acids highly-loaded in pattern 1 is in agreement with previous reports in animal models showing that dietary trans-fatty acids can impair Δ^6 -desaturase activity, the rate limiting step in PUFAs metabolism (27). Since blood trans-fatty acids are indicators of dietary intake, this pattern could reflect high intake of these fats leading to a decrease in Δ^6 -desaturase activity. Under these circumstances conversion of α -linolenic acid would be impaired, resulting in high levels of α -linolenic acid and low levels of long chain n-3 fatty acids, eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA) (Figure 2). The non-significant positive association of pattern 1 with prostate cancer risk parallels previous work in this cohort (6, 8). It is possible that the combination of

different fatty acids as summarized by this data-derived pattern may be diluting the previously-identified signals via adding noise resulting from incorporating into the summary pattern fatty acids that are unrelated to risk.

Pattern 2 is suggestive of higher activity of the *de novo* lipogenesis pathway (the endogenous synthesis of even chain saturated fatty acids from acetyl CoA) resulting in higher levels of 14C and 16C SFAs, coupled with higher activity of desaturases and lower activity of elongases in both PUFA metabolism (resulting in higher levels of γ -linolenic acid and lower levels of linoleic acid) and the *de novo* lipogenesis pathway (resulting in higher levels of 14C and 16C MUFAs as opposed to 18C and longer SFAs) (Figure 2). Although the desaturase enzymes, namely 6-desaturase and 5-desaturase in PUFAs metabolism and 9-desaturase in *de novo* lipid synthesis, have different specific functions, they share a regulatory mechanism; suppressed activity by PUFAs (28). Our finding that pattern 2 is positively related to prostate cancer risk, supported the hypothesis that *de novo* lipogenesis plays a prominent role in prostate carcinogenesis. This hypothesis was also supported by our previous study documenting that two single nucleotide polymorphisms in Fatty Acid Synthase gene were related to prostate cancer risk (29) and that blood levels of MUFAs are positively associated with prostate cancer risk (30).

While multiple studies have previously investigated the relation of individual fatty acids with prostate cancer risk (3, 4, 6, 8–15), only two have summarized fatty acid levels into patterns (21, 31). Shannon and colleagues identified six fatty acid patterns using PCA that explained 62.5% of the variance (21), where two of these patterns, one negatively associated with PUFAs and the other positively associated with 16C SFA and MUFAs and negatively with 18C SFA, have very similar composition to the patterns identified in our study. However, in the Shannon *et al* report, only the pattern with high SFAs and low MUFAs was associated with higher prostate cancer risk. No other patterns were related to prostate cancer risk. In a separate report from the EPIC cohort, investigators used a Treelet Transform (TT) algorithm on 26 plasma fatty acids (31). TT is a dimension-reduction method that can be viewed as a combination of PCA and hierarchical clustering methods resulting in smaller number of grouped variables without contribution from the remaining variables (32). Of the four patterns identified, a pattern characterized by high levels of n-3 long chain PUFAs was related to higher risk of prostate cancer and the remaining three patterns were not related to prostate cancer.

A comparison of our results with the emerging literature on the identification of fatty acid patterns as predictors of chronic disease risk highlights more the limitations of this approach than its potential benefits. While there is some degree of subjectivity in selecting the number of patterns and in assigning an interpretation when using PCA, when applied to food intakes this technique has produced remarkably consistent patterns across studies in Western populations regardless of sex or age (18, 19, 33–36). However, for fatty acid patterns neither the number of patterns nor their composition has been consistent across the studies that have used this approach to examine prostate cancer risk. The same is true for the studies that have used PCA to derive fatty acid patterns and related the later to cardio-metabolic outcomes (22, 23). In the case of prostate cancer, the results of pattern-based analyses essentially mirror previous results for individual fatty acids, observed in the EPIC studies (9, 31) and

our previous finding on the positive relation between MUFAs and prostate cancer (30), which closely approximates our findings for pattern 2 in direction but not in magnitude. While the literature in this area is still emerging, the current evidence does not suggest that identifying patterns is more informative than investigating fatty acids individually or grouped according to shared metabolism. However, these findings do not preclude this strategy as a useful tool in characterizing exposure patterns in biomarker panels when metabolic pathways or biologic relations between analytes are less well understood than fatty acid metabolism.

It is important to consider the limitations and strengths of our study. A potential limitation is that unmeasured and residual factors associated with blood fatty acid levels may be responsible for the observed associations. Nevertheless, we evaluated a number of variables as potential confounders and did not find any appreciable impact on the result upon adjustment for these variables. Another limitation is that, as discussed above, PCA involves arbitrary but important decisions in determining the number of factors to extract which could account for some of the discrepancies observed across studies. Our study also has several strengths including its prospective design with a long period between blood draw and prostate cancer diagnosis (a median of 9 years), which minimizes the possibility of reverse causation. The high follow up rates in the PHS cohort decrease the possibility of selection bias and the large number of cases provided sufficient statistical power.

In summary, we identified two major whole blood fatty acid patterns in the PHS cohort. These patterns could be interpreted based on the current understanding of fatty acid metabolism and regulation. The patterns, respectively, suggestive of interactions between trans-fatty acids and PUFA, and indicative of higher activity of desaturases and lower activity of elongases in both PUFA metabolism and the *de-novo* lipogenesis pathway, were both positively related to prostate cancer risk. While fatty acid patterns may have captured complex interactions between individual fatty acids, analyzing patterns does not appear to provide significant advantages over analyzing specific fatty acids or fatty acid groupings based on known metabolic pathways in relation to prostate cancer risk.

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All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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Figure 1. Heatmap for correlation matrix of 28 circulating fatty acids in the whole blood measured among 476 controls in the Physicians' Health Study.

Figure 2.

Major metabolic pathways in fatty acid metabolism: A) *de novo* lipogenesis and B) Polyunsaturated fatty acid metabolism. *De novo* lipogenesis converts excess acetyl CoA, from carbohydrate metabolism, into fatty acids that are then esterified to storage triglycerides. This metabolic pathway involves synthase, elongase and desaturase. Polyunsaturated fatty acid (PUFA) metabolism converts C18PUFA (AA and ALA) into long chain PUFA by desaturation and elongation. n-3 long chain PUFA undergo multiple steps including beta-oxidation to generate DHA.

AA: arachidonic acid; ALA: α -linolenic acid; DGLA: Dihomo- γ -linolenic acid; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; GLA: γ -linolenic acid; LA: linoleic acid; n-3 PUFA: n-3 polyunsaturated fatty acid; n-6 PUFA: n-6 polyunsaturated fatty acid.

Table 1

Factor loading matrix for the major factors (fatty acids patterns) identified by using blood fatty acids data in the Physicians' Health Study¹

Blood fatty acids	Common Names	Pattern 1	Pattern 2
<i>n-6 polyunsaturated fatty acid</i>			
18:2n-6cc	Linoleic acid	-	-0.58
18:3n-6c	γ -linolenic acid	-	0.42
20:2n-6c	Eicosadienoic acid	-	-
20:3n-6c	Dihomogammalinolenic acid	-	-
20:4n-6c	Arachidonic acid	-0.62	-
22:2n-6c		-	-
22:4n-6c	Aolenic acid	-	-0.43
<i>n-3 polyunsaturated fatty acid</i>			
18:3n-3c	α -linolenic acid	0.44	-
20:3n-3c		-	-
20:5n-3c	EPA	-0.54	-
22:5n-3c	DPA	-0.54	-
22:6n-3c	DHA	-0.45	-
<i>Trans-unsaturated fatty acid</i>			
18:1n-12t	Petroselaic acid	0.86	-
18:1n-9t	Elaidic acid	0.87	-
18:1n-7t	Vaccenic acid	0.65	-0.43
18:2n-6t	Linolelaic acid	0.84	-
18:2n-6ct		0.82	-
18:2n-6tc		0.78	-
<i>Saturated fatty acid</i>			
14:0	Myristic acid	-	0.75
16:0	Palmitic acid	-	0.86
18:0	Stearic acid	-	-0.53
22:0	Docosanoic acid	-	-0.58
<i>Monounsaturated fatty acid</i>			
14:1n-5c	Myristoleic acid	-	0.82
16:1n-7c	Palmitoleic acid	-	0.86
18:1n-9c	Oleic acid	0.47	0.64
18:1n-7c	Asclepic acid	-	-
20:1n-12c		-	-
20:1n-9c	Gondoic acid	-	-

¹ Blood fatty acids patterns were derived from 28 blood individual fatty acids among 476 prostate cancer cases and matched controls through principal component analysis.

DHA: docosahexaenoic acid; DPA: docosapentaenoic acid; EPA: eicosapentaenoic acid

Table 2

Clinical characteristics of prostate cancer cases and matched control subjects

	Cases (n = 476)	Controls (n = 476)	P value ¹
Age at baseline (years) ²	58 [53 – 64]	58 [53 – 63]	
Length of follow-up (years) ²	9 [7 – 11]	-	
Age at diagnosis (years) ²	67 [62 – 72]	-	
Disease presentation at diagnosis, %			
PSA screening	30	-	
Disease/metastatic symptoms	22	-	
Digital rectal exam screening	17	-	
Unspecified or missing	31	-	
Tumor Stage at diagnosis (TNM) %			
T1/T2	82	-	
T3	8	-	
T4/N1/M1	7	-	
Undetermined	4	-	
Gleason grade at diagnosis, %			
< 7	63	-	
= 7	25	-	
8	11	-	
Undetermined	2	-	
Date of diagnosis, %			
Before December 31, 1990	33	-	
On or after December 31, 1990	67	-	
Smoking Status, %			
Current	8	8	
Former	42	42	
White / Caucasian, %	95	93	0.51
Height (m) ²	1.78 [1.75 – 1.83]	1.78 [1.73 – 1.83]	0.12
BMI (kg/m ²) ²	24.4 [23.1 – 25.8]	24.2 [22.8 – 25.8]	0.13
Regular multivitamin use, %	21	24	0.47
Vigorous exercise twice per week or more, %	58	55	0.41
Alcohol use once per day or more, %	32	30	0.47
Blood fatty acid pattern ^{2,3}			
Pattern 1	-0.11 [-0.65, 0.62]	-0.23 [-0.72, 0.46]	0.13
Pattern 2	-0.0008 [-0.67, 0.70]	-0.16 [-0.75, 0.53]	0.03

¹P values were computed using the Wilcoxon sign rank test for continuous variables and the McNemar's test for categorical variables. Cases and controls were individually matched on age at baseline, smoking status at baseline and length of follow-up.

²Values expressed as Median [25th – 75th percentile].

³Blood fatty acids patterns were derived from 28 blood individual fatty acids among 476 prostate cancer cases and matched controls through principal component analysis.

Table 3

Relative risk (95% confidence intervals) of prostate cancer by control quintiles of blood fatty acid pattern

Quintile of blood fatty acid patterns					
	Q1	Q2	Q3	Q4	Q5
Pattern 1					
Cases / controls	88 / 95	76 / 95	103 / 96	91 / 95	118 / 95
Adjusted RR (95%CI) ²	1.00 (referent)	0.87 (0.58–1.30)	1.16 (0.78–1.73)	1.04 (0.68–1.59)	1.34 (0.90–2.00)
Pattern-adjusted RR (95%CI) ³	1.00 (referent)	0.88 (0.59–1.31)	1.16 (0.78–1.72)	1.07 (0.70–1.64)	1.37 (0.91–2.05)
Pattern 2					
Cases / controls	74 / 95	89 / 95	88 / 96	109 / 95	116 / 95
Adjusted RR (95%CI) ²	1.00 (referent)	1.19 (0.79–1.81)	1.23 (0.80–1.90)	1.49 (0.97–2.29)	1.55 (1.00–2.41)
Pattern-adjusted RR(95%CI) ³	1.00 (referent)	1.25 (0.82–1.90)	1.30 (0.84–2.02)	1.59 (1.03–2.47)	1.63 (1.04–2.55)

¹ Calculated with median fatty acid pattern in each quintile as a continuous variable.

² Adjusted for matching factors (age, smoking status at baseline, and length of follow-up) and BMI.

³ Adjusted with matching factors, BMI, and the other pattern (continuous).

RR: Relative risk; CI: Confidence interval

Table 4

Adjusted relative risk (95% confidence intervals) of prostate cancer across quintiles of blood fatty acid patterns according to tumor stage, grade, and aggressiveness^{1,2}

	Adjusted OR _{Q5vs.Q1} (95%CI)	Adjusted OR _{Q5vs.Q1} (95%CI)	P, heterogeneity
<i>Tumor Stage</i>	<i>T1/T2 (n = 388 cases)</i>	<i>T3/T4/M1 (n = 69 cases)</i>	
Pattern 1	1.49 (0.94–2.34)	0.92 (0.29–2.91)	0.71
Pattern 2	1.75 (1.06–2.89)	1.47 (0.42–5.10)	0.39
<i>Tumor Grade</i>	<i>Gleason < 7 (n = 298 cases)</i>	<i>Gleason ≥ 7 (n = 168 cases)</i>	
Pattern 1	1.81 (1.08–3.03)	1.09 (0.53–2.26)	0.10
Pattern 2	1.68 (0.85–2.98)	1.91 (0.87–4.17)	0.46
<i>Tumor Aggressiveness</i> ³	<i>Non-aggressive (n = 325 cases)</i>	<i>Aggressive (n = 151 cases)</i>	
Pattern 1	1.52 (0.93–2.49)	1.08 (0.53–2.22)	0.58
Pattern 2	1.64 (0.94–2.86)	1.71 (0.78–3.74)	0.63
<i>Follow-up time</i> ⁴	<i>< 10 years (n=304 cases)</i>	<i>10 years (n=172 cases)</i>	
Pattern 1	1.27 (0.77–2.09)	1.92 (0.89–4.15)	0.51
Pattern 2	1.31 (0.75–2.26)	2.69 (1.15–6.27)	0.31

¹ Adjusted for matching factors (age, smoking status at baseline, and length of follow-up), BMI, and the other pattern (continuous).

² For men in the highest quintile of the specific fatty acid pattern in comparison to men in the lowest quintile.

³ Tumor aggressiveness was defined if ultimately died, had metastasis, had stage T3/T4/M1, or Gleason ≥ 8 at diagnosis.

⁴ Follow-up time was time interval between blood draw and prostate cancer diagnosis.

RR: Relative risk; CI: Confidence interval