Research Article

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Associations of Serum Sex Steroid Hormone and 5α-Androstane-3α,17β-Diol Glucuronide Concentrations with Prostate Cancer Risk Among Men Treated with Finasteride

Alan R. Kristal[1,2], Cathee Till[1], Catherine M. Tangen[1], Phyllis J. Goodman[1], Marian L. Neuhouser[1,2], Frank Z. Stanczyk[3], Lisa W. Chu[4], Sherfaraz K. Patel[3], Ian M. Thompson[5], Juergen K. Reichardt[12], Ashraful Hoque[6], Elizabeth A. Platz[7], William D. Figg[8], Adrie Van Bokhoven[9], Scott M. Lippman[10], and Ann W. Hsing[11]

Abstract

Background: Finasteride, an inhibitor of 5α-reductase (type II), lowers intraprostatic dihydrotestosterone (DHT), which is reflected in serum as reduced 5α-androstane-3α,17β-diol glucuronide (3α-dG). It also modestly increases serum testosterone (T), estrone (E_1), and estradiol (E_2). In this altered hormonal milieu, it is unknown whether serum concentrations of these hormones are associated with prostate cancer risk.

Methods: In this nested case–control study of men in the finasteride arm of the Prostate Cancer Prevention Trial, sex steroid hormones and sex hormone binding globulin were measured at baseline and approximately 3-year posttreatment in 553 prostate cancer cases and 694 controls.

Results: Median posttreatment changes in concentrations of 3α-dG, T, E_1, and E_2 were −73.8%, +10.1%, +11.2%, and +7.5% (all $P < 0.001$), respectively. Neither the pre- nor posttreatment concentrations of 3α-dG, nor its change, were associated with risk. Pretreatment, high concentrations of E_1 and low concentrations of T were associated with increased cancer risk [OR; 95% confidence interval (CI) quartile 4 vs. 1: 1.38 (0.99–1.93) $P_{trend} = 0.03$; 0.64 (0.43–0.93) $P_{trend} = 0.07$, respectively]. Posttreatment, high concentrations of both E_1 and E_2 were associated with increased cancer risk [OR; 95% CI quartile 4 vs. 1: 1.54 (1.09–2.17) $P_{trend} = 0.03$; 1.49 (1.07–2.07) $P_{trend} = 0.02$, respectively].

Conclusions: Among finasteride-treated men, concentrations of 3α-dG were not associated with total or Gleason grades 2 to 6, 7 to 10, or 8 to 10 cancer. High serum estrogens may increase cancer risk when intraprostatic DHT is pharmacologically lowered.

Impact: Low posttreatment serum estrogens may identify men more likely to benefit from use of finasteride to prevent prostate cancer. *Cancer Epidemiol Biomarkers Prev; 21(10); 1823–32. ©2012 AACR.*

There is general consensus that normal, physiologic variability in blood concentrations of sex steroid hormones is not associated with the risk of prostate cancer (1). However, both finasteride, a type II steroid 5α-reductase inhibitor, and dutasteride, a dual type I and type II steroid 5α-reductase inhibitor, dramatically change the intraprostatic hormonal milieu. By inhibiting intraprostatic conversion of testosterone (T) to dihydrotestosterone (DHT), these drugs substantially lower the concentration of intraprostatic DHT (2) and modestly increase the concentrations of blood T (3); whether estradiol (E_2) and estrone (E_1) are affected remains uncertain. In the Prostate Cancer Prevention Trial (PCPT), which tested whether finasteride could prevent prostate cancer, finasteride reduced the risk of total prostate cancer by 25% but also increased the risk of high-grade cancer (4). This paradoxical finding remains unexplained. It could be attributable to increasing the detection of high-grade cancer, as finasteride improves the sensitivities of screening tests [digital rectal examination (DRE)] (5) and prostate-specific antigen (PSA; ref. 6) and diagnostic biopsies (7); however, it is also possible that low intraprostatic DHT provides a growth advantage for aggressive tumors (8).

Authors' Affiliations: [1]Division of Public Health Sciences, Fred Hutchinson Cancer Research Center; [2]Department of Epidemiology, University of Washington School of Public Health, Seattle, Washington; [3]Department of Obstetrics & Gynecology, University of Southern California Keck School of Medicine, Los Angeles, California; [4]National Cancer Institute, Division of Cancer Epidemiology; [5]Department of Urology, University of Texas Health Sciences Center at San Antonio, San Antonio; [6]Department of Clinical Cancer Prevention, MD Anderson Cancer Center, Houston, Texas; [7]Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore; [8]Medical Oncology Branch, National Cancer Institute, Bethesda, Maryland; [9]Department of Pathology, University of Colorado, Denver, Colorado; [10]Moores Cancer Center, University of California San Diego; [11]Cancer Prevention Institute of California, California; and [12]Faculty of Medicine, Health and Molecular Sciences, James Cook University, QLD, Australia

Corresponding Author: Alan R. Kristal, Cancer Prevention Program, Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue N, Mail Stop M4-B402, PO Box 19024, Seattle, WA 98109. Phone: 206-667-4686; Fax: 1-206-667-7850; E-mail: akristal@fhcrc.org

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Here, we investigate whether, among men who were compliant with finasteride treatment, the pre- and posttreatment concentrations of steroid hormones and their treatment-associated changes are associated with prostate cancer risk. We use serum 5α-androstane-3α,17β-diol glucuronide (3α-dG), a distal metabolite of DHT, as a surrogate measure of intraprostatic DHT (9, 10), because direct assay of prostate tissue was not feasible. Our primary hypothesis is that among men treated with finasteride, low posttreatment concentration of 3α-dG, reflecting a larger reduction in intraprostatic DHT, would be associated with lower overall prostate cancer risk. Secondarily, given the association of finasteride with high-grade disease, we also consider whether low posttreatment 3α-dG was associated with an increased risk of high-grade disease. In more exploratory analyses, we examine whether the pre- and posttreatment concentrations of T, E_1, and E_2 were associated with prostate cancer risk. Findings from this study could make it feasible to identify men who would maximally benefit from the use of finasteride for cancer prevention, and could provide insight into the etiology of the increased risk of high-grade cancer among men treated with finasteride.

Materials and Methods

Study design and study population

Data are from the PCPT, a randomized, placebo-controlled trial that tested whether finasteride, a steroid 5α-reductase type II inhibitor, could reduce the 7-year period prevalence of prostate cancer. Details about study design and participant characteristics have been described previously (4). Briefly, 18,880 men age 55 years and older with normal DRE and PSA levels of 3 ng/mL or below, as well as no history of prostate cancer, severe lower urinary tract symptoms or clinically significant coexisting conditions, were randomized to receive finasteride (5 mg/d) or placebo. During the PCPT, men underwent DRE and PSA determinations annually, and a prostate biopsy was recommended for participants with an abnormal DRE or if a PSA adjusted for the effect of finasteride was 4.0 ng/mL or greater. At the final study visit at year 7, all men not previously diagnosed with prostate cancer were requested to undergo an end-of-study prostate biopsy. All biopsies consisted of a minimum of 6 cores collected under transrectal ultrasonographic guidance and were reviewed for adenocarcinoma by both the pathologist at the local study site and a central pathology laboratory with concordance achieved in all cases. Clinical stage was assigned locally and tumors were graded centrally using the Gleason scoring system. All men gave informed consent and study procedures were approved by Institutional Review Boards at each study center, the Southwest Oncology Group (SWOG, San Antonio, TX), and the Fred Hutchinson Cancer Research Center (Seattle, WA).

Case and control selection

The study reported here is part of a large nested case–control study designed to examine multiple hypotheses about prostate cancer biology and risk (11). Cancer cases and controls in this report were from the finasteride treated study arm. Cases ($n = 676$) had biopsy-confirmed cancer identified before study unblinding, and blood samples both at baseline and before cancer diagnosis. Controls ($n = 759$) were disease-free at the end-of-study biopsy and had both baseline and follow-up blood samples. Controls were frequency matched to cases on distributions of age (±5 years) and having a first-degree relative with prostate cancer, and included all non-Whites. There were more controls than cases because men diagnosed with cancer in the first 2 years, or before a follow-up blood was collected, were excluded. Men who were not compliant with the study treatment, defined as either (i) reporting not using the drug at the time of the posttreatment serum collection ($n = 155$) or (ii) having a posttreatment finasteride blood concentration of zero ($n = 33$), were excluded, leaving 553 cases and 694 control participants in the study.

Data collection and laboratory methods

Information on age, race, diabetes status, family history of prostate cancer in first-degree relatives, and history of smoking were collected at baseline using self-administered questionnaires. Participants' height and weight were measured at baseline, and body mass index (BMI) was calculated as weight (kg)/height (m^2).

Nonfasting blood was collected approximately 3 months before randomization and annually thereafter until diagnosis or the study end. Venous blood was drawn into glass collection tubes without anticoagulant, refrigerated, and shipped to a central repository where they were centrifuged, aliquoted, and stored at −70°C. Concentrations of T, 3α-dG, E_1, E_2, and sex hormone binding globulin (SHBG) were measured at baseline and at year 3 postbaseline. For the approximately 5% of men missing a year 3 blood sample, the sample closest in time was used (range years 1–7).

Hormone measurements

Total T, 3α-dG, E_1, E_2, and SHBG were quantified in serum by highly specific immunoassays at the Reproductive Endocrine Research Laboratory, University of Southern California Keck School of Medicine (F.Z. Stanczyk). Total T and SHBG were measured by a direct solid-phase, competitive chemiluminescent enzyme immunoassay and a direct solid-phase, 2-site chemiluminescent immunometric assay, respectively, using the Immulite 2000 analyzer (Siemens Healthcare Diagnostics, Deerfield, IL). The sensitivity of the T assay is 20 ng/dL and that of the SHBG assay is 1 nmol/L. The interassay CVs for T were 11.9%, 7.6%, and 9.1% at concentrations of 124, 539, and 1,058 ng/dL, respectively, and for SHBG were 5.2%, 5.2%, and 6.6% at 21, 63, and 80 nmol/L, respectively. 3α-dG was measured manually by direct competitive radioimmunoassay, using kits obtained from Beckman-Coulter). This assay was validated extensively and measures the predominant form of 3α-dG, which contains the

glucuronide at carbon 17 instead of carbon 3 (12). The 3α-dG assay sensitivity is 0.5 ng/mL and the interassay CVs were 2.7% and 9.0% at concentrations of 4.5 and 6.4 ng/mL, respectively. Estrogens were measured by radioimmunoassay after organic solvent extraction and Celite column partition chromatography, as described previously (13). The E_1 and E_2 assay sensitivities are 2 and 4 pg/mL, respectively. The interassay CVs were 11%, 12%, and 9% at concentrations of 24, 61, and 159 pg/mL, respectively, for E_1, and 10% at concentrations of 22, 66, and 183 pg/ml for E_2.

Free and bioavailable (non-SHBG-bound) T and E_2 were calculated using a validated method (14) based on measured total T and E_2 levels, respectively, and SHBG, assuming an average concentration for albumin (15, 16). This method has been found to have high validity (14). Assays were not successful for small numbers of samples, and thus the numbers with baseline, follow-up, and change measures differs slightly for each analyte. Quality control (QC) samples from pooled serum from healthy volunteers, split into 6 pools, were also included in each analytical batch. Between 1 and 6 samples from the same pool were placed randomly within each box of samples. QC data were monitored regularly and laboratory personnel were blinded to sample type. Coefficients of variation for 3α-dG, T, E_1, E_2, and SHBG were 14.0%, 10.5%, 15.2%, 14.9%, and 12.2%, respectively.

Statistical analysis

Paired *t* tests were used to test whether the absolute or percentage differences in 3α-dG, T (total and free), E_1, E_2 (total and free), and SHBG concentrations between baseline and posttreatment were significantly different from zero. Baseline-adjusted change was calculated from a linear regression using change as the dependent variable and baseline as the independent variable; the residual from this model was added to the population mean change. Spearman rank-order correlations were used to assess the associations among treatment-associated changes in all measures, and were computed both unadjusted and, using residuals from linear regression models, adjusted for SHBG. Unconditional and polytomous logistic regression analyses were used to estimate ORs and 95% confidence intervals (CI) for the associations of steroid concentrations, and changes in these concentrations, with total, low- and high-grade cancer. Each measure was categorized into quartiles based on distributions in controls. Models were adjusted for SHBG, as well as frequency matching variables and variables associated with prostate cancer risk in this cohort, including age (continuous), race (Caucasian, other), family history of prostate cancer in first-degree relatives (yes, no), body mass index (continuous), and serum cholesterol. Diabetes, although associated with cancer risk, could not be included in stratified models because of its low frequency; however, findings did not change when men with diabetes were excluded. Low-grade cancer was defined as Gleason Score 2 to 6, and high-grade cancer was defined as Gleason Score 7 to 10 and, to capture a more rare but more phenotypically uniform group of highly aggressive cancers (17) as Gleason Score 8 to 10. There were no significant differences between findings for Gleason 7 to 10 and 8 to 10 cancers, in part because of the relatively small number of Gleason 8 to 10 cancers, and the results for both are described together in the text as "high-grade" cancer. All statistical tests were 2-sided, with a statistical significance level set at $P = 0.05$. All statistical analyses were carried out using SAS statistical software (version 9.2; SAS Corporation).

Results

Table 1 gives baseline demographic and health-related characteristics in case and control participants. Cases and controls did not differ significantly by BMI, smoking status, alcohol consumption, or physical activity, nor did they differ by age or family history of prostate cancer because of matching. Compared with cases, PSA levels and years of education were significantly lower in controls. The much higher proportion of non-White men in the control group was because of purposeful oversampling.

Table 2 gives mean and median levels of serum androgens, estrogens, and SHBG at baseline and follow-up, along with the absolute, percentage, and baseline-adjusted differences between these 2 time points. For all measures, the changes between baseline and follow-up were statistically significant ($P < 0.001$). As expected, the largest change was a mean 74% reduction in serum 3α-dG. There were small increases in median concentrations of serum T, SHBG, E_1, and E_2, which ranged from 6.0% to 11.2% and were attenuated for free compared with total T and E_2.

Table 3 gives the correlations among changes in 3α-dG, serum steroids, and SHBG. Changes in 3α-dG were not correlated with changes in other steroids or SHBG. Changes in T were moderately and positively correlated with changes in E_1, E_2, and SHBG, and changes in E_1 and E_2 were strongly correlated with each other. Correlations were similar when adjusted for SHBG (data not shown).

In this subset of men in the PCPT who were finasteride-compliant approximately 3 years postrandomization, neither absolute, percentage, nor baseline-adjusted changes in sex steroids or 3α-dG were associated with risks of total, low-, or high-grade cancer (data not shown).

Table 4 gives the covariate-adjusted associations of pretreatment steroid concentrations with prostate cancer risk. Compared with men in the lowest quartile of T, those in the highest quartile had a 36% (95% CI, 57%–7%) reduced risk of total cancer. This association was similar for low- and high-grade cancer but was not linear; reduced risks were limited to those in the highest quartile only. The association of free T with cancer risk was substantially attenuated and not significant. There was also a 38% (−1%−93%) increased risk of cancer, comparing men in the highest to lowest quartiles of E_1. The association was limited to low-grade cases.

Table 5 gives the covariate-adjusted associations of posttreatment steroid and SHBG concentrations with prostate cancer risk. Neither T, free T, nor 3α-dG was associated with the risk of total, low-, or high-grade cancer. Concentrations of E_1, E_2, and free E_2 were positively associated with cancer risk: comparing the fourth to first quartiles (Q4 vs. Q1) risks were increased by 54% (9%–117%), 49% (7%–107%), and 34% (−4%–87%), respectively. For all associations, trends were significant (all $P_{trend} < 0.03$) and similar for low- and high-grade disease.

In additional analyses not shown, the results for pre- and posttreatment T, free T, SHBG, and 3α-dG did not change when models were further adjusted for other steroids. Results for pre- and posttreatment E_1, E_2, and free E_2 did not change when additionally adjusted for T and 3α-dG. Associations of pretreatment E_1 and E_2 with cancer risk did not change when mutually adjusted for each other, but posttreatment associations for both were attenuated and no longer significant.

Discussion

In this nested case–control study among treatment-compliant men in the PCPT, there was a 74% reduction in serum 3α-dG after approximately 3 years of finasteride treatment. Neither pretreatment, posttreatment, nor the magnitude of change in 3α-dG were associated with the risk of total or Gleason Score 2 to 6, 7 to 10, or 8 to 10 cancer. Finasteride treatment also modestly increased serum T, free T, E_1, E_2, and free E_2. Pretreatment, men in the highest quartiles of T and E_1 had a 36% lower and a 38% higher risk of prostate cancer, respectively. Posttreatment, men in the highest quartiles of E_1, E_2, and free E_2 had 54%, 47%, and 34% increased risks of prostate cancer, respectively. The magnitudes of change in steroid concentrations were not associated with cancer risk.

The lack of association between posttreatment 3α-dG and cancer risk was unexpected. We had hypothesized that larger decreases in 3α-dG, reflecting larger reductions in intraprostatic DHT after treatment, would indicate

Table 1. Demographic and health-related characteristics at baseline, among treatment-compliant men in the Prostate Cancer Prevention Trial finasteride arm

	Control ($n = 694$)	Case ($n = 553$)	
	Mean (SD)	Mean (SD)	*P* value
Age, y[a]	63.8 (5.6)	64.1 (5.7)	0.35
Body mass index, kg/m^2	27.7 (4.1)	27.4 (3.8)	0.30
Prostate specific antigen, ng/mL	1.2 (0.7)	1.6 (0.7)	<0.0001
Cholesterol, mg/dL	216.6 (37.9)	216.7 (41.1)	
	N (%)	*N* (%)	
Family history of prostate cancer[a]	148 (21.3)	127 (23.0)	0.49
Diabetes	51 (7.3)	32 (5.8)	0.27
Race			
Non-Hispanic White	514 (74.1)	512 (92.6)	<0.0001
African-American[b]	93 (13.4)	29 (5.2)	
Other	87 (12.5)	12 (2.2)	
Education, y			
≤12	146 (21.1)	96 (17.4)	0.01
13–15	217 (31.3)	146 (26.4)	
≥16+	330 (47.6)	311 (56.2)	
Smoking			
Never	244 (35.2)	193 (34.9)	0.12
Current	58 (8.4)	30 (5.4)	
Former	392 (56.5)	330 (59.7)	
Alcohol intake, g/d			
0	161 (23.2)	132 (23.9)	0.88
>0–<30	472 (68.0)	369 (66.7)	
≥30	61 (8.8)	52 (9.4)	
Body mass index, kg/m^2			
Normal (<25)	184 (26.7)	146 (26.6)	0.99
Overweight (25–<30)	344 (50.0)	277 (50.5)	
Obese (≥30)	160 (23.3)	126 (23.0)	

[a]Matching variable for control selection.
[b]Non-White controls were oversampled.

Table 2. Serum concentrations of 5α-androstane-3α,17β-diol glucuronide, sex steroid hormones, and sex hormone binding globulin, before and after finasteride treatment, among treatment-compliant men in the Prostate Cancer Prevention Trial finasteride arm

	N	Median (IQR[a])	Mean (SD)
3α-dG, ng/mL[b]			
Baseline	1,247	5.7 (3.9,8.0)	6.8 (5.1)
Posttreatment	1,247	1.5 (1.0,2.1)	1.9 (1.7)
Absolute difference[c,d]	1,247	−4.1 (−6.1,−2.4)	−4.9 (4.7)
Absolute difference, baseline adjusted[c,d]	1,247	−5.2 (−5.6,−4.7)	−4.9 (1.5)
% Difference[c,e]	1,247	−73.8 (−81.5,−62.6)	−67.1 (32.6)
Testosterone, ng/dL			
Baseline	1,246	361 (285,448)	381 (138)
Posttreatment	1,246	398 (316,496)	421 (157)
Absolute difference[c,d]	1,246	37.0 (−31.0,103.0)	39.9 (121.0)
Absolute difference, baseline adjusted[c,d]	1,246	29.6 (−33.6,96.4)	39.9 (116.5)
% Difference[c,e]	1,246	10.1 (−8.1,31.7)	14.7 (36.1)
Free testosterone, ng/dL			
Baseline	1,246	8.4 (6.8,10.0)	8.7 (2.7)
Posttreatment	1,246	9.1 (7.3,10.9)	9.4 (3.0)
Absolute difference[c,d]	1,246	0.7 (−1.00,2.2)	0.7 (2.7)
Absolute difference, baseline adjusted[c,d]	1,246	0.5 (−0.9,2.0)	0.7 (2.5)
% Difference[c,e]	1,246	7.5 (−10.4,29.4)	12.5 (36.8)
Estrone, pg/mL			
Baseline	1,227	44.4 (35.8,55.2)	46.5 (15.6)
Posttreatment	1,227	49.2 (39.9,59.8)	51.8 (17.0)
Absolute difference[c,d]	1,227	4.7 (−3.3,13.2)	5.2 (15.2)
Absolute difference, baseline adjusted[c,d]	1,227	3.7 (−3.8,11.8)	5.2 (14.0)
% Difference[c,e]	1,227	11.2 (−7.2,35.5)	16.5 (35.3)
Estradiol, pg/mL			
Baseline	1,236	33.5 (26.7,40.5)	34.6 (11.7)
Posttreatment	1,236	35.6 (29.3,43.3)	37.4 (12.4)
Absolute difference[c,d]	1,236	2.6 (−3.4,8.1)	2.7 (11.5)
Absolute difference, baseline adjusted[c,d]	1,236	1.9 (−3.4,7.4)	2.7 (10.4)
% Difference[c,e]	1,236	7.5 (−8.8,28.1)	11.7 (32.4)
Free estradiol, pg/mL			
Baseline	1,236	0.9 (0.7,1.1)	0.9 (0.3)
Posttreatment	1,236	0.9 (0.8,1.2)	1.0 (0.3)
Absolute difference[c,d]	1,236	0.0 (−0.1,0.2)	0.1 (0.3)
Absolute difference, baseline adjusted[c,d]	1,236	0.0 (−0.1,0.2)	0.1 (0.3)
% Difference[c,e]	1,236	5.3 (−10.7,26.1)	9.6 (31.8)
Sex hormone binding globulin, nmol/L			
Baseline	1,247	36.0 (28.0,45.7)	38.5 (16.2)
Posttreatment	1,247	38.3 (29.4,48.7)	40.8 (16.3)
Absolute difference[c,d]	1,247	2.0 (−2.7,6.8)	2.3 (10.4)
Absolute difference, baseline adjusted[c,d]	1,247	1.3 (−3.1,6.4)	2.3 (9.9)
% Difference[c,e]	1,247	6.0 (−7.1,20.4)	8.9 (25.7)

[a]Interquartile range.
[b]5α-androstane-3α,17β-diol glucuronide.
[c]$P < 0.0001$.
[d]Posttreatment−baseline.
[e]100 × (posttreatment−baseline)/baseline.

response to finasteride treatment and thereby be associated with larger reductions in prostate cancer risk. This finding suggests that, at least among treatment-compliant men, the concentration of intraprostatic DHT was reduced below a threshold, beyond which its further reduction did not affect cancer risk.

Table 3. Spearman correlations among changes in 5α-androstane-3α,17β-diol glucuronide, sex steroid hormones, and sex hormone binding globulin, pre- and post-finasteride treatment, among treatment-compliant men in the Prostate Cancer Prevention Trial finasteride arm

	Δ3α-dG[a]	ΔT	ΔE_1	ΔE_2	Δ*Free T*	Δ*Free* E_2
ΔT[b]	0.04[c]					
	(0.11)					
ΔE_1[d]	0.08	0.23				
	(<0.01)	(<0.0001)				
ΔE_2[e]	0.03	0.36	0.54			
	(0.29)	(<0.0001)	(<0.0001)			
ΔFree T	0.06	0.92	0.22	0.33		
	(<0.04)	(<0.0001)	(<0.0001)	(<0.0001)		
Δ*Free* E_2	0.04	0.26	0.52	0.95	0.32	
	(0.20)	(<0.0001)	(<0.0001)	(<0.0001)	(<0.0001)	
ΔSHBG[f]	−0.03	0.37	0.06	0.17	0.05	−0.09
	(0.22)	(<0.0001)	(<0.03)	(<0.0001)	(0.05)	(<0.001)

[a]5α-androstane-3α,17β-diol glucuronide.
[b]Testosterone.
[c]Spearman correlation (*P* value), pairwise deletion of missing values, $N = 1{,}226$–$1{,}247$.
[d]Estrone.
[e]Estradiol.
[f]Sex hormone binding globulin.

One of the main controversies about the use of steroid 5-α reductase inhibitors for the primary prevention of prostate cancer is whether or not the observed increased risk of high-grade cancer after treatment in 2 clinical trials was causal or because of diagnostic bias (4, 18). Some investigators have hypothesized that reduced intraprostatic DHT suppresses growth of androgen-dependent cancer clones, which allows the preferential growth of androgen-independent, aggressive cancers (19). In contrast, several studies have suggested that high-grade cancers are more easily detected in finasteride-treated men, because their prostates are reduced in size and a larger proportion of the gland is sampled during biopsy (20). If low intraprostatic DHT causes increased risk of high-grade disease, we would expect that men with the lowest posttreatment 3α-dG concentration would have a greater risk of high-grade disease. In contrast, in this study there was a nonsignificant but large 81% (−9% to 260%, $P_{trend} = 0.04$) increased risk of Gleason Score 8 to 10 cancer among men in the highest quartile of 3α-dG. Thus, although this study does not address directly whether or not the association of 5-α reductase inhibitors with high-grade cancer is causal, it does not support the hypothesis that the reduction in DHT after treatment allows the preferential growth of high-grade disease.

The association of high pretreatment T with decreased risk is somewhat inconsistent with study findings overall and difficult to interpret. This association was limited to men in the highest quartile and not consistent with the lack of an association for free T. Furthermore, because neither posttreatment T nor free T were associated with risk, we judge this likely to be a chance finding. The association of high pretreatment E_1 with increased cancer risk is also difficult to interpret. Pretreatment, estrogen associations were limited to E_1 with low-grade disease, compared with significant associations of posttreatment E_1 and E_2 with both low- and high-grade disease. Also, the pretreatment estrogen findings given here on treatment-compliant men differed somewhat from those previously published from a larger sample of PCPT participants that did not exclude noncompliant men (21), in which both E_1 and E_2 were associated with increased risk of low-grade disease. Further research will be needed to clarify these findings.

The associations of high posttreatment estrogens with increased cancer risk are noteworthy because trends were significant and the ORs were similar for low- and high-grade disease. The mechanism underlying these associations is unclear. It is possible that estrogens influence prostate cancer risk when intraprostatic DHT is pharmacologically reduced, or the association could be indirect; for example, genetic and/or environmental characteristics that increase estrogen levels (e.g., through increased aromatase activity) may in some way modify the response to finasteride. Unfortunately, this nested case–control study does not allow us to directly measure the effect of finasteride in men with low and high posttreatment estrogen concentrations, because controls were matched on treatment arm. Overall, these findings on posttreatment estrogens support a new area of research to investigate the effects of estrogens on prostate tissues in a low androgen environment.

Table 4. Associations of pre-finasteride-treatment 5α-androstane-3α,17β-diol glucuronide, sex steroid hormones, and sex hormone binding globulin with prostate cancer risk

		Overall cancer		Gleason Score 2–6		Gleason Score 7–10		Gleason Score 8–10	
	Range of values	*N* case/ control	OR (95% CI)[2]	*N* case/ control	OR (95% CI)[2]	*N* case/ control	OR (95% CI)[2]	*N* case/ control	OR (95% CI)[b]
3α-dG[a], ng/mL	<(3.9)	156/169	Ref.	96/169	Ref.	58/169	Ref.	21/169	Ref.
	(3.9) to <(5.7)	144/166	0.93 (0.67–1.28)	84/166	0.87 (0.60–1.27)	56/166	0.98 (0.63–1.52)	19/166	0.91 (0.47–1.78)
	(5.7) to <(8.0)	142/167	0.87 (0.63–1.20)	73/167	0.72 (0.49–1.06)	57/167	0.96 (0.62–1.48)	19/167	0.90 (0.46–1.75)
	≥(8.0)	130/172	0.80 (0.58–1.11)	74/172	0.73 (0.50–1.07)	52/172	0.88 (0.57–1.37)	17/172	0.82 (0.41–1.63)
	P_{slope}		0.17		0.07		0.58		0.58
Testosterone, ng/dL	<(289)	167/170	Ref.	90/170	Ref.	71/170	Ref.	27/170	Ref.
	(289) to <(363)	128/165	0.78 (0.56–1.09)	71/165	0.78 (0.52–1.15)	53/165	0.79 (0.51–1.22)	13/165	0.50 (0.25–1.02)
	(363) to <(457)	159/166	0.92 (0.66–1.29)	95/166	0.97 (0.66–1.42)	58/166	0.84 (0.54–1.30)	21/166	0.77 (0.40–1.47)
	≥ (457)	118/173	0.64 (0.43–0.93)	71/173	0.63 (0.40–1.00)	41/173	0.57 (0.34–0.97)	15/173	0.50 (0.23–1.12)
	P_{slope}		0.07		0.15		0.07		0.18
Free testosterone, ng/dL	<(6.8)	147/170	Ref.	82/170	Ref.	61/170	Ref.	23/170	Ref.
	(6.8) to <(8.5)	167/166	1.16 (0.84–1.60)	92/166	1.12 (0.77–1.63)	69/166	1.19 (0.79–1.81)	21/166	1.00 (0.53–1.90)
	(8.5) to <(10.2)	139/166	0.99 (0.72–1.38)	86/166	1.05 (0.71–1.54)	48/166	0.88 (0.56–1.38)	18/166	0.95 (0.49–1.86)
	≥ (10.2)	119/172	0.83 (0.59–1.17)	67/172	0.77 (0.52–1.16)	45/172	0.83 (0.53–1.32)	14/172	0.78 (0.37–1.61)
	P_{slope}		0.21		0.22		0.25		0.49
Estrone, pg/mL	<(35.8)	137/166	Ref.	72/166	Ref.	60/166	Ref.	17/166	Ref.
	(35.8) to <(44.0)	128/172	0.93 (0.67–1.29)	67/172	0.93 (0.62–1.39)	55/172	0.91 (0.59–1.39)	19/172	1.06 (0.53–2.13)
	(44.0) to <(54.5)	138/163	1.12 (0.81–1.57)	85/163	1.32 (0.89–1.95)	51/163	0.94 (0.61–1.46)	21/163	1.33 (0.67–2.63)
	≥ (54.5)	161/167	1.38 (0.99–1.93)	99/167	1.68 (1.14–2.48)	54/167	1.00 (0.64–1.55)	19/167	1.12 (0.55–2.28)
	P_{slope}		0.03		0.00		0.98		0.62
Total estradiol, pg/mL	<(26.7)	138/164	Ref.	69/164	Ref.	66/164	Ref.	19/164	Ref.
	(26.7) to <(33.3)	142/168	1.02 (0.73–1.41)	85/168	1.24 (0.83–1.84)	49/168	0.72 (0.46–1.11)	21/168	1.01 (0.52–1.98)
	(33.3) to <(40.4)	146/166	1.13 (0.81–1.57)	92/166	1.44 (0.97–2.14)	46/166	0.73 (0.46–1.14)	16/166	0.81 (0.40–1.66)
	≥ (40.4)	145/172	1.11 (0.79–1.55)	81/172	1.25 (0.83–1.88)	61/172	0.95 (0.62–1.46)	20/172	0.96 (0.48–1.91)
	P_{slope}		0.45		0.22		0.82		0.76
Free estradiol, pg/mL	<(0.73)	136/169	Ref.	68/169	Ref.	63/169	Ref.	19/169	Ref.
	(0.73) to <(0.90)	123/165	0.92 (0.66–1.29)	71/165	1.09 (0.73–1.63)	49/165	0.78 (0.50–1.20)	15/165	0.77 (0.38–1.58)
	(.90) to <(1.10)	137/166	1.17 (0.83–1.63)	92/166	1.65 (1.11–2.45)	40/166	0.69 (0.43–1.09)	21/166	1.15 (0.59–2.25)
	≥ (1.10)	132/170	1.12 (0.80–1.57)	71/170	1.27 (0.84–1.91)	55/170	0.94 (0.61–1.46)	13/170	0.71 (0.33–1.51)
	P_{slope}		0.31		0.09		0.65		0.64

[a]5α-androstane-3α,17β-diol glucuronide.

[b]Adjusted for age, race, family history of prostate cancer, SHBG, body mass index, and serum cholesterol.

Table 5. Associations of post-finasteride-treatment 5α-androstane-3α,17β-diol glucuronide, sex steroid hormones, and sex hormone binding globulin with prostate cancer risk

		Overall cancer		Gleason Score 2–6		Gleason Score 7–10		Gleason Score 8–10	
	Range of values	*N* case/control	OR (95% CI)[b]	*N* case/control	OR (95% CI)[b]	*N* case/control	OR (95% CI)[b]	*N* case/control	OR (95% CI)[b]
3α-dG[a], ng/mL	<(1.0)	135/164	Ref.	82/164	Ref.	49/164	Ref.	15/164	Ref.
	(1.0) to <(1.4)	127/171	0.89 (0.63–1.24)	78/171	0.88 (0.60–1.30)	45/171	0.88 (0.55–1.40)	11/171	0.70 (0.31–1.58)
	(1.4) to <(2.1)	137/170	0.99 (0.71–1.38)	78/170	0.90 (0.61–1.33)	54/170	1.11 (0.71–1.75)	17/170	1.14 (0.54–2.40)
	≥ (2.1)	130/169	0.96 (0.68–1.34)	64/169	0.74 (0.49–1.11)	60/169	1.28 (0.82–2.00)	25/169	1.81 (0.91–3.60)
	P_{slope}		0.96		0.18		0.17		0.04
Testosterone, ng/dL	<(318)	137/168	Ref.	76/168	Ref.	55/168	Ref.	19/168	Ref.
	(318) to <(398)	132/169	0.94 (0.67–1.32)	71/169	0.87 (0.58–1.30)	58/169	1.10 (0.71–1.72)	16/169	0.94 (0.46–1.94)
	(398) to <(494)	120/168	0.83 (0.58–1.19)	71/168	0.82 (0.54–1.25)	45/168	0.86 (0.53–1.40)	15/168	0.92 (0.43–1.98)
	≥ (494)	139/169	1.11 (0.75–1.64)	83/169	1.06 (0.66–1.68)	50/169	1.14 (0.67–1.94)	18/169	1.38 (0.60–3.15)
	P_{slope}		0.84		0.92		0.92		0.52
Free testosterone, ng/dL	<(7.3)	138/168	Ref.	72/168	Ref.	62/168	Ref.	24/168	Ref.
	(7.3) to <(9.1)	138/170	0.95 (0.68–1.33)	77/170	0.97 (0.65–1.44)	54/170	0.89 (0.58–1.38)	12/170	0.55 (0.26–1.16)
	(9.1) to <(11.)	146/169	1.09 (0.78–1.51)	94/169	1.26 (0.85–1.86)	50/169	0.90 (0.58–1.40)	16/169	0.83 (0.41–1.65)
	≥ (11.2)	106/167	0.83 (0.59–1.19)	58/167	0.81 (0.53–1.24)	42/167	0.81 (0.51–1.30)	16/167	0.91 (0.45–1.83)
	P_{slope}		0.51		0.69		0.40		0.92
Estrone, pg/mL	<(39.4)	109/167	Ref.	56/167	Ref.	47/167	Ref.	11/167	Ref.
	(39.4) to <(49.0)	139/167	1.27 (0.90–1.78)	79/167	1.37 (0.91–2.07)	57/167	1.24 (0.79–1.94)	23/167	2.19 (1.03–4.67)
	(49.0) to <(59.2)	128/171	1.19 (0.84–1.68)	78/171	1.41 (0.93–2.13)	47/171	1.01 (0.64–1.61)	14/171	1.28 (0.56–2.93)
	≥ (59.2)	145/162	1.54 (1.09–2.17)	84/162	1.74 (1.15–2.63)	55/162	1.35 (0.85–2.13)	20/162	2.11 (0.97–4.59)
	P_{slope}		0.03		0.01		0.35		0.21
Total estradiol, pg/mL	<(29.1)	121/169	Ref.	72/169	Ref.	44/169	Ref.	14/169	Ref.
	(29.1) to <(35.2)	117/168	1.04 (0.74–1.47)	58/168	0.86 (0.57–1.31)	53/168	1.31 (0.82–2.08)	17/168	1.33 (0.63–2.81)
	(35.2) to <(42.4)	122/162	1.15 (0.81–1.62)	72/162	1.15 (0.77–1.72)	46/162	1.18 (0.73–1.90)	16/162	1.26 (0.59–2.70)
	≥ (42.4)	167/171	1.49 (1.07–2.07)	98/171	1.45 (0.98–2.13)	65/171	1.62 (1.03–2.56)	21/171	1.64 (0.79–3.41)
	P_{slope}		0.02		0.03		0.06		0.22
Free estradiol, pg/mL	<(0.77)	124/170	Ref.	69/170	Ref.	49/170	Ref.	14/170	Ref.
	(0.77) to <(0.92)	106/167	0.89 (0.63–1.26)	61/167	0.93 (0.62–1.41)	41/167	0.86 (0.53–1.38)	14/167	1.02 (0.47–2.23)
	(0.92) to <(1.14)	145/162	1.33 (0.95–1.86)	80/162	1.35 (0.91–2.02)	60/162	1.35 (0.87–2.11)	21/162	1.65 (0.80–3.38)
	≥ (1.14)	152/171	1.34 (0.96–1.87)	90/171	1.44 (0.97–2.14)	58/171	1.28 (0.81–2.00)	19/171	1.47 (0.70–3.07)
	P_{slope}		0.02		0.02		0.11		0.18

[a]5α-androstane-3α,17β-diol glucuronide.

[b]Adjusted for age, race, family history of prostate cancer, SHBG, body mass index, and serum cholesterol.

Strengths of this study include the use of highly sensitive and specific assays for serum steroids, the large sample size, the use of prostate biopsy to verify absence or presence of cancer, and the exclusion of men not compliant with finasteride treatment. One important weakness of this study is our assumption that the reduction in 3α-dG after finasteride treatment accurately reflects the reduction in intraprostatic DHT. It is also uncertain whether, when measured 3 years after the initiation of finasteride treatment, the associations of steroids with cancer risk are the same as those that would be observed if steroids were measured at other times after treatment initiation. The reduction in serum DHT after finasteride treatment is roughly 90% 1 year posttreatment and does not change thereafter; however, PSA falls by approximately 45% at 1 year to a maximum of 60% at 3 years posttreatment (22). Thus, it is not entirely clear whether the posttreatment steroid concentrations used in this study precisely reflect changes that occurred soon after treatment initiation. We also relied upon self-report and/or a single blood finasteride concentration to determine treatment adherence. Nevertheless, when nonadherent men were included in the analysis there was a significant positive association of posttreatment 3α-dG with cancer risk, suggesting that this approach was valid. Additional weaknesses include the small number of men with high-grade disease, and the inability to directly calculate the conditional effects of finasteride on posttreatment estrogen concentrations.

In conclusion, we found no support for the hypothesis that lower 3α-dG after finasteride treatment, reflecting a larger reduction in intraprostatic DHT, is associated with lower risks of total or high-grade prostate cancer. There was some evidence that high pretreatment T and E_1 concentrations predict reduced and increased prostate cancer risk, respectively, but we consider this weak. There was stronger and more consistent evidence that high posttreatment concentrations of E_1, E_2, and free E_2 are associated with increased low- and high-grade prostate cancer risk. It is possible that estrogens play a significant role in prostate cancer risk only when intraprostatic DHT is lowered pharmacologically. Further research is needed to evaluate whether low posttreatment serum estrogens could be used to identify men most likely to benefit from finasteride for prostate cancer prevention.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: A. R. Kristal, C. M. Tangen, P. J. Goodman, M. L. Neuhouser, I. M. Thompson, Jr., J. Reichardt, A. M. Hoque, W. D. Figg, A. W. Hsing

Development of methodology: A. R. Kristal, P. J. Goodman, A. W. Hsing

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A. R. Kristal, P. J. Goodman, F. Z. Stanczyk, S. K. Patel, I. M. Thompson, Jr., A. van Bokhoven, A. W. Hsing

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A. R. Kristal, C. A. Till, C. M. Tangen, P. J. Goodman, M. L. Neuhouser, F. Z. Stanczyk, L. W. Chu, A. M. Hoque, S. M. Lippman, A. W. Hsing

Writing, review, and/or revision of the manuscript: A. R. Kristal, C. M. Tangen, P. J. Goodman, M. L. Neuhouser, F. Z. Stanczyk, L. W. Chu, I. M. Thompson, Jr., J. Reichardt, A. M. Hoque, E. A. Platz, W. D. Figg, A. van Bokhoven, S. M. Lippman, A. W. Hsing

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A. R. Kristal, P. J. Goodman, L. W. Chu, A. van Bokhoven, A. W. Hsing

Study supervision: A. R. Kristal, P. J. Goodman, I. M. Thompson, Jr., A. W. Hsing

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References

1. Endogenous Hormones and Prostate Cancer Collaborative Group, Roddam A, Allen N, Appleby P, Key T. Endogenous sex hormones and prostate cancer: a collaborative analysis of 18 prospective studies. J Natl Cancer Inst 2008;100:170.
2. Rittmaster RS, Hahn RG, Ray P, Shannon JB, Wurzel R. Effect of dutasteride on intraprostatic androgen levels in men with benign prostatic hyperplasia or prostate cancer. Urology 2008;72:808–12.
3. Roehrborn C, Lee M, Meehan A, Waldstreicher J. Effects of finasteride on serum testosterone and body mass index in men with benign prostatic hyperplasia. Urology 2003;62:894–9.
4. Thompson IM, Goodman PJ, Tangen CM, Lucia MS, Miller GJ, Ford LG, et al. The influence of finasteride on the development of prostate cancer. N Engl J Med 2003;349:215–24.
5. Thompson IM, Tangen CM, Goodman PJ, Lucia MS, Parnes HL, Lippman SM, et al. Finasteride improves the sensitivity of digital rectal examination for prostate cancer detection. J Urol 2007;177:1749–52.
6. Thompson IM, Chi C, Ankerst DP, Goodman PJ, Tangen CM, Lippman SM, et al. Effect of finasteride on the sensitivity of PSA for detecting prostate cancer. J Natl Cancer Inst 2006;98:1128–33.
7. Redman MW, Tangen CM, Goodman PJ, Lucia MS, Coltman CA, Thompson IM. Finasteride does not increase the risk of high-grade prostate cancer: a bias-adjusted modeling approach. Cancer Prev Res 2008;1:174–81.
8. Thomas LN, Douglas RC, Lazier CB, Gupta R, Norman RW, Murphy PR, et al. Levels of 5α-reductase type 1 and type 2 are increased in localized high grade compared to low grade prostate cancer. J Urol 2008;179:147–51.
9. Horton R. Testicular steroid transport, metabolism and effects. Principles and practice of endocrinology and metabolism. Philadelphia: Lippincott; 1990.

10. Thigpen A, Silver R, Guileyardo J, Casey ML, McConnell J, Russell D. Tissue distribution and ontogeny of steroid 5 alpha-reductase isozyme expression. J Clin Invest 1993;92:903–10.
11. Goodman PJ, Tangen CM, Kristal AR, Thompson IM, Platz EA, Figg WD, et al. Transition of a clinical trial into translational research: The Prostate Cancer Prevention Trial experience. Cancer Prev Res 2010; 3:1523–33.
12. Narang R, Rao JN, Savjani G, Peterson J, Gentzchein E, Stanczyk FZ. Radioimmunoassay kit for the quantitative measurement of androstanediol glucuronide in unextracted serum. Proceedings of the 17th National Meeting of the Clinical Ligand Assay Society; 1991.
13. Goebelsmann U, Bernstein GS, Gale JA, Kletzky OA, Nakamura RM, Coulson AH, et al.. Serum gonadotropin, testosterone, estradiol and estrone levels prior to and following bilateral vasectomy. In: Lepow I. H., Crozier R., editors. Vasectomy: immunologic and pathophysiologic effects in animals and man. New York: Academic Press; 1979. pp. 165–75.
14. Rinaldi S, Geay A, Dechaud H, Biessy C, Zeleniuch-Jacquotte A, Akhmedkhanov A, et al. Validity of free testosterone and free estradiol determinations in serum samples from postmenopausal women by theoretical calculations. Cancer Epidemiol Biomarkers Prev 2002;11: 1065–71.
15. Södergard R, Bäckström T, Shanbhag V, Carstensen H. Calculation of free and bound fractions of testosterone and estradiol-17β to human plasma proteins at body temperature. J Steroid Biochem 1982;16: 801–10.
16. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for estimation of free testosterone in serum. J Clin Endocrinol Metab 1999;84:3666–72.
17. Tefilli MV, Gheiler EL, Tiguert R, Sakr W, Grignon DJ, Banerjee M, et al. Should Gleason score 7 prostate cancer be considered a unique grade category? Urology 1999;53:372–7.
18. Andriole G, Bostwick D, Brawley O, Gomella L, Marberger M, Montorsi F, et al. Effect of dutasteride on the risk of prostate cancer. N Engl J Med 2010;362:1192–202.
19. Scardino PT. The prevention of prostate cancer—the dilemma continues. N Engl J Med 2003;349:297–9.
20. Cohen Y, Liu K, Heyden N, Carides A, Anderson K, Daifotis A, et al. Detection bias due to the effect of finasteride on prostate volume: a modeling approach for analysis of the Prostate Cancer Prevention Trial. J Natl Cancer Inst 2007;99:1366–74.
21. Yao S, Till C, Kristal AR, Goodman PJ, Hsing AW, Tangen CM, et al. Serum estrogen levels and prostate cancer risk in the prostate cancer prevention trial: a nested case–control study. Cancer Causes Control 2011;22:1121–31.
22. Vaughan D, Imperato-McGinley J, McConnell J, Matsumoto A, Bracken B, Roy J, et al. Long-term (7 to 8-year) experience with finasteride in men with benign prostatic hyperplasia. Urology 2002;60:1040–4.