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ORIGINAL PAPER

Serum estrogen levels and prostate cancer risk in the prostate cancer prevention trial: a nested case–control study

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Abstract
Objective Finasteride reduces prostate cancer risk by blocking the conversion of testosterone to dihydrotestosterone. However, whether finasteride affects estrogens levels or change in estrogens affects prostate cancer risk is unknown.
Methods These questions were investigated in a case–control study nested within the prostate cancer prevention trial (PCPT) with 1,798 biopsy-proven prostate cancer cases and 1,798 matched controls.
Results Among men on placebo, no relationship of serum estrogens with risk of prostate cancer was found. Among those on finasteride, those in the highest quartile of baseline estrogen levels had a moderately increased risk of Gleason score < 7 prostate cancer (for estrone, odds ratio [OR] = 1.51, 95% confidence interval [CI] = 1.06–2.15; for estradiol, OR = 1.50, 95% CI = 1.03–2.18). Finasteride treatment increased serum estrogen concentrations; however, these changes were not associated with prostate cancer risk.

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Conclusion Our findings confirm those from previous studies that there are no associations of serum estrogen with prostate cancer risk in untreated men. In addition, finasteride results in a modest increase in serum estrogen levels, which are not related to prostate cancer risk. Whether finasteride is less effective in men with high serum estrogens, or finasteride interacts with estrogen to increase cancer risk, is uncertain and warrants further investigation.

Keywords Prostate cancer · Etiology · Estrogen · Estradiol · Nested case–control study

Introduction

Prostate cancer is the most common non-skin malignancy among men in the United States, with an estimated 217,730 incident cases and 32,050 deaths in 2010 [1]. Relatively few etiologic factors for prostate cancer have been conclusively identified. The difficulty in identification of these risk factors may be best illustrated by studies on the relationship between sex steroid hormones and prostate cancer risk. Prostate cancer is generally deemed androgen-related, and almost always responds initially to androgen-deprivation therapy [2]. However, numerous epidemiologic studies on circulating androgen levels and prostate cancer risk have been inconclusive, and a recent pooled-analysis of 18 prospective studies found no association between androgens and prostate cancer risk [3]. Nevertheless, the prostate cancer prevention trial's (PCPT) finding that finasteride, a 5α-reductase inhibitor suppressing production of active 5α-dihydrotestosterone (DHT), significantly reduced the risk of prostate cancer, provides compelling evidence for the importance of androgens in prostate carcinogenesis [4].

In comparison to androgens, the role of estrogens in prostate carcinogenesis is more elusive. As men age and their risk of prostate cancer increases, testosterone concentrations decline while estradiol remains stable, resulting in an elevating ratio of estradiol to testosterone [5–7]. The temporal coincidence of this shifting hormonal relationship and prostate cancer risk may suggest a possible relationship between estrogens and carcinogenesis [8]. This hypothesis is further supported by findings in the Noble rat [9] and the aromatase knockout mice models [10] that androgen plus estrogen, but not androgen alone, induces prostate malignancy. However, previous observational studies on circulating estrogens and prostate cancer risk have not reported positive correlations as summarized in pooled- and meta-analyses [3, 11, 12]. In fact, estrogens have been inversely linked to prostate cancer risk in at least three prospective studies [13–15]. More well-designed epidemiologic studies are warranted to clarify the relationships between estrogens and prostate cancer risk.

The relationships between estrogens and prostate cancer risk after finasteride treatment may differ from those among men not on the drug, because the intervention alters the course of prostate carcinogenesis. Moreover, finasteride has a direct influence on estrogen concentrations by suppressing the conversion of testosterone (T) to DHT, increasing the amount of T available for aromatization to estrogens [16]. It is unknown whether finasteride-associated increase in estrogen levels has any effect on prostate cancer risk.

Latent prostate cancer is common among older men, and this undiagnosed disease will cause misclassification of disease status in studies on prostate cancer etiology. This bias is minimized in the PCPT, because participants were screened annually and those not diagnosed with cancer during the trial were recommended for biopsy at the end of the study. Therefore, the PCPT provides an ideal study population to refine etiologic risk factors for early stage prostate cancer. In this nested case–control study, we investigate the relationships between serum estrone and estradiol and risk of prostate cancer overall and by Gleason grade, the effects of finasteride treatment on serum estrogens concentrations, and associations of the changes in estrogen concentrations due to finasteride treatment with prostate cancer risk.

Methods and materials

Study design and population

All data and biospecimens used in this study were previously collected and stored in the biorepository of the PCPT. The PCPT was a Phase III double-blinded, placebo-controlled trial, administered through the Southwest Oncology Group, that tested whether finasteride could reduce the period prevalence of prostate cancer during a 7-year intervention. Details regarding study design and population characteristics have been described previously [4]. Briefly, 18,882 men aged 55 years or older with a normal digital rectal examination (DRE), a prostate-specific antigen (PSA) level of $\leq$ 3 ng/ml, and no prior history of prostate cancer, severe benign prostate hyperplasia, or other clinically significant coexisting conditions, were randomized to receiving finasteride (5 mg/day) or placebo. Participants underwent DRE and PSA testing annually, and prostate biopsy was recommended for participants with an abnormal DRE or a PSA of $\geq$ 4.0 ng/ml. The PSA level prompting a biopsy recommendation in the finasteride group was adjusted to yield a similar number of biopsy recommendations in both study groups. After 7 years on study, all men, including those with a PSA $\leq$ 4.0 ng/ml and normal DRE and who were not previously diagnosed

with prostate cancer, were offered an end-of-study biopsy. All biopsies were performed under transrectal ultrasonographic guidance and included a minimum of six cores. Biopsies were reviewed by both the pathologist at the local study site and 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 [4]. 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.

At the termination of the PCPT after 7 years, a total of 1,809 men were biopsy-proven to develop prostate cancer, and consisted the case pool for this nested case–control study. Biopsy-negative controls were frequency matched to cases on age in 5-year increments, treatment arm (finasteride vs. placebo) and positive family history (first degree relative with prostate cancer). Controls were oversampled to include all non-whites to increase power for analyses by race/ethnicity. The final sample size for this study, after accounting for missing estrogen data, was 1,798 cases and 1,798 controls.

Data and biospecimen collection

Following informed consent and enrollment, data on sociodemographic characteristics, including age, race, education, physical activity, smoking, alcohol consumption, 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 the square of height (m^2) and categorized as < 25 kg/m^2 (normal), 25–29.9 kg/m^2 (overweight), and ≥ 30 kg/m^2 (obese). Non-fasting blood samples were collected from all participants at the baseline visit and annually thereafter. Detailed procedures for blood collection, processing, and storage have been described previously [17]. A portion of the serum was used for PSA testing and the reminder was aliquoted and stored at -70°C until analysis.

Measurement of serum estrone and estradiol concentrations

For men in the finasteride arm, baseline levels were measured based on serum samples collected approximately 3 months prior to randomization. For the majority of the men in the placebo arm, to reduce intra-individual variability and to conserve limited pre-randomization samples, 0.5 ml serum samples collected at baseline and at Year 3 ($n = 1{,}667$) was pooled before estrogen analysis. For a small subset of men without Year 3 samples available, samples collected at an alternate year were used ($n = 180$ cases and $n = 56$ controls at Year 1–7). We tested the intra-class correlation for serum levels of estrone and estradiol based on 150 men on the placebo with separate measurement at baseline and at Year 3, which were 0.62 and 0.74, respectively. For those men, the mean of the two assays were used in the analysis. There was another subset of 162 men who were measured based on a single pre-randomization sample. When prostate cancer was diagnosed within 3 years from randomization ($n = 201$ from both treatment arms), post-randomization samples collected at an earlier time point before diagnosis was used. Serum concentrations of estrone and estradiol were determined by radioimmunoassay (RIA). Before RIA, purification steps including an organic solvent extraction and Celite column partition chromatography were performed to increase assay sensitivity and specificity, which are essential for measurement of the usually low concentrations of estrogens in men [13]. The coefficients of variation (CV) for estrone and estradiol assays were calculated based on blind pools of control samples included in the analysis. Across different pools, the intra-batch CV% ranged from 8.7–19.1% for estrone and 8.6–20.6% for estradiol. The inter-batch CV% ranged from 9.1–14.6% for estrone and 10.4–13.7% for estradiol. The sensitivities of the estrone and estradiol RIAs were 10 and 5 pg/ml, respectively.

Statistical analysis

Standard univariate approaches including chi-square test for categorical variables and t test for continuous variables were used for comparisons of descriptive characteristics between cases and controls. Pearson correlation coefficients were used to examine associations between continuous variables. To examine the effects of finasteride treatment on circulating estrogen concentrations, the absolute change as a linear outcome was modeled by treatment arm (finasteride vs. placebo) with control for baseline value and age. The linear regression coefficient and corresponding p-value were used as indicators for age- and baseline-adjusted treatment effects. Similar regression models were used with log-transformed ratio of concentrations at follow-up to these at baseline, which after back transformation are reported as percentage change.

To estimate prostate cancer risk associated with serum concentrations of estrogens, they were categorized into quartiles based on their distributions among controls. To estimate prostate cancer risk associated with changes of serum estrogen concentrations after finasteride treatment, both absolute change and percent change were categorized into four categories, described as decrease, minimum change, moderate increase, and large increase, based on a priori cut-off points derived from examining the

distribution of change. In addition, change was also categorized into quartiles based on the distribution among controls; however, results were similar and are thus not presented. Removing potential outliers that fell off three times of the interquartile range from the median, or were determined to be physiologically inappropriate, did not substantially alter the results. Therefore, results based on all measures including these potential outliers are presented.

Unconditional logistic regression models were used to derive odds ratios (ORs) and 95% confidence intervals (CIs) for overall prostate cancer risk, and polytomous logistic regression models were used for low-grade (Gleason score < 7) and high-grade (Gleason score ≥ 7) prostate cancer compared to controls. All of these analyses were performed separately by treatment groups. Covariates included in the models were age (continuous), BMI (continuous), race (white vs. non-white), and sex hormone-binding globulin (SHBG) (continuous). Additional adjustment for testosterone concentrations was performed for all models. Potential effect modification by testosterone levels, BMI, race, family history of prostate cancer, diabetes and cause of cancer diagnosis, i.e., whether cancer was diagnosed after an elevated PSA or abnormal DRE (for-cause) or at the end of the trial without cause (not-for-cause), were tested by including a multiplicative term in the models. Analyses were performed using SAS 9.0 (SAS Institute, Cary, NC). All *p*-values were 2-sided with a significance level of 0.05.

Results

Demographic and lifestyle characteristics of the study population are given in Table 1. PSA levels at baseline were significantly higher in cases than in controls. The majority of men were overweight or obese. The proportion of non-white men in controls was higher than in cases due to sampling strategy. Case and controls did not differ by smoking status; but as reported previously [18], cases were slightly more likely to consume alcohol than controls. Only a small proportion of men had diabetes at baseline, with a higher proportion in controls than in cases.

Among men in the placebo group, there were no significant associations between baseline estrone or estradiol levels and overall prostate cancer risk (Table 2). Results remained similar when stratified by Gleason grade, with the exception of a reduced risk of high-grade prostate cancer in the second quartile of estrone level (OR = 0.57, 95% CI = 0.36–0.89). When Gleason grade of 8–10 was used as a definition of high-grade cancer, the results were similar to those of Gleason grade of 7–10 (data not shown). There were no significant effect modifications by testosterone levels, BMI, race, diabetes, family history of prostate cancer, or cause of cancer diagnosis (data not shown).

Associations between baseline levels of estrone and estradiol among men in the finasteride group are shown in Table 3. Compared to men in the lowest quartile of estrone levels, those in the highest quartiles had 43% increased risk of low-grade prostate cancer risk. Similarly, men in the highest quartile of estradiol levels had 34% increased risk of low-grade cancer risk. Revising the definition of high-grade cancer by Gleason grade 8–10 did not substantially change the results (data not shown). The increased risk became slightly stronger after control for testosterone, and were strongest among those without a positive family history of prostate cancer (for estrone, OR = 1.77, 95% CI = 1.18–2.66, P for trend < 0.01; for estradiol, OR = 1.79, 95% CI = 1.17–2.75, P for trend = 0.01). Nevertheless, interaction testings for testosterone, BMI, race, diabetes, family history of prostate cancer or cause of cancer diagnosis showed no effect modification (data not shown).

Table 4 summarizes the effects of finasteride treatment on serum estrogen concentrations. Overall, concentrations of estrogens at year 3 of the trial were increased significantly in the finasteride but not in the placebo group. These effects were similar after stratification for case–control status or tumor grade, with the exception of a significant decrease in placebo men who developed low-grade cancer. After adjustment for age and baseline estrogen concentrations, overall treatment effects were +11.9% for estrone and +9% for estradiol, and did not differ by case–control status or grade; although not all tests reached statistical significance among low-grade and high-grade cases due to limited sample size.

There were no significant associations of cancer risk in the finasteride group with either the absolute or the percent change in estrone or estradiol following treatment, with an exception of an inverse trend between percentage increase in estradiol concentrations and risk of low-grade cancer (Table 5). However, the same inverse association was not found with the absolute change and the odds ratio contrasting the highest to lowest quartile was not statistically significant.

Discussion

Among men randomized to the placebo group in the PCPT, there were no associations of baseline levels of estrogens with prostate cancer risk. Among those randomized to the finasteride group, high estrogen levels at baseline were associated with a moderately increased risk of low-grade but not high-grade prostate cancer. Serum concentrations

Table 1 Demographic and lifestyle characteristics of cases and controls in the PCPT

	All (n = 3,596) Mean ± SD	Control (n = 1,798) Mean ± SD	Case (n = 1,798) Mean ± SD	p-value[a]
Age at baseline, years	63.6 ± 5.5	63.6 ± 5.5	63.7 ± 5.5	0.61
Baseline PSA, ng/ml	1.4 ± 0.8	1.2 ± 0.7	1.6 ± 0.7	<0.01
BMI at baseline, kg/m^2	27.5 ± 4.0	27.6 ± 4.0	27.4 ± 4.0	0.12
	N (%)[b]	N (%)[b]	N (%)[b]	
Age at baseline, year[c]				1.00
55–59	956 (26.6)	478 (26.6)	478 (26.6)	
60–64	1,166 (32.4)	583 (32.4)	583 (32.4)	
65–69	886 (24.6)	443 (24.6)	443 (24.6)	
70+	588 (16.4)	294 (16.4)	294 (16.4)	
BMI				0.08
Normal (< 25 kg/m^2)	941 (26.4)	444 (24.9)	497 (27.9)	
Overweight (25–29.9 kg/m^2)	1,854 (52.0)	942 (52.9)	912 (51.1)	
Obese (≥ 30 kg/m^2)	768 (21.6)	394 (22.1)	374 (21.0)	
Race[d]				<0.01
Non-hispanic white	3,098 (86.2)	1,429 (79.5)	1,669 (92.8)	
Non-hispanic black	254 (7.1)	171 (9.5)	83 (4.6)	
Hispanic	177 (4.9)	140 (7.8)	37 (2.1)	
Other	67 (1.9)	58 (3.2)	9 (0.5)	
Family history[c]				0.94
No	2,832 (78.8)	1,417 (78.8)	1,415 (78.7)	
Yes	764 (21.2)	381 (21.2)	383 (21.3)	
Smoking status				0.51
Never smoker	1,254 (34.9)	615 (34.2)	639 (35.5)	
Current smoker	263 (7.3)	139 (7.7)	124 (6.9)	
Former smoker	2,079 (57.8)	1,044 (58.1)	1,035 (57.6)	
Alcohol consumption				0.04
<1 drink/week	1,508 (44.8)	800 (46.6)	708 (42.9)	
1–6 drinks/week	909 (27.0)	457 (26.6)	452 (27.4)	
7–13 drinks/week	505 (15.0)	241 (14.0)	264 (16.0)	
14 + drinks/week	444 (13.2)	219 (12.8)	225 (13.6)	
Diabetes				<0.01
No	3,378 (94.0)	1,664 (92.6)	1,714 (95.3)	
Yes	217 (6.0)	133 (7.4)	84 (4.7)	
Treatment arm[c]				0.95
Placebo	2,072 (57.6)	1,035 (57.6)	1,037 (57.7)	
Finasteride	1,524 (42.4)	763 (42.4)	761 (42.3)	
Gleason score				–
<7	–	–	1,230 (71.4)	
≥7	–	–	493 (28.6)	

[a] p-values are based on student t test for continuous variables and chi-square test for unordered categorical variables between cases and controls. For ordered categorical variables, ordinal numbers were assigned to levels and then treated as continuous variables to give trend p-values

[b] For specific variables, men with missing data are not shown. The count and percentage are based on those with known values

[c] Cases and controls are matched on age, family history, and treatment arms

[d] Controls are oversampled to include all non-White men. *SD* standard deviation

of both estrone and estradiol increased modestly following finasteride treatment; however, this increase was not associated with prostate cancer risk.

Previous epidemiologic studies provide inconsistent results on the relationships between circulating levels of estrogens and prostate cancer risk. While most studies

Table 2 Prostate cancer risk by quartiles of serum estrone and estradiol concentrations in the placebo arm of the PCPT

	All prostate cancer			Gleason score < 7			Gleason score ≥ 7		
	N[a] (case/control)	OR[b] (95% CI)	OR[c] (95% CI)	N[a] (case/control)	OR[b] (95% CI)	OR[c] (95% CI)	N[a] (case/control)	OR[b] (95% CI)	OR[c] (95% CI)
Estrone (pg/ml)									
Q1 (< 36.6)	296/292	1.00	1.00	219/292	1.00	1.00	64/292	1.00	1.00
Q2 (36.6–44.4)	241/267	0.89 (0.70–1.13)	0.89 (0.70–1.13)	197/267	0.99 (0.76–1.28)	0.98 (0.76–1.27)	33/267	0.57 (0.36–0.89)	0.57 (0.36–0.89)
Q3 (44.5–54.1)	249/239	1.07 (0.84–1.37)	1.06 (0.83–1.36)	189/239	1.11 (0.86–1.45)	1.09 (0.84–1.42)	52/239	0.99 (0.66–1.48)	1.00 (0.66–1.50)
Q4 (≥ 54.2)	234/214	1.19 (0.93–1.54)	1.17 (0.90–1.51)	166/214	1.17 (0.88–1.54)	1.13 (0.85–1.49)	56/214	1.21 (0.80–1.82)	1.23 (0.81–1.86)
Trend *p*-value		0.11	0.16		0.20	0.33		0.18	0.18
Estradiol (pg/ml)									
Q1 (≤ 26.7)	272/280	1.00	1.00	209/280	1.00	1.00	54/280	1.00	1.00
Q2 (26.8–33.0)	267/262	1.08 (0.85–1.37)	1.06 (0.83–1.36)	208/262	1.09 (0.84–1.41)	1.07 (0.82–1.39)	49/262	0.98 (0.64–1.50)	0.98 (0.64–1.51)
Q3 (33.1–39.4)	237/251	1.02 (0.79–1.31)	1.00 (0.77–1.29)	163/251	0.92 (0.70–1.20)	0.88 (0.67–1.17)	58/251	1.20 (0.80–1.82)	1.21 (0.79–1.85)
Q4 (> 39.4)	252/230	1.23 (0.95–1.59)	1.20 (0.91–1.56)	195/230	1.25 (0.95–1.65)	1.19 (0.89–1.59)	48/230	1.11 (0.71–1.73)	1.11 (0.70–1.76)
Trend *p*-value		0.18	0.29		0.28	0.48		0.46	0.47

For the majority of the men in the placebo arm, serum samples collected at baseline and at Year 3 was pooled before estrogen analysis. For a subset of men ($n = 150$) in the placebo arm, separate assays were run for baseline and at Year 3, and the mean of the two assays were used in the analysis

[a] The total number of cases or controls does not add up to those shown in Table 1 due to missingness of the covariates

[b] Odds ratios are adjusted for age, body mass index, race (white versus nonwhite), and baseline serum sex hormone-binding globulin concentrations

[c] Odds ratios are adjusted for age, body mass index, race (white versus nonwhite), and baseline serum sex hormone-binding protein and testosterone concentrations. *OR* odds ratio. *CI* confidence interval

Table 3 Prostate cancer risk by quartiles of serum estrone and estradiol concentrations at baseline in the finasteride arm of the PCPT

	All prostate cancer			Gleason score < 7			Gleason score ≥ 7		
	N[a] (case/control)	OR[b] (95% CI)	OR[c] (95% CI)	N[a] (case/control)	OR[b] (95% CI)	OR[c] (95% CI)	N[a] (case/control)	OR[b] (95% CI)	OR[c] (95% CI)
Estrone (pg/ml)									
Q1 (≤ 35.5)	172/176	1.00	1.00	97/176	1.00	1.00	69/176	1.00	1.00
Q2 (35.6–43.3)	164/181	0.96 (0.70–1.30)	0.98 (0.72–1.33)	98/181	1.01 (0.71–1.46)	1.04 (0.73–1.49)	60/181	0.87 (0.58–1.31)	0.88 (0.59–1.33)
Q3 (43.4–53.2)	189/186	1.12 (0.83–1.52)	1.15 (0.85–1.56)	115/186	1.22 (0.86–1.73)	1.25 (0.88–1.78)	68/186	1.00 (0.67–1.49)	1.02 (0.68–1.52)
Q4 (> 53.2)	220/208	1.29 (0.95–1.73)	1.35 (0.99–1.83)	133/208	1.43 (1.01–2.02)	1.51 (1.06–2.15)	73/208	1.01 (0.68–1.50)	1.05 (0.70–1.59)
Trend *P*-value		0.05	0.03		0.02	0.01		0.80	0.65
Estradiol (pg/ml)									
Q1 (≤ 26.7)	184/182	1.00	1.00	94/182	1.00	1.00	85/182	1.00	1.00
Q2 (26.8–33.0)	177/185	0.98 (0.73–1.32)	1.01 (0.75–1.37)	110/185	1.22 (0.85–1.73)	1.27 (0.89–1.81)	57/185	0.67 (0.45–0.99)	0.68 (0.46–1.02)
Q3 (33.1–39.4)	180/177	1.06 (0.78–1.44)	1.12 (0.82–1.53)	117/177	1.39 (0.97–1.98)	1.48 (1.03–2.13)	52/177	0.64 (0.42–0.97)	0.67 (0.44–1.02)
Q4 (> 39.4)	212/209	1.13 (0.83–1.52)	1.23 (0.89–1.69)	126/209	1.34 (0.94–1.91)	1.50 (1.03–2.18)	79/209	0.86 (0.59–1.27)	0.93 (0.62–1.40)
Trend *P*-value		0.36	0.16		0.09	0.03		0.46	0.71

[a] The total number of cases or controls does not add up to those shown in Table 1 due to missingness of the covariates

[b] Odds ratios are adjusted for age, body mass index, race (white versus nonwhite), and baseline serum sex hormone-binding globulin concentrations

[c] Odds ratios are adjusted for age, body mass index, race (white versus nonwhite), and baseline serum sex hormone-binding protein and testosterone concentrations. *OR* odds ratio, *CI* confidence interval

Table 4 Changes of serum estrone and estradiol concentrations between baseline and follow-up in the PCPT

	Finasteride arm[a]			Placebo arm[a]			Adjusted treatment effect			
	Baseline	Follow-up	Change	Baseline	Follow-up	Change	Absolute changes[b]		Percent increase[c]	
	Mean ± SD	Mean ± SD	Mean ± SE	Mean ± SD	Mean ± SD	Mean ± SE	β ± SE	*p*-value	% (95% CI)	*p*-value
All										
N	1,497	1,496	1,490	150	150	150				
Estrone, pg/ml	46.6 ± 15.7	51.2 ± 16.9	4.6 ± 0.4	48.5 ± 16.6	46.6 ± 14.7	−1.9 ± 1.1	5.8 ± 1.2	<0.01	11.9 (7.2–16.9)	<0.01
N	1,507	1,506	1,500	150	150	150				
Estradiol, pg/ml	34.5 ± 11.5	36.9 ± 12.2	2.5 ± 0.3	32.5 ± 9.3	32.5 ± 9.1	−0.1 ± 0.5	3.4 ± 0.9	<0.01	9.0 (4.6–13.6)	<0.01
Controls										
N	755	753	750	110	110	110				
Estrone, pg/ml	46.1 ± 15.7	50.9 ± 17.0	4.7 ± 0.6	48.7 ± 17.5	47.7 ± 15.1	−1.0 ± 1.3	4.7 ± 1.4	<0.01	9.3 (3.8–15.0)	<0.01
N	757	756	752	110	110	110				
Estradiol, pg/ml	34.6 ± 12.7	36.6 ± 11.8	2.0 ± 0.4	32.1 ± 9.7	32.0 ± 9.1	−0.1 ± 0.6	3.3 ± 1.0	<0.01	8.4 (3.2–13.8)	<0.01
Cases										
N	743	743	740	40	40	40				
Estrone, pg/ml	47.0 ± 15.6	51.5 ± 16.8	4.5 ± 0.6	48.1 ± 14.0	43.7 ± 13.1	−4.5 ± 1.6	8.6 ± 2.2	<0.01	19.4 (9.9–29.7)	<0.01
N	750	750	748	40	40	40				
Estradiol, pg/ml	34.4 ± 10.3	37.3 ± 12.7	2.9 ± 0.4	33.6 ± 8.4	33.6 ± 9.2	0.03 ± 0.9	3.2 ± 1.7	0.06	9.2 (1.0–18.0)	0.03
Low-grade cases										
N	443	442	441	29	29	29				
Estrone, pg/ml	47.2 ± 15.2	51.6 ± 17.2	4.7 ± 0.7	50.0 ± 14.4	43.9 ± 13.6	−6.1 ± 2.0	9.8 ± 2.6	<0.01	21.6 (10.3–33.9)	<0.01
N	447	445	445	29	29	29				
Estradiol, pg/ml	34.8 ± 9.8	37.4 ± 13.1	2.6 ± 0.5	33.9 ± 9.0	33.9 ± 9.8	0.1 ± 1.0	2.8 ± 2.1	0.18	7.4 (−1.9–17.7)	0.12
High-grade cases										
N	269	271	269	11	11	11				
Estrone, pg/ml	46.6 ± 16.3	51.4 ± 16.2	4.9 ± 16.8	43.3 ± 12.1	43.0 ± 12.1	−0.3 ± 2.0	6.8 ± 4.4	0.12	15.6 (−1.3–35.4)	0.07
N	272	274	272	11	11	11				
Estradiol, pg/ml	33.7 ± 10.8	37.5 ± 12.1	3.8 ± 10.5	32.8 ± 7.0	32.7 ± 7.7	−0.1 ± 2.2	4.4 ± 3.0	0.14	13.3 (−2.6–31.8)	0.11

[a] Estrogen concentrations at baseline and at follow-up of approximately three years after on the study are presented in raw values. The changes are calculated as values at follow-up minus values at baseline

[b] Treatment effect is calculated as regression coefficient (β) of treatment arm (finasteride vs. placebo) in the linear regression model with absolute changes of estrogen concentrations as the dependent variable. The *p*-values compare the mean changes in estrogen concentrations between the treatment arms, with adjustment for baseline concentrations and age

[c] Treatment effect is calculated as regression coefficient of treatment arm in the linear regression model with log-transformed ratio of follow-up to baseline values as the dependent variable. The percent increase presented in the table is derived by back-transforming the regression coefficient. *SD* standard deviation, *SE* standard error

found null associations [19–26], which may have driven a same conclusion in several meta- and pooled-analysis [3, 11, 12], three studies showed reduced risk among those with high estrogen levels [13–15]. In contrast, a recent case-cohort study with 275 prostate cancer cases reported an increased risk among those in the highest 3 quartiles of estrone levels, but there were no associations with estradiol levels [27]. This inconsistency may be due to the balance of the opposing effects of estrogens in the prostate, and the lack of assessment of estrogen receptor activity in the prostate gland. It has been hypothesized that estrogens may play dual and opposing roles in prostatic homeostasis and carcinogenesis [28]. Estrogens can cause abnormal proliferation, inflammation, and prostate malignancy, mediated by estrogen receptor α (ER-α), but they may also confer important beneficial effects, including anti-proliferation, anti-inflammation, and anti-carcinogenesis, mediated by ER-β. The balance between activities of the two ER subtypes may dictate prostatic responses to estrogens [29]. The exact mechanisms for coordination between these opposing effects of estrogens and disruption during prostate carcinogenesis are unclear.

Among men in the finasteride group, high estrogen levels at baseline were associated with an increased risk of low-grade prostate cancer. It is possible that finasteride could modify the relationships between estrogens and prostate cancer risk, although a mechanism for this is not obvious. Alternatively, estrogen may modify the efficacy of finasteride for prevention of low-grade prostate cancer. Because of the nested case–control design, we could not distinguish these possibilities. This issue needs to be investigated in the entire PCPT cohort and would require

Table 5 Prostate cancer risk by absolute change and percent change in serum estrone and estradiol concentrations from baseline to follow-up in the finasteride arm of the PCPT

	All prostate cancer			Gleason score < 7			Gleason score ≥ 7		
	N (case/control)	OR[a] (95% CI)	OR[b] (95% CI)	N (case/control)	OR[a] (95% CI)	OR[b] (95% CI)	N (case/control)	OR[a] (95% CI)	OR[b] (95% CI)
Absolute change of estrone concentrations from baseline to follow-up[c] (pg/ml)									
<−5.0	151/160	1.00	1.00	87/160	1.00	1.00	56/160	1.00	1.00
−5.0–5.0	239/228	1.02 (0.76–1.38)	1.02 (0.76–1.37)	148/228	1.08 (0.77–1.53)	1.07 (0.76–1.52)	78/228	0.93 (0.62–1.40)	0.92 (0.62–1.39)
5.1–12.0	149/158	0.94 (0.68–1.31)	0.94 (0.67–1.30)	84/158	0.91 (0.62–1.33)	0.90 (0.61–1.32)	63/158	1.11 (0.72–1.70)	1.10 (0.71–1.69)
>12.0	195/198	1.01 (0.74–1.37)	1.00 (0.73–1.36)	119/198	1.05 (0.73–1.50)	1.04 (0.72–1.48)	70/198	1.00 (0.66–1.52)	0.99 (0.65–1.50)
Trend *p*-value		0.91	0.85		0.95	0.90		0.78	0.84
Percent change of estrone concentrations from baseline to follow-up									
<−10%	163/170	1.00	1.00	95/170	1.00	1.00	59/170	1.00	1.00
−10–10%	213/195	1.08 (0.80–1.45)	1.08 (0.80–1.46)	131/195	1.12 (0.79–1.58)	1.12 (0.79–1.59)	71/195	1.02 (0.67–1.53)	1.02 (0.68–1.54)
10.1–20%	85/104	0.84 (0.58–1.22)	0.83 (0.57–1.21)	55/104	0.91 (0.59–1.39)	0.90 (0.59–1.38)	29/104	0.82 (0.49–1.37)	0.81 (0.48–1.36)
>20%	273/275	0.98 (0.74–1.30)	0.97 (0.73–1.29)	157/275	0.95 (0.68–1.32)	0.94 (0.68–1.31)	108/275	1.10 (0.75–1.61)	1.09 (0.75–1.59)
Trend *p*-value		0.63	0.58		0.49	0.45		0.65	0.71
Absolute change of estradiol concentrations from baseline to follow-up (pg/ml)									
<−4.0	167/180	1.00	1.00	104/180	1.00	1.00	55/180	1.00	1.00
−4.0–4.0	218/234	0.89 (0.66–1.19)	0.87 (0.65–1.17)	136/234	0.88 (0.63–1.23)	0.86 (0.61–1.21)	69/234	0.87 (0.58–1.32)	0.85 (0.56–1.29)
4.1–8.0	181/148	1.15 (0.84–1.58)	1.12 (0.82–1.54)	103/148	1.03 (0.72–1.48)	1.00 (0.70–1.45)	72/148	1.44 (0.94–2.20)	1.39 (0.91–2.14)
>8.0	176/184	0.93 (0.68–1.26)	0.91 (0.66–1.24)	99/184	0.82 (0.58–1.18)	0.80 (0.56–1.15)	74/184	1.22 (0.81–1.85)	1.19 (0.78–1.80)
Trend *p*-value		0.92	0.97		0.47	0.39		0.09	0.11
Percent change of estradiol concentrations from baseline to follow-up									
<−10%	132/145	1.00	1.00	86/145	1.00	1.00	43/145	1.00	1.00
−10–10%	315/308	1.04 (0.78–1.40)	1.03 (0.77–1.39)	195/308	0.98 (0.70–1.37)	0.97 (0.69–1.35)	100/308	1.04 (0.69–1.58)	1.03 (0.68–1.56)
10.1–20%	120/106	1.14 (0.79–1.64)	1.12 (0.77–1.61)	70/106	1.01 (0.67–1.53)	0.99 (0.65–1.50)	47/106	1.40 (0.85–2.29)	1.37 (0.83–2.25)
>20%	167/184	0.90 (0.65–1.25)	0.88 (0.64–1.23)	87/184	0.71 (0.48–1.04)	0.69 (0.47–1.01)	77/184	1.33 (0.85–2.07)	1.30 (0.83–2.03)
Trend *p*-value		0.54	0.45		0.06	0.05		0.09	0.11

[a] Odds ratios are adjusted for age, body mass index, race (white versus nonwhite), and baseline serum sex hormone-binding globulin concentrations

[b] Odds ratios are adjusted for age, body mass index, race (white versus nonwhite), and baseline serum sex hormone-binding protein and testosterone concentrations

[c] Blood samples collected at the third year after on the study were used as the follow-up measurement. *OR* odds ratio *CI* confidence interval

measurement of serum estrogen levels from all participants, which is currently available only from cases and controls included in this nested study. We examined the linear correlations of baseline concentrations of estrone and estradiol with the change of 5α-androstane-3α,17β-diol glucuronide (3α-diol G), a metabolite of DHT that reflects concentrations of intra-prostatic DHT and thus indirectly measures the finasteride treatment effect, following finasteride treatment. There were significant inverse associations between baseline estrogens and change in 3a–dG ($r = -0.09$ for estrone and $r = -0.11$ for estradiol, both $P < 0.001$). However, because the strength of these correlations was weak, it does not support a strong or direct effect of estrogens on finasteride treatment efficacy. It is also possible that the observed increased risk was biased by factors potentially related to estrogen. Estrogen concentrations are positively associated with obesity; however, the effects of finasteride on cancer risk did not differ by obesity [30]. The possibility that whether other factors such as diet or physical activity modify the effects of finasteride treatment have not yet been evaluated.

Baseline estrogens may have been associated with high PSA and therefore bias in cancer detection; but there were no associations of estrogen concentrations with baseline PSA (Spearman correlation coefficient ranges from −0.08 to 0.05 in cases or controls from the placebo or finasteride arm, with or without adjustment for age and BMI). Lastly, we could not rule out the possibility that these findings were due to chance alone. However, the hypotheses were all a priori and the associations were observed consistently for estrone and estradiol with significant trends, which lower the likelihood of chance findings. Overall, we cannot explain the findings of a positive association of baseline estrogens with cancer risk in finasteride-treated men, which warrants further research.

We had hypothesized that the magnitudes of increases in estrogens following finasteride treatment may have increased prostate cancer risk. However, with only a single exception, neither the changes in estrogens nor the post-treatment estrogen concentrations were associated with cancer risk. There was a suggestive trend of increased risk of low-grade cancer with percentage increase in estrone; however, because the odds ratio contrasting extreme quartiles was not statistically significant and there was no association of absolute change with risk, we deem this as a chance finding. Moreover, the magnitude of changes in estrogens in the finasteride arm following treatment were modest (mean change, 4.6 pg/ml for estrone and 2.5 pg/ml for estradiol), compared to the inter-quartile differences in at baseline (for estrone, Q1 ≤ 35.5 pg/ml, Q4 > 53.2 pg/ml; for estradiol, Q1 ≤ 26.7 pg/ml, Q4 > 39.4 pg/ml). Thus, it is unlikely that changes in estrogens of these small magnitudes have physiological or clinical significance.

The study benefits from vigorous annual screening by DRE and PSA test, and an end-of-study biopsy offered to all men who were cancer-free at the exit of the study. These measures greatly reduced the likelihood of undiagnosed prostate cancer in the control group and minimize misclassification. Moreover, the PCPT adapted a centralized and standardized approach for cancer grading, minimizing misclassification. Other strengths include purification steps before RIA for estrogen measurement and a large sample size nested within a completed multi-center trial. However, several limitations should also be considered in this study. First, non-fasting blood samples were collected at different daily times and thus variations in estrogen levels throughout the day could not be controlled for; however, these variations were not likely to be systematically different between cases and controls [17]. Second, circulating estrogens may not reflect intraprostatic levels [31]. Local expression of aromatase and production of estrogen in the prostate further complicates the relevance of circulating levels to prostate cancer etiology [32]. However, this issue is not specific to our study but common to all studies examining circulating biomarkers, and measurement of intra-prostatic hormone levels is currently not feasible for large-scale epidemiologic studies.

In summary, we found no evidence for an association of estrogen levels with prostate cancer risk among men not on finasteride. Among those taking finasteride, there may be a positive association of pretreatment estrogen concentrations and risk of low-grade prostate cancer; however, we know of no mechanisms that could explain such an association. These results add to a body of previous published studies finding no associations of circulating estrogen concentrations with prostate cancer risk. The moderate increase in risk of low-grade prostate cancer associated with high baseline levels of estrogen among men taking finasteride warrants further investigation.

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