Research Article

Cancer Prevention Research

Serum Oxidized Protein and Prostate Cancer Risk within the Prostate Cancer Prevention Trial

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Abstract

To evaluate the role of oxidative stress in prostate cancer risk, we analyzed serum levels of protein carbonyl groups in 1,808 prostate cancer cases and 1,805 controls, nested in the Prostate Cancer Prevention Trial, a randomized, placebo-controlled trial that found finasteride decreased prostate cancer risk. There were no significant differences in protein carbonyl levels in baseline samples between those later diagnosed with prostate cancer and those without at the end of study biopsy. Adjusted odds ratios and 95% confidence intervals (95% CI) for the 4th quartile of protein carbonyl level for the combined, placebo, and finasteride arms were 1.03 (95% CI, 0.85-1.24), 0.88 (95% CI, 0.69-1.12), and 1.27 (95% CI, 0.94-1.71), respectively. There were no significant associations between carbonyl level and risk when analyzing high-grade and low-grade disease separately, nor did finasteride affect protein oxidation levels. The results of this large nested case-control study do not support the hypothesis that oxidative stress, at least as measured by protein carbonyl level, plays a role in prostate cancer. *Cancer Prev Res; 3(4); 478–83. ©2010 AACR.*

Introduction

Increases in the generation of reactive oxygen species and decreases in antioxidant enzyme activities with aging have been reported in the prostate (1, 2) and are also observed in age-related disorders such as atherosclerosis, Alzheimer's disease, and cataracts (3). Several studies have shown that proteins are a target for reactive oxidants in cells and that oxidized proteins accumulate during aging, oxidative stress, and in some pathologic conditions (4). However, only a limited number of studies have actually evaluated oxidative damage in relation to exposures thought to increase reactive oxygen species or have assessed its relationship with prostate carcinogenesis (3). In this nested case-control study, we measured protein carbonyls, a marker for oxidative damage, in serum samples from men who participated in the Prostate Cancer Prevention Trial (PCPT) and had received either finasteride or placebo treatment from 1993 to 2003 (5). The goal of this investigation was to determine whether baseline levels of serum levels of oxidized proteins are associated with an increased risk of prostate cancer or high-grade disease. We also examined associations between serum protein carbonyl levels and other factors thought to be associated with oxidative stress levels.

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Materials and Methods

Study design and study population

Data and biospecimens used in this study came from the PCPT, a large, phase III, double-blind, placebo-controlled trial that tested whether finasteride could reduce the period prevalence of prostate cancer during the 7-y intervention. Details about study design and population characteristics have been described previously (5). Briefly, a total of 18,880 men ages 55 y or older, with a normal digital rectal examination (DRE), a prostate-specific antigen (PSA) level of ≤3 ng/mL, and no prior history of prostate cancer, severe benign prostate hyperplasia, or other clinically significant diseases, were randomized to receive finasteride (5 mg/d) or placebo. Participants underwent DRE and PSA test annually, and a prostate biopsy was recommended for participants with an abnormal DRE or a PSA of ≥4.0 ng/mL. The PSA level prompting a biopsy recommendation in the finasteride group was adjusted so as to result in a similar number of biopsy recommendations in both study groups. After 7 y on-study, all men with PSA values consistently ≤4.0 ng/mL and nonsuspicious DREs who were not previously diagnosed with prostate cancer were offered an end-of-study biopsy. All biopsies were done under transrectal ultrasonographic guidance and included a minimum of six cores. All prostate biopsies were reviewed by both the pathologist at the local study site and a pathologist

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at a central PCPT pathology laboratory to confirm the diagnosis of adenocarcinoma. Discordant pathology diagnoses were reviewed by a referee pathologist, and concordance was achieved in all cases. Clinical stage was assigned locally and the Gleason scoring system was used centrally to grade the tumor. Low-grade prostate cancer was defined as tumors with Gleason score <7 and high-grade prostate cancer, with Gleason score ≥7. In this study, we used a nested case-control design to evaluate whether higher levels of serum oxidized proteins were associated with higher overall prostate cancer risk and high-grade disease and whether the effects of finasteride on prostate cancer risk differed between men with high and low levels of serum oxidized protein. Cases were defined as men with biopsy-proven prostate cancer and controls were biopsy negative, both having available serum samples for oxidized protein analysis. Controls were frequency matched to cases on age in 5-y increments, PCPT treatment arm (finasteride versus placebo), and positive family history (first-degree relative with prostate cancer). Controls were oversampled to include all nonwhites to increase power for analyses by race/ethnicity. The final sample size for this study was 1,808 cases and 1,805 controls.

Data collection

Following each PCPT participant's informed consent and enrollment, data on sociodemographic characteristics, including age, race, education, physical activity, smoking, fruit intake, vegetable intake, treatment arm (finasteride/placebo), and family history of prostate cancer, were collected. Height and weight were measured at the baseline clinic visit and weight was measured annually thereafter. Body mass index (BMI) was calculated as weight (kg) divided by height (m^2) and categorized as <25 (normal), 25 to 30 (overweight), and ≥30 (obese). A food frequency questionnaire was administered at the participant's first annual visit and was completed by 88% of the participant population, from which daily fruit and vegetable intakes were calculated.

Biospecimen collection, processing, and storage

Blood samples were collected into vacutainers without anticoagulant but with a gel to separate serum from clot from all cases and controls 3 mo before randomization and annually. Samples were centrifuged after 30 to 60 min at room temperature and sera stored at −70°C. Detailed procedures for blood collection, processing, and storage have been described previously (6).

Serum oxidized protein measurement

The levels of serum protein carbonyl groups were assessed using a noncompetitive ELISA, as previously described (7). Briefly, the oxidized protein standard was prepared by incubation of bovine serum albumin with 0.73 mol/L H_2O_2 and 0.42 mmol/L Fe^{2+} for 1 h at 37°C and carbonyl content was measured spectrophotometrically (8). Total protein concentration in the serum was measured using a bicinchoninic acid kit (Sigma) and the samples were diluted with PBS to a final protein concentration of 4 mg/ mL. After derivatization with 2,4-dinitrophenylhydrazine, the plate was coated with 200 μL of sample (1 μg) and incubated overnight at 4°C in the dark. Biotinylated primary anti–2,4-dinitrophenol antibody (Molecular Probes) was followed by the addition of the streptavidin-biotinylated horseradish peroxidase conjugate (Amersham). Color was developed by adding the tetramethyl benzidine liquid substrate system (Sigma) and the reaction was stopped with H_2SO_4. The absorbance was measured with a microplate reader at 450 nm. Serum protein carbonyl concentration was expressed as nanomoles of carbonyl per milliliter of serum. Each sample was analyzed in duplicate. To account for plate variation, values were adjusted for a plate-specific control. Two pooled serum samples were used for additional quality control. These samples were blinded and interspersed among the study participant samples. The coefficient of variation for QC pool 1 (n = 49) was 16.3% and 15.2% for pool 2 (n = 53).

Statistical analysis

Characteristics of cases and controls were compared using χ^2 test for categorical variables and t test for continuous variables. Serum concentrations of protein carbonyls were categorized into quartiles based on the distribution in the controls. We calculated odds ratios (OR) and 95% confidence intervals (95% CI) for prostate cancer risk using multiple logistic regression analysis and polychotomous logistic regression models to calculate ORs for low-grade and high-grade prostate cancer compared with controls. These analyses were adjusted for age (continuous), race (white versus nonwhite), education (high school degree or less, some college or college degree, and advanced degree), physical activity (moderate or active versus sedentary or light), smoking (nonsmoker, current, and past), daily fruit intake (<1 serving, 1 to <2 servings, and 2+ servings), daily vegetable intake (<1 serving, 1 to <2 servings, 2 to <3 servings and 3+ servings), treatment arm (finasteride versus placebo), and family history of prostate cancer in first-degree relatives (yes versus no). We also conducted stratified analyses to assess the interaction between oxidized protein concentration and finasteride use. All P values were two-sided and were considered statistically significant at $P < 0.05$. All analyses were done using SAS (version 9.0).

Results

The characteristics of the cases and controls are shown in Table 1. Due to the sampling scheme, 93% of cases and 79% of controls were Caucasian. There were no significant differences between cases and controls according to age, BMI, smoking status, education, alcohol consumption, physical activity, and daily vegetable and fruit intake.

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Table 1. Characteristics of cases and controls

Variables	Control (*n* = 1,805) Mean (SD)	Case (*n* = 1,808) Mean (SD)	*P**
Age at baseline† (y)	63.58 (5.55)	63.62 (5.54)	0.60
Oxidized protein			
Oxidized protein, nmol/mL	19.81 (3.74)	19.83 (4.39)	0.91
	***n* (%)**	***n* (%)**	
Physical activity			
Sedentary	314 (17.4)	310 (17.2)	0.52
Light	742 (41.3)	748 (41.5)	
Moderate	553 (30.8)	593 (32.9)	
Active	188 (10.5)	150 (8.3)	
Alcohol drinking (g/d)			
0	415 (23.0)	410 (22.6)	0.60
<30	1,236 (68.5)	1,232 (68.1)	
≥30	154 (8.5)	166 (9.1)	
BMI			
Normal (BMI <25)	449 (25.1)	499 (27.9)	0.54
Overweight (BMI 25 to <30)	944 (52.8)	917 (51.2)	
Obese (BMI ≥30)	394 (21.8)	376 (20.8)	
Education			
Grade school or some high school	77 (4.3)	72 (4.0)	0.12
High school graduate or GED	273 (15.1)	236 (13.1)	
Voc/training school, some college, college grad	1,454 (80.6)	1,499 (82.9)	
Family history of prostate cancer†	384 (21.3)	384 (21.2)	0.98
Finasteride arm†	765 (42.4)	764 (42.3)	0.94
Fruit consumption (servings/d)			
<1	561 (34.7)	530 (34.0)	0.79
1 to <2	581 (36.0)	570 (36.6)	
2+	473 (29.3)	457 (29.4)	
Race‡			
Nonwhite	372 (20.6)	130 (7.2)	<0.0001
Nonhispanic white	1,433 (79.4)	1,678 (92.8)	
Smoking status			
Never	619 (34.3)	644 (35.6)	0.48
Former	1,047 (58.0)	1,041 (57.6)	
Current	139 (7.7)	123 (6.8)	
Vegetable consumption (servings/d)			
<1	211 (13.1)	200 (12.9)	0.94
1 to <2	595 (36.8)	562 (36.1)	
2 to <3	428 (26.5)	441 (28.3)	
3+	381 (23.6)	354 (22.7)	

*P values were calculated based on a two-sided t test, comparing cases to controls; unordered categorical P values were calculated based on a χ^2 test; ordered categorical variables were assigned values 1/2/3, etc., and then treated as continuous variables to calculate P-trend values.

†Controls were frequency matched to cases based on age, family history of prostate cancer, and treatment arm (finasteride or placebo).

‡Minorities were oversampled for the control population.

The mean levels of serum protein carbonyls between cases (19.83 ± 4.39 nmol/mL) and controls (19.81 ± 3.74 nmol/mL) were also similar. When we compared protein carbonyl levels in cases and controls stratified by variables that are thought to be associated with oxidative stress, no significant associations were found in either cases or controls (Table 2). We also measured serum protein carbonyl levels in the second year of intervention, but did not

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observe any differences compared with baseline by treatment arm (data not shown).

There was no significant association between serum protein carbonyl levels and risk of prostate cancer in either the placebo or the finasteride arm separately or in the entire study population (Table 3). Adjusted ORs and 95% CIs for the 4th quartile of protein carbonyl level for the combined, placebo, and finasteride arms were 1.03 (95% CI, 0.85-1.24), 0.88 (95% CI, 0.69-1.12), and 1.27 (95% CI, 0.94-1.71), respectively. Although the association was stronger in the finasteride arm, it was not statistically significant (Table 3). Results were similar when we restricted our analysis to white participants only (data not shown).

When examining associations between oxidized protein levels and prostate cancer grade, no statistically significant relationships were observed (Table 4). ORs and 95% CIs for high-grade cancer within the 4th quartile of protein carbonyl level for the combined, placebo, and finasteride arms were 1.02 (95% CI, 0.77-1.36), 0.84 (95% CI, 0.56-1.27), and 1.25 (95% CI, 0.83-1.87), respectively (Table 4). Results were similar when analyses were restricted to white participants only (data not shown).

Table 2. Mean serum protein carbonyl levels among cases and controls stratified by select variables

Variables	Controls			Cases		
	n	Protein carbonyls (nmol/mL) Mean (SD)	*P**	*n*	Protein carbonyls (nmol/mL) Mean (SD)	*P**
Age (y)						
<63	831	19.84 (3.77)	0.80	800	19.64 (3.88)	0.09
≥63	974	19.79 (3.71)		1,008	19.98 (4.76)	
Fruit intake (servings/d)						
<1	561	19.51 (3.74)	0.01	530	19.50 (3.33)	0.13
1 to <2	581	20.18 (3.78)		570	20.03 (5.83)	
2+	473	19.62 (3.71)		457	19.64 (3.73)	
Vegetable intake (servings/d)						
<1	211	19.78 (3.84)	0.10	200	19.20 (3.48)	0.25
1 to <2	595	19.89 (3.71)		562	19.71 (4.09)	
2 to <3	428	20.01 (3.83)		441	19.79 (3.56)	
3+	381	19.38 (3.67)		354	20.00 (6.33)	
BMI						
Normal (BMI <25)	449	19.79 (3.86)	0.28	499	19.79 (3.69)	0.97
Overweight (BMI 25 to <30)	944	19.92 (3.77)		917	19.85 (5.01)	
Obese (BMI 30+)	394	19.57 (3.55)		376	19.82 (3.62)	
Alcohol intake (g/d)						
0	415	19.69 (3.72)	0.75	410	19.85 (3.42)	0.95
>0 to <30	1,236	19.85 (3.73)		1,232	19.81 (3.84)	
30+	154	19.84 (3.83)		166	19.93 (8.51)	
Smoking						
Never	619	19.90 (3.87)	0.63	644	19.66 (3.56)	0.29
Former	1,047	19.80 (3.69)		1,041	19.87 (4.93)	
Current	139	19.57 (3.52)		123	20.31 (3.42)	
Physical activity						
Sedentary	314	19.80 (3.66)	0.97	310	19.72 (3.55)	0.58
Light	742	19.85 (3.69)		748	19.89 (3.84)	
Moderate	553	19.84 (3.80)		593	19.91 (5.51)	
Active	188	19.71 (3.83)		150	19.40 (3.57)	
Gleason grade						
<7				1,235	19.73 (3.74)	0.31
7+				495	19.97 (5.77)	
Treatment arm						
Placebo	1,040	19.82 (3.79)	0.93	1,044	19.73 (3.87)	0.27
Finasteride	765	19.80 (3.67)		764	19.97 (5.02)	

*For factors with two levels, *P* values were based on a two-sided *t* test comparing mean protein values between levels; for factors with three or more levels, *P* values were based on a homogeneity of variance *F* test.

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Table 3. ORs of prostate cancer by serum protein carbonyl levels

Protein carbonyls (nmol/mL)*	Combined			Finasteride arm			Placebo arm		
	Cases, *n* = 1,801	Controls, *n* = 1,797	OR (95% CI)	Cases, *n* = 760	Controls, *n* = 760	OR (95% CI)	Cases, *n* = 1,041	Controls, *n* = 1,037	OR (95% CI)
Quartile 1 (<17.35)	468	449	1.00	186	203	1.00	282	246	1.00
Quartile 2 (17.5-19.5)	419	451	0.93 (0.77-1.12)	180	176	1.16 (0.86-1.57)	239	275	0.79 (0.61-1.01)
Quartile 3 (19.6-22.1)	475	446	1.09 (0.91-1.32)	208	197	1.26 (0.94-1.68)	267	249	0.97 (0.76-1.25)
Quartile 4 (>22.1)	439	451	1.03 (0.85-1.24)	186	184	1.27 (0.94-1.71)	253	267	0.88 (0.69-1.12)
P-trend			0.42			0.09			0.66

NOTE: All ORs were adjusted for age, race (white vs nonwhite), education (<HS, HS, ≥HS), smoking status (current, past, never), and physical activity (moderate or active vs sedentary or light). *P*-trend values were calculated by assigning values of 1/2/3/4 to OP quartiles and then treating as continuous.

*Quartile values were calculated based on controls only.

Discussion

There were no significant associations between prostate cancer risk or its aggressiveness and serum levels of oxidized protein as measured by protein carbonyls in this large nested case-control study. Whereas the cancer risk associated with the highest oxidized protein levels was slightly elevated in the finasteride arm, the association did not reach statistical significance. Finasteride did not seem to affect serum protein carbonyl levels when comparing baseline measurements to those obtained after 2 years on finasteride. There were also no significant associations between factors considered to be associated with increased oxidative stress and oxidized protein levels.

In our prior large study of breast cancer, protein oxidation, defined as high plasma levels of protein carbonyl groups, was significantly associated with a 60% increased risk of breast cancer (7). However, in contrast to the present study, blood samples were collected, on average, 3 mo after diagnosis and thus could have been affected by disease. Previous smaller studies have provided conflicting evidence on the association between protein oxidation and cancer risk, with positive results for Hodgkin's lymphoma (9) and bladder cancer (10), but not for lung (11) or brain (12) cancer. None of these studies were prospective.

Whereas aging is accompanied by increasing levels of oxidative damage, including protein oxidation (reviewed in ref. 13), no associations with age were observed in

Table 4. ORs of low-grade and high-grade prostate cancer by serum protein carbonyl levels

	Grade	Protein carbonyl (nmol/mL)*							
		Q1 (<17.35)	Q2 (17.5-19.5)		Q3 (19.6-22.1)		Q4 (>22.1)		*P*-trend
		n	*n*	OR† (95% CI)	*n*	OR† (95% CI)	*n*	OR† (95% CI)	
Combined arm	Low-grade (*n* = 1,229)	328	284	0.89 (0.72-1.09)	318	1.04 (0.85-1.28)	299	1.00 (0.82-1.24)	0.6
	High-grade (*n* = 495)	127	114	0.96 (0.72-1.28)	134	1.12 (0.85-1.48)	120	1.02 (0.77-1.36)	0.61
Finasteride arm	Low-grade (*n* = 449)	114	110	1.14 (0.81-1.60)	112	1.12 (0.8-1.57)	113	1.28 (0.91-1.79)	0.19
	High-grade (*n* = 278)	67	60	1.11 (0.74-1.68)	84	1.39 (0.95-2.05)	67	1.25 (0.83-1.87)	0.16
Placebo arm	Low-grade (*n* = 780)	214	174	0.75 (0.58-0.98)	206	0.99 (0.76-1.29)	186	0.86 (0.66-1.12)	0.66
	High-grade (*n* = 217)	60	54	0.82 (0.55-1.24)	50	0.85 (0.56-1.30)	53	0.84 (0.56-1.27)	0.47

NOTE: Low-grade, Gleason <7; high-grade, Gleason ≥7. All ORs were adjusted for age, race (white vs nonwhite), education (<HS, HS, ≥HS), smoking status (current, past, never), and physical activity (moderate or active vs sedentary or light). *P*-trend values were calculated by assigning values of 1/2/3/4 to OP quartiles and then treating as continuous.

*Quartile values were calculated based on controls only.

†OR for low-grade and high-grade cancers were modeled by polychotomous logistic regression; both cancers are contrasted with controls in the same model.

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our study, perhaps due to the narrow age range of our participants. Significantly higher levels of oxidized proteins in smokers than in nonsmokers have been observed (11, 14), but we found no association between serum protein carbonyl levels and smoking status. Conflicting data have been observed for an association with fruit and vegetable intake (7, 15–18). The lack of an association between these factors believed to be associated with increased oxidative stress and serum oxidized protein concentrations suggests that this measure may not be sensitive to environmental factors that increase oxidative stress.

In summary, in this large study using blood samples collected before diagnosis, we found no association between serum protein carbonyl levels and prostate cancer risk. Among controls, oxidized protein levels were not significantly associated with factors thought to be associated with oxidative stress. It is possible that serum levels of oxidized proteins do not accurately reflect oxidative damage in the prostate, which may have a more inflammatory environment; studies examining prostate tissue for oxidative damage may help clarify the role of oxidative stress in prostate cancer etiology.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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