A Prospective Study of *Trans*-Fatty Acid Levels in Blood and Risk of Prostate Cancer

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Abstract

Background: **Previous studies suggest a positive association between markers of *trans*-fatty acid intake and prostate cancer. We therefore prospectively evaluated the association between blood *trans*-fatty acid levels and risk of prostate cancer.**

Methods: **We conducted a nested case-control study among 14,916 apparently healthy men who provided blood samples in 1982. Blood fatty acid levels were determined for 476 men diagnosed with prostate cancer during a 13-year follow-up and their matched controls. Controls were individually matched to cases according to age and smoking status at baseline. Conditional logistic regression was used to estimate the relative risk and 95% confidence interval of total, nonaggressive (stage A/B and low grade), and aggressive (stage C/D, high grade, subsequent distant metastasis or death) prostate cancer associated with blood levels of specific *trans*-fatty acids.**

Results: **Blood levels of all the *trans*-fatty acids examined were unrelated to total prostate cancer risk. When results were divided according to tumor aggressiveness, blood levels of 18:1n-9*t*, all the 18:2*t* examined, and total *trans*-fatty acids were positively associated to nonaggressive tumors. The relative risks (95% confidence intervals; *P* trend) comparing top with bottom quintile *trans*-fatty acid levels were 2.16 (1.12-4.17; 0.11) for 18:1n-9*t*, 1.97 (1.03-3.75; 0.01) for total 18:2*t*, and 2.21 (1.14-4.29; 0.06) for total *trans*-fatty acids. None of the *trans* fats examined was associated with aggressive prostate tumors.**

Conclusion: **Blood levels of *trans* isomers of oleic and linoleic acids are associated with an increased risk of nonaggressive prostate tumors. As this type of tumors represents a large proportion of prostate cancer detected using prostate-specific antigen screening, these findings may have implications for the prevention of prostate cancer.** (Cancer Epidemiol Biomarkers Prev 2008;17(1):95–101)

Introduction

Trans-fatty acids are unsaturated fatty acids with at least one double bond in the *trans*, rather than the *cis*, configuration. Most of the *trans*-fatty acids in diet come from foods containing partially hydrogenated vegetable oils, mainly from semisolid fats used in margarines and commercial food preparation (1). *Trans* fats resulting from bacterial action in ruminant animals are also consumed in smaller amounts from meats and dairy products. *Trans*-fatty acids have been implicated in the development of coronary heart disease and type 2 diabetes (1) and may also play a role in the etiology of prostate cancer. Intake of *trans* fats increases systemic inflammation (2-4) and insulin resistance (5, 6), both of which may play a role in prostate carcinogenesis (7, 8). Additionally, these fats interfere with the transcription of genes that may be important in the initiation and progression of prostate cancer and other epithelial tumors (9, 10). Five studies have explored the association between *trans*-fatty acids and prostate cancer (11-15), and of these, only two have examined whether the potential effect of *trans* fats differed according to tumor clinical characteristics (12, 13). We have previously examined the association between whole blood (16) levels of polyunsaturated fatty acids and prostate cancer risk. Here, we report the results of a prospective case-control study nested within the Physicians' Health Study that examines the association between levels of *trans*-fatty acids in whole blood, a suitable biomarker of intake (17), and risk of prostate cancer overall and separately for clinically aggressive and nonaggressive tumors.

Materials and Methods

This study is based in the Physician's Health Study (18, 19), a randomized trial of aspirin and β-carotene among 22,071 U.S. male physicians, ages 40 to 84 in 1982, without a history of cardiovascular disease, cancer, or other major illnesses. Written informed consent was obtained from each participant and the study was approved by the Human Research Committee at Brigham and Women's Hospital. Participants completed prerandomization questionnaires where they reported information on risk factors for cardiovascular disease and cancer, including anthropometric characteristics, a

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Table 1. Baseline characteristics of prostate cancer cases and control subjects

	Cases (n = 476)	Controls (n = 476)	P*
Age at baseline (y) †	58 (53-64)	58 (53-63)	‡
Length of follow-up (y) †	9 (7-11)	—	
Age at diagnosis (y)†	67 (62-72)	—	
Disease status at diagnosis (%)			
Localized (stage A or B)	61	—	
Advanced (stage C or D)	23	—	
Undetermined	16	—	
Tumor grade at diagnosis (%)			
Gleason <7 or well or moderately differentiated	65	—	
Gleason ≥7 or poorly differentiated	33	—	
Undetermined	2	—	
Date of diagnosis (%)			
Before October 1, 1990	33	—	
On or after October 1, 1990	67	—	
Smoking status (%)			‡
Current	8	8	
Former	42	42	
White/Caucasian (%)	95	93	0.51
Height (m) †	1.78 (1.75-1.83)	1.78 (1.73-1.83)	0.12
BMI (kg/m^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
Whole milk once per day or more (%)	7	8	0.71
Low-fat milk once per day or more (%)	27	23	0.17
Blood *trans*-fatty acids (% of total fatty acids)			
Total 18:1 *trans*-fatty acids	1.07 (0.89-1.42)	1.09 (0.87-1.37)	0.47
Total 18:2 *trans*-fatty acids	0.56 (0.45-0.65)	0.53 (0.44-0.66)	0.08
Total *trans*-fatty acids	1.82 (1.56-2.28)	1.81 (1.51-2.18)	0.26

*P values were computed using the Wilcoxon sign rank test for continuous variables and the McNemar's test for categorical variables.
† Values expressed as median (25th-75th percentile).
‡ Cases and controls were individually matched on these variables.

short dietary questionnaire, and physical activity habits. Follow-up questionnaires were mailed at 6 and 12 months after randomization and yearly thereafter. Blood specimens were collected before randomization from 14,916 (68%) physicians and stored at −82°C. The current report concerns newly reported prostate cancer cases during the first 13 years of follow-up among the men who provided a baseline blood sample. During this period, >99% of surviving participants were still reporting morbidity events and vital status was known for the entire cohort.

Selection of Cases and Controls. For each new report of prostate cancer, we requested hospital records and pathology reports for review by study physicians to confirm diagnosis and abstract the tumor stage and grade at diagnosis. Histologic grade was recorded as well-differentiated, moderately differentiated, or poorly differentiated tumors or following the Gleason scoring system, as available in the pathology reports. Stage was recorded according to the modified Whitemore-Jewett classification scheme (20). Cases without pathologic staging were classified as indeterminate stage unless there was clinical evidence of distant metastases. Cases were classified according to their clinical aggressiveness. Nonaggressive cases were defined as those with localized (stage A or B) and low-grade (Gleason <7 or well or moderately differentiated) tumors at diagnosis. Cases were considered aggressive when they presented as advanced disease (stage C or D) or high grade (Gleason ≥7 or poorly differentiated) at diagnosis or subsequently developed distant metastases or died from prostate cancer.

We selected a control subject for each confirmed case among the men who had provided a blood sample, had not had a partial or total prostatectomy, and had not reported prostate cancer at the time that the case's diagnosis was reported. Controls were individually matched by age (within 1 year for men <55 years and within 5 years for men ≥55 years) and smoking status at baseline (current, former, or never). Of the 758 cases accrued through 1995, 505 had enough blood for fatty acid level determination. Case and control subjects whose blood sample was received >6 days after it was drawn were excluded from analyses, leaving 476 cases and their matched controls.

Laboratory Analyses. Blinded samples from cases and controls were analyzed together in random order to reduce any effect of interassay variability. Fatty acids were extracted from whole blood and quantified by gas-liquid chromatography on a fused silica capillary *cis/trans* column (SP2560, Supelco) as previously described (17). Peak retention times were identified by injecting known standards (Nu-Check Prep) and analyzed with the ChemStation A.08.03 software (Agilent Technologies). The fatty acid levels in each sample were expressed as the percentage of total fatty acids. Coefficients of variation for all fatty acid peaks were measured by analyzing quality control samples indistinguishable from study samples randomly distributed throughout the study samples. The coefficients of variation for *trans*-fatty acids ranged from 1.24% for 16:1n-7*t* to 4.97% for 18:2n-6*tc*. For each participant, we took the sum of the peaks for 18:1n-12*t*, 18:1n-9*t*, and 18:1n-7*t*; 18:2n-6*tt*,

18:2n-6*tc*, and 18:2n-6*ct*; and all the *trans*-fatty acids measured to estimate the 18:1, 18:2, and total *trans*-fatty acid levels, respectively.

Statistical Analyses. We calculated median values and proportions of the baseline characteristics of cases and controls. Differences in these characteristics were tested using the McNemar's test for categorical variables and the Wilcoxon sign rank test for continuous variables. Cases and controls were divided into five groups according to quintiles of *trans*-fatty acid levels among the controls. Then, we used conditional logistic regression models to estimate the risk of prostate cancer in a given quintile of fatty acid level relative to the risk in the lowest quintile while simultaneously accounting for the matching factors. We considered the potential confounding effects of baseline characteristics by adding to the initial model terms for variables associated with prostate cancer and fatty acid levels at $P < 0.20$ and evaluating whether adding these variables changed the initial fatty acid estimates by >10%. Because linoleic and α-linolenic acids share common food sources with *trans* fats, we also did additional analyses adjusting for blood levels of these fatty acids alone and simultaneously. Additionally, because fasting status could theoretically affect blood levels of fatty acids, we also evaluated fasting status as a potential confounder. None of the variables evaluated [height, body mass index (BMI; kg/m^2), low-fat milk intake, blood levels of linoleic and α-linolenic acids, and fasting status] changed the initial estimates substantially and therefore results are presented with adjustment for matching factors only.

Lastly, we refitted the regression models in subgroups defined by tumor aggressiveness, diagnosis before (through September 30, 1990) or after (since October 1, 1990) the widespread use of prostate-specific antigen screening, age at diagnosis (<65 and ≥65 years), baseline BMI (<25 and ≥25 kg/m^2), and randomized aspirin assignment. Tests for linear trend were conducted in all

Table 2. RR (95% CIs) for prostate cancer by control quintiles of whole blood *trans*-fatty acid levels

	Quintile of fatty acid level					*P* trend*
	1 (reference)	2	3	4	5	
16:1 *trans*						
16:1n-7*t*						
Concentration (%)	0.13	0.16	0.19	0.20	0.23	
Cases/controls	89/93	89/97	116/94	87/95	95/97	
Adjusted RR†	1.00	0.93 (0.63-1.39)	1.29 (0.87-1.91)	0.94 (0.61-1.44)	1.03 (0.67-1.58)	0.87
18:1 *trans*						
18:1n-12*t*						
Concentration (%)	0.18	0.24	0.30	0.36	0.50	
Cases/controls	92/97	105/94	92/96	82/94	105/95	
Adjusted RR†	1.00	1.17 (0.79-1.73)	1.00 (0.67-1.50)	0.92 (0.62-1.38)	1.15 (0.78-1.71)	0.73
18:1n-9*t*						
Concentration (%)	0.29	0.39	0.47	0.59	0.79	
Cases/controls	87/97	87/95	108/95	71/96	123/93	
Adjusted RR†	1.00	1.01 (0.68-1.52)	1.27 (0.85-1.90)	0.82 (0.53-1.26)	1.49 (1.00-2.22)	0.10
18:1n-7*t*						
Concentration (%)	0.20	0.27	0.31	0.37	0.45	
Cases/controls	112/96	94/96	83/93	83/95	104/96	
Adjusted RR†	1.00	0.83 (0.55-1.25)	0.75 (0.49-1.15)	0.74 (0.49-1.12)	0.90 (0.60-1.36)	0.63
Total 18:1 *trans*						
Concentration (%)	0.71	0.94	1.10	1.30	1.67	
Cases/controls	86/97	105/96	97/92	74/97	114/94	
Adjusted RR†	1.00	1.25 (0.82-1.90)	1.21 (0.80-1.84)	0.87 (0.57-1.33)	1.41 (0.93-2.12)	0.34
18:2 *trans*						
18:2n-6*t*,*t*						
Concentration (%)	0.11	0.15	0.17	0.21	0.27	
Cases/controls	80/96	83/94	98/95	118/96	97/95	
Adjusted RR†	1.00	1.10 (0.72-1.68)	1.26 (0.83-1.90)	1.53 (1.00-2.33)	1.28 (0.83-1.96)	0.14
18:2n-6*c*,*t*						
Concentration (%)	0.15	0.18	0.21	0.24	0.28	
Cases/controls	82/96	77/94	96/95	120/97	101/94	
Adjusted RR†	1.00	0.94 (0.63-1.41)	1.18 (0.79-1.77)	1.46 (0.99-2.16)	1.30 (0.86-1.97)	0.07
18:2n-6*t*,*c*						
Concentration (%)	0.09	0.13	0.15	0.18	0.24	
Cases/controls	78/95	88/95	104/96	108/95	98/95	
Adjusted RR†	1.00	1.11 (0.74-1.67)	1.36 (0.90-2.05)	1.41 (0.94-2.14)	1.28 (0.85-1.93)	0.22
Total 18:2 *trans*						
Concentration (%)	0.36	0.46	0.53	0.63	0.77	
Cases/controls	82/96	76/93	97/96	126/97	95/94	
Adjusted RR†	1.00	0.96 (0.63-1.46)	1.21 (0.81-1.83)	1.57 (1.05-2.36)	1.24 (0.82-1.89)	0.11
Total *trans*						
Concentration (%)	1.27	1.57	1.81	2.11	2.62	
Cases/controls	80/97	114/94	89/94	76/97	117/94	
Adjusted RR†	1.00	1.46 (0.98-2.19)	1.15 (0.76-1.74)	0.96 (0.62-1.47)	1.53 (1.01-2.32)	0.23

*Calculated with median fatty acid concentration in each quintile as a continuous variable.
† Adjusted for matching factors: age, smoking status at baseline, and length of follow-up.

Table 3. Adjusted RR (95% CIs) for nonaggressive and aggressive prostate cancer by control quintiles of whole blood *trans*-fatty acid levels

Fatty acid	Quintile of fatty acid level					Q5 95% CI	*P* trend*
	1 (reference)	2	3	4	5		
Nonaggressive tumors (n = 209 cases) †							
Total *trans*-fatty acids	1.00	1.51	1.52	0.93	2.21 ‡	(1.14-4.29)	0.06
16:1n-7*t*	1.00	1.22	1.32	1.02	1.17	(0.61-2.24)	0.79
Total 18:1 *trans*-fatty acids	1.00	1.42	1.49	0.80	1.96 ‡	(1.01-3.80)	0.18
18:1n-12*t*	1.00	1.35	1.13	1.17	1.42	(0.76-2.67)	0.37
18:1n-9*t*	1.00	1.38	1.54	0.66	2.16 ‡	(1.12-4.17)	0.11
18:1n-7*t*	1.00	0.81	0.73	0.72	1.17	(0.62-2.18)	0.66
Total 18:2 *trans*-fatty acids	1.00	1.20	1.22	2.17 ‡	1.97 ‡	(1.03-3.75)	0.01
18:2n-6*t*,*t*	1.00	1.28	1.41	2.15 ‡	1.80	(0.93-3.50)	0.04
18:2n-6*c*,*t*	1.00	0.88	1.08	1.85 ‡	1.55	(0.82-2.94)	0.03
18:2n-6*t*,*c*	1.00	1.13	1.59	1.58	1.84	(0.96-3.52)	0.05
Aggressive tumors (n = 221 cases)§							
Total *trans*-fatty acids	1.00	1.38	1.01	1.08	1.15	(0.64-2.08)	0.93
16:1n-7*t*	1.00	0.80	1.29	0.99	0.82	(0.43-1.54)	0.85
Total 18:1 *trans*-fatty acids	1.00	1.22	1.28	0.92	1.15	(0.62-2.03)	0.99
18:1n-12*t*	1.00	0.96	0.88	0.80	0.87	(0.49-1.53)	0.54
18:1n-9*t*	1.00	0.77	1.25	1.00	1.16	(0.66-2.02)	0.50
18:1n-7*t*	1.00	0.95	0.81	0.87	0.70	(0.39-1.28)	0.24
Total 18:2 *trans*-fatty acids	1.00	0.78	1.14	1.33	0.91	(0.49-1.70)	0.91
18:2n-6*c*,*t*	1.00	0.92	1.44	1.24	1.24	(0.67-2.29)	0.36
18:2n-6*t*,*c*	1.00	1.16	1.19	1.43	1.00	(0.56-1.77)	0.96

NOTE: Adjusted for matching factors: age, smoking status at baseline, and length of follow-up.
*Calculated with median fatty acid concentration in each quintile as a continuous variable.
† Defined as localized disease (stage A or B) and low-grade tumors (Gleason <7 or well or moderately differentiated) at diagnosis.
‡ $P < 0.05$, when compared with men in the lowest quintile of blood levels for the specific *trans*-fatty acid.
§Defined as advanced disease (stage C or D) or high grade (Gleason ≥7 or poorly differentiated) at diagnosis or subsequent development of distant metastases or death from prostate cancer.

models by using the median fatty acid levels in each quintile as a continuous variable. Tests for interaction by BMI, fasting status, or aspirin assignment in the trial were conducted by adding to the model for *trans* fat levels a term for the variable of interest and a cross-product term between *trans* fat levels (median level in each quintile as a continuous variable) and this variable. All statistical analyses were done using Statistical Analysis System, version 9.1 (SAS Institute). Results were considered to be statistically significant when $P < 0.05$, two tailed.

Results

At baseline, blood levels of 18:2 *trans* fats were slightly higher in men who later developed prostate cancer compared with their controls (Table 1). There were no appreciable differences in the blood levels of 18:1 *trans* or total *trans* fats or in other subject characteristics. Blood levels of 18:1 *trans* and 18:2 *trans* were highly correlated with each other and with the levels of total *trans*-fatty acids. *Trans*-fatty acid levels were positively related to blood levels of α-linolenic acid but unrelated to the levels of linoleic acid. Blood levels of *trans*-fatty acids were slightly higher in blood samples obtained with less than 8 h of fasting when compared with samples with 8 or more hours of fasting. The median (25th percentile-75th percentile) blood level of total *trans*-fatty acids as percent of total fatty acids was 1.83% (1.55-2.27%) for nonfasted samples and 1.78% (1.51-2.06%) for fasted samples (P = 0.01).

Blood levels of 16:1*t* and 18:1 *trans*-fatty acids were unrelated to total prostate cancer risk (Table 2). Among the 18:2 *trans* fats, there was a suggestion of a linear trend toward increasing risk of prostate cancer with increasing blood levels of *c*,*t* linolelaidic acid levels (18:2n-6*c*,*t*; P trend = 0.07). Blood levels of the remaining 18:2 *trans*-fatty acids, as well as the sum of all *trans* fats, were unrelated to total prostate cancer risk.

To assess whether blood *trans*-fatty acid levels might be influenced by preclinical cancer, we then divided the analyses by date of diagnosis. Blood levels of 18:2 *trans* fats were positively associated with prostate cancer diagnosed later in follow-up (on or after October 1, 1990) but not to cases occurring earlier. This tendency was strongest for levels of *c*,*t* linolelaidic acid (18:2n-6*c*,*t*). For tumors diagnosed later in follow-up, the relative risks [RR; 95% confidence interval (95% CI)] of prostate cancer for men in successively higher quintiles of blood 18:2n-6*c*,*t* were 1.11 (0.67-1.83), 1.13 (0.68-1.88), 1.57 (0.97-2.54), and 1.74 (1.04-2.93) when compared with men in the lowest quintile (P trend = 0.01). For tumors diagnosed earlier, the RR (95% CI) comparing men in the highest with men in the lowest quintile of 18:2n-6*c*,*t* was 0.71 (0.35-1.47; P trend = 0.74). There were no clear differences in association between specific 16:1 *trans*-fatty acids and 18:1 *trans*-fatty acids and overall prostate cancer risk according to the date of diagnosis.

We then examined the association between blood *trans*-fatty acid levels and prostate cancer separately for nonaggressive and clinically aggressive tumors (Table 3). Blood levels of total *trans*-fatty acids, as well as of some individual *trans*-fatty acids, were positively associated with the risk of developing nonaggressive tumors but unrelated to the risk of developing aggressive prostate cancer. Men in the highest quintile of total *trans*-fatty acid levels had a >2-fold risk of developing

nonaggressive prostate cancer when compared with men in the lowest quintile (P = 0.02) with a tendency of a positive linear trend. The association between elaidic acid (18:1n-9*t*) and nonaggressive prostate tumors was similar to the association between total *trans* fats and these tumors. There was also a positive association between blood levels of all 18:2 *trans* isomers examined and total 18:2 *trans* fats with risk of nonaggressive prostate tumors. Men in the highest two quintiles of total 18:2 *trans* fats had approximately twice the risk of nonaggressive prostate cancer than men in the lowest quintile and there was a statistically significant positive linear trend (P trend = 0.01). There were no associations between blood levels of the *trans*-fatty acids examined and risk of advanced, high-grade, or aggressive prostate cancer. The associations of blood levels of 18:2 *trans* fats and total *trans* fats with localized (stage A/B) and low-grade (Gleason <7 or well or moderately differentiated) tumors resembled their associations with nonaggressive tumors. The RR (95% CI) comparing men in the highest with men in the lowest quintile of total *trans*-fatty acids was 2.21 (1.28-3.83) for localized tumors and 1.82 (0.98-3.37) for low-grade tumors.

Lastly, we evaluated whether the association between *trans*-fatty acids in blood and prostate cancer, overall and for nonaggressive tumors, differed according to age at diagnosis (<65 and ≥65 years), fasting status, random assignment into the aspirin arm of the trial, or baseline BMI (<25 and ≥25). The association between 18:2 *trans* fats and prostate cancer seemed to be stronger among overweight and obese men than among normal weight men. The RRs (95% CI) of overall prostate cancer among normal weight men in successively higher quintiles of total 18:2 *trans* levels in blood were 1.19 (0.70-2.04), 1.09 (0.66-1.82), 1.39 (0.84-2.29), and 1.21 (0.71-2.05) when compared with men in the lowest quintile (P trend = 0.40). The corresponding RRs (95% CI) among overweight and obese men were 0.72 (0.36-1.42), 1.48 (0.74-2.97), 2.06 (1.02-4.16), and 1.31 (0.66-2.62; P trend = 0.14). Similarly, the association between *trans* fats and prostate cancer seemed to be stronger among men assigned to the aspirin placebo arm of the trial than among men assigned to aspirin. The RRs (95% CI) of prostate cancer among

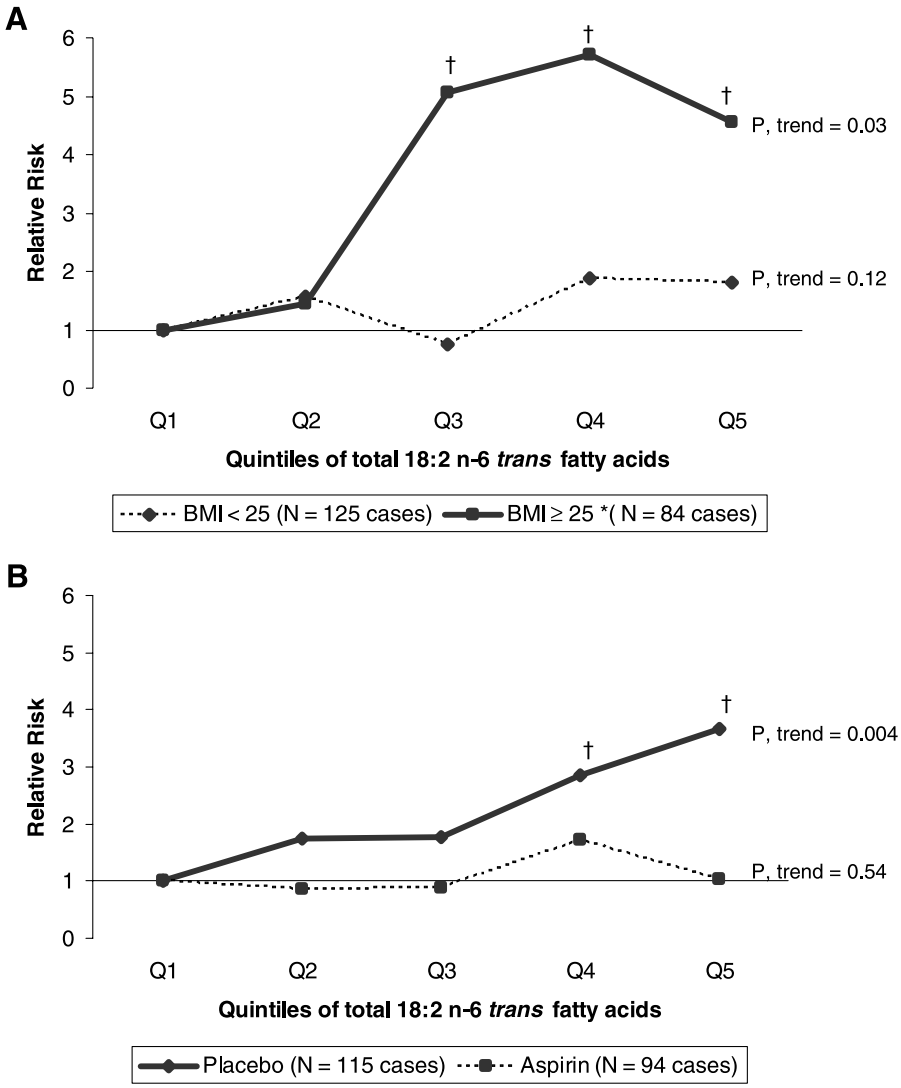

Figure 1. Adjusted RR of nonaggressive prostate cancer by control quintiles of total 18:2n-6 *trans*-fatty acids according to baseline BMI (**A**) and random aspirin assignment (**B**). Adjusted for matching factors: age, smoking status at baseline, and length of follow-up; †, $P < 0.05$, when compared with men in the lowest quintile.

men assigned to aspirin placebo in successively higher quintiles of total 18:2 *trans* levels in blood were 1.00, 1.58 (0.84-3.00), 2.31 (1.23-4.33), 2.19 (1.20-4.00), and 2.65 (1.41-4.98; P trend = 0.003), whereas among the men assigned to aspirin the RR (95% CI) comparing the highest with the lowest quintile of total 18:2 *trans* was 0.63 (0.34-1.15; P trend = 0.40). For both BMI and aspirin, these apparent differences were more evident when only nonaggressive tumors were considered (Fig. 1), although tests for interaction were not statistically significant ($P > 0.05$ in all cases). On the other hand, there were no appreciable differences in the relations between individual *trans*-fatty acids and prostate cancer risk according to fasting status or age at diagnosis (P interaction >0.05 in all cases). The RR (95% CI) comparing the highest with the lowest quintile of total *trans*-fatty acids was 1.54 (0.95-2.50) in the nonfasting samples and 1.94 (0.69-5.46) in the fasting samples and 1.84 (0.93-3.63) among men <65 years and 1.35 (0.80-2.29) among men ≥65 years.

Discussion

We prospectively evaluated the association between blood levels of *trans*-fatty acids, a reliable biomarker of intake (17), and prostate cancer and found that the levels of elaidic acid (18:1n-9*t*) and of the three 18:2n-6 *trans* isomers examined were associated with an increased risk of developing localized and low-grade prostate tumors. Further, blood *trans*-fatty acid levels were unrelated to the risk of developing aggressive tumors (i.e., advanced stage or high grade at diagnosis or subsequently lethal disease). These results suggest that some specific *trans* fats may be involved in early stages of prostate carcinogenesis but do not contribute importantly to disease progression or that they increase risk only for specific tumor types.

Only three studies have previously examined the association between *trans* fat intake and prostate cancer risk. In the Netherlands Cohort Study, baseline intake of total *trans* fats was unrelated to the risk of total, localized, and advanced prostate cancer within 6 years of follow-up (12). These results agree with ours, as in our study, blood *trans*-fatty acid levels were unrelated to overall prostate cancer risk during the first 8 years of follow-up and the association we observed with specific 18:2 *trans*-fatty acids was due to cases occurring later in follow-up. In an Australian retrospective case-control study that included only cases with Gleason ≥5, intakes of 16:1, 18:1, and 18:2 *trans* fats were unrelated to prostate cancer (13). However, intake of margarine, the most important source of *trans* fats in the Australian diet (21), was associated with a greater risk of prostate cancer (13). The nutrient results of this study are difficult to interpret especially because the food frequency questionnaire used has not been validated. Nevertheless, their results for margarine are consistent with our results for *trans*-fatty acids and nonaggressive tumors, as the majority (74%) of cases in the Australian study had a Gleason score of 5 or 6 and, therefore, would have likely been classified as nonaggressive tumors in our study.

Liu et al. (15) examined the association between intakes of *trans* fats and prostate cancer risk in a case-control study limited to advanced prostate cancer (defined as Gleason ≥7, tumor stage ≥T2c, or prostate-specific antigen at diagnosis ≥10 ng/mL). This study did not observe an association between *trans* fat intake in the year before diagnosis and advanced prostate cancer, which is consistent with our results for aggressive disease. However, they reported a positive association limited to men carrying a functional variant of the *RNASEL* gene, a gene involved in inflammation and previously linked to prostate cancer risk (15).

Two biomarker studies have examined the association between tissue levels of *trans*-fatty acids and prostate cancer. Bakker et al. (11) reported a nonsignificant ecological correlation (r = 0.50) between adipose tissue total *trans*-fatty acid levels in eight European countries and age-standardized prostate cancer incidence. In a prospective case-control study nested within the CARET trial, King et al. (14) found positive associations between serum levels of some, but not all, *trans*-fatty acids and prostate cancer risk. In their study, high serum levels of 18:1n-7*t* and 18:2n-6*c*,*t* were associated with an increased risk of prostate cancer, which was stronger for low-grade (Gleason <7) tumors than for high-grade tumors (14). Our results are in close agreement with those findings.

Increasing evidence suggests that inflammation and insulin resistance may be important in prostate carcinogenesis (22, 23) and both of these factors can be influenced by *trans*-fatty acid intake. *Trans*-fatty acids have been associated with increased circulating levels of tumor necrosis factor soluble receptors 1 and 2 in observational studies (3) and increased C-reactive protein, interleukin-6, and other markers of inflammation in controlled feeding studies (2, 4). Intake of *trans* fats increased insulin resistance in controlled feeding trials (5, 6) and has been associated to increased risk of type 2 diabetes in observational studies (24). Further, *trans*-fatty acids can decrease the activity of the Δ5 and Δ6 desaturases (25, 26), which may also contribute to increased insulin resistance (27).

In addition, specific *trans*-fatty acids down-regulate peroxisome proliferator-activated receptor-γ *in vivo* (10). This is a particularly important finding given that peroxisome proliferator-activated receptor-γ can modulate both insulin resistance and inflammation (28) and is expressed in prostate tumors where its agonists have generally antiproliferative effects (28-31). Moreover, the association between 18:2 *trans* fats and prostate cancer was apparently stronger among overweight and obese men, who may already be subject to increased insulin resistance and chronic inflammation (32, 33), and among men who were not assigned to aspirin in the trial in our study and among carriers of the variant genotype of the *RNASEL* gene in other study (15), supporting the notion that *trans*-fatty acids may influence prostate carcinogenesis through these mechanisms.

Strengths of our study include its prospective design and high follow-up rates, which decrease the possibility that our findings could be the result of a bias. In addition, blood samples were collected before prostate cancer diagnosis, in most cases several years in advance, thus decreasing the possibility that elevated *trans*-fatty acid levels among the cases were the result of the disease process. In addition, the large number of cases allowed us to examine the associations separately for nonaggressive and aggressive prostate cancer. Limitations of this study include that we only had a baseline measurement

of blood *trans* fat levels. However, this results in misclassification of the long-term fatty acid levels that is nondifferential with respect to case or control status, thereby attenuating the observed associations. In addition, as is true for all observational studies, there is the possibility that some unmeasured factor associated with blood fatty acid levels may be responsible for the observed associations. Nevertheless, we evaluated several variables as potential confounders and found that adjusting for these variables had minimal effect on the results.

In conclusion, we found that blood levels of *trans* isomers of oleic and linoleic acids were related to the risk of developing nonaggressive (localized and low grade) prostate tumors. These results contrast with our previous work showing that blood levels of the *cis* isomer of linoleic acid are associated with a lower prostate cancer risk (16). As nonaggressive tumors represent a large proportion of prostate cancer detected using prostate-specific antigen screening (34, 35), our findings may have implications for the prevention of prostate cancer on a population level if they are confirmed in future studies. These findings should also further encourage the ongoing efforts by some cities in the United States and by other developed nations to eliminate artificial *trans* fats from the food supply as they point to yet another potential health hazard of consuming these fats.

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