Endogenous Sex Hormones and Prostate Cancer Risk: A Case-Control Study Nested within the Carotene and Retinol Efficacy Trial

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Abstract

To examine whether endogenous androgens influence the occurrence of prostate cancer, we conducted a nested case-control study among participants enrolled in the Carotene and Retinol Efficacy Trial. We analyzed serum samples of 300 cases diagnosed between 1987 and 1998, and 300 matched controls. Higher concentrations of testosterone (T) were not associated with increased prostate cancer risk. Relative to men with levels in the lowest fourth of the distribution, men in the upper fourth of total T had a risk of 0.82 [95% confidence interval (CI), 0.52–1.29]. The corresponding relative risks for free T (0.72; 95% CI, 0.45–1.14), percentage of free T (0.74; 95% CI, 0.46–1.19), and total T:sex hormone binding globulin ratio (0.52; 95% CI, 0.32–0.83) similarly were not elevated. Higher concentrations of androstenedione, dehydroepiandrosterone sulfate, and 3α-androstanediol glucuronide were weakly associated with risk. Relative risks associated with being in the highest fourth for androstenedione, dehydroepiandrosterone sulfate, and 3α-androstanediol glucuronide were 1.20 (95% CI, 0.76–1.89), 1.38 (95% CI, 0.86–2.21), and 1.27 (95% CI, 0.80–2.00), respectively. Men in the upper fourth of total estradiol (E2), free E2 and percentage of free E2 had relative risks of 0.71 (95% CI, 0.42–1.13), 0.52 (95% CI, 0.33–0.82), and 0.65 (95% CI, 0.40–1.05), respectively. The inverse association between E2 and prostate cancer risk was largely restricted to men with blood collection within 3 years of diagnosis. Our results add to the evidence that serum testosterone is unrelated to prostate cancer incidence. The suggestions that intraprostatic androgen activity may increase risk and that serum estrogens may decrease risk, warrant additional study.

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Introduction

It seems plausible that endogenous androgens play a role in the pathogenesis of prostate cancer, because: (*a*) growth and maintenance of prostatic tissue require androgens; (*b*) large doses of androgens can induce prostate cancer in rodents (1); (*c*) prostate cancer incidence is very low among castrated men; (*d*) androgens stimulate the *in vitro* proliferation of human prostate cancer cells (2); and (*e*) surgical or medical castration of men with prostate cancer often causes tumor regression (3). There have been 10 prospective studies (4–14) using stored plasma or serum to evaluate the association of endogenous sex hormones, sex hormone binding globulin (SHBG), and the androgen metabolite, 3α-androstanediol glucuronide (3α-diol G), and the risk of prostate cancer. Whereas some of these studies have reported a small positive association between testosterone (T) levels and risk of prostate cancer (5, 6, 12), many of these studies have reported no association (4, 8–10) or even an inverse relation (11). With regarding to circulating estradiol (E2), two studies showed a reduction in risk with increasing levels (6,7), whereas three others did not (4, 5, 11). Results from a combined analysis of these studies showed that among the various endogenous hormones analyzed, only 3α-diol G exhibited a difference between prostate cancer cases and controls (15). However, with the exception of the study of Gann *et al.* (6), the sample size of the prior studies have been small, and it remains uncertain whether serum androgen concentrations (beyond some minimum threshold level) have an influence on the etiology of prostate cancer. To additionally investigate the potential association of serum hormones and prostate cancer risk, we conducted a case-control study nested within the Carotene Retinol Efficacy Trial.

Materials and Methods

Study Population. A description of the study population has been published previously (16). The β-Carotene and Retinol Efficacy Trial (CARET) is a double-blind, randomized, placebo-controlled trial to test the effect of β-carotene (30 mg/day) and retinyl palmitate (25,000 IU/day) on lung cancer incidence in male ($n = 7,965$) and female ($n = 6,289$) heavy smokers and in male asbestos workers ($n = 4,060$; Ref. 17). The heavy smoker population included persons 50–69 years of age who were current or former (quit <6 years earlier) cigarette smokers with at least 20 pack-years (product of the number of packs of 20 cigarettes/day and the number of years of smoking history) of cigarette smoking. The asbestos-exposed population included men 45–69 years of age who were current or former smokers with either occupational exposure to asbestos at least 15 years before randomization or a chest X-ray positive for asbestos-related lung disease. A total of 18,314 participants were enrolled in CARET at six study centers from 1985 to 1994, and followed routinely for compliance, side effects, and cancer (as frequently as every 3 months or as infrequently as every year). When a CARET participant reported a new cancer

diagnosis at a follow-up visit or telephone contact, the diagnosis was confirmed by requesting the relevant medical records. Those records were reviewed by the members of the Endpoint Review Committee for adjudication and for a final diagnosis. All of the cases of prostate cancer included in this analysis had pathological confirmation of the diagnosis. However, in many cases the only record that was available was a pathology report from a needle biopsy. Race of men was self-identified.

A total of 300 participants with prostate cancer reported during 1987–1998 (and confirmed by August 1999) was selected for the present study. Fifty-three other participants with prostate cancer potentially were eligible, but limited availability of blood samples on these men precluded their inclusion in this study. None of the cases were known to have had lung cancer, whereas 13 of them had been diagnosed with another malignancy before the diagnosis of prostate cancer. A medical oncologist reviewed the medical records and, when possible, determined the Gleason score and both clinical and pathological stage of the tumor, using the American Joint Committee on Cancer Staging system of 1992. Stage could not be determined in the 126 cases who had only a needle biopsy. A blinded rereview of a number of cases found 100% agreement in pathological staging from surgical specimens. For the present analysis, "aggressive" cancers were defined as stage C or D (extraprostatic) at the time of diagnosis, or stage A or B but with a Gleason score ≥ 7 (or with "poorly differentiated" tumors). Approximately 46% of the cases whose stage/grade could be assessed met our criteria for "aggressive."

Controls were chosen from male CARET participants who were alive and free of any cancer, except nonmelanoma skin cancer, and were matched to the cases on race, age at enrollment (within 5 years), time of day of blood draw (within 2 h), months from enrollment in CARET to blood draw, enrollment study center, and year of randomization to the CARET intervention. Three cases had no suitable control with the same year of randomization, and for these we selected a control that was randomized within a year of the case (*i.e.,* $+/-$ 365 days). In all but 3 instances, controls were matched to cases on 5-year intervals of age at randomization (45–49, 50–54, and so forth). For these 3 cases a control in the same 5-year age group was not available, and so controls who were within 5 years of age of the case were selected instead.

For the 300 prostate cancer cases, we selected serum samples drawn between 6 months and 8.5 years before diagnosis, with a mean of 3 years. For 5 cases, we could not find a control with serum available from the same visit, and for these, we selected a control sample from the draw nearest to the one used for the case. The blood drawn within the 2-h criterion could not be met for 9 cases. For these we attempted to match on AM/PM, and were successful for 5 cases (ranging between 3 and 5 h apart). For the remaining 4 cases, the blood draws were between 3 and 8 h apart. Controls were followed-up in CARET through the date of diagnosis of the paired case, at a minimum. All of the participants provided informed consent, and the Institutional Review Offices of all of the participating centers approved the study.

Hormone Measurements. Paired cases and controls were assayed in the same run to avoid bias due to run-to-run variation. The technologist performing the assays was blinded as to the case-control status. For purposes of quality control, in-house pooled serum or charcoal-stripped serum, supplemented with pure steroids, was assayed along with the samples at the beginning and end of each run. Because protein is generally more labile than steroid hormones (levels of the latter have been reported to remain constant in sera frozen for up to 15 years; Ref. 4), our laboratory assayed SHBG and albumin first (albumin concentration was used in the calculation of free T and E2).

We measured SHBG with a competitive radioimmunoassay using the DSL (Diagnostic Systems Laboratories Inc., Webster, TX) Double Antibody kits. The plasma sample was incubated with I^{125}-labeled SHBG and goat anti-SHBG antiserum. The labeled SHBG competes with the endogenous SHBG for a fixed number of antibody binding sites. After incubation, the bound SHBG was separated from the unbound by precipitation with donkey antigoat γ globulin in the presence of polyethylene glycol. I^{125}-labeled SHBG bound to the tube was counted in a gamma counter. The radioactivity is inversely related to the amount of endogenous SHBG in the plasma. Concentration was determined by comparison with a standard curve. The minimum detectable concentration was 5 nmol/liter. The intra- and interassay percentage coefficient of variations were 2–5% and 3–7%, respectively. Linearity was verified between 0 and 80 nmol/liter. Albumin concentration was determined on a Cobas Mira Plus Centrifugal Chemistry Analyzer using Roche Albumin Reagent (Indianapolis, IN).

Androstenedione (A), T, and E2 were measured by RIA after organic solvent extraction and Celite partition chromatography. These purification steps were performed to increase assay sensitivities for low levels of steroid hormones (men have low levels of E2), and to increase specificity by discriminating the parent androgens and estrogens from their metabolites. Before performance of these assays in the laboratory of C. C., successful correlation studies were conducted on split samples between laboratories of C. C. and F. Z. S. The assay procedures involved aliquoting 0.8 ml of plasma; adding 1000 dpm of [^{3}H]A, [^{3}H]T, and [^{3}H]E_2 (NEN Research, Boston) as internal standards to assess procedural loss; incubating for 30 min at 37°C to allow equilibration of the internal standards with the steroid-binding proteins present; extracting the unbound steroids with hexane:ethyl acetate (3:2); removing and drying down the organic layer under N_2 at 37°C; applying the extract reconstituted in isooctane on a Celite partition column to separate A, T, E2, and interfering steroids by differential elution [100% isooctane to elute A, 40% (v/v) toluene in isooctane to elute T, and 40% (v/v) ethyl acetate in isooctane to elute E_2]; reconstituting residues in assay buffer; and serially diluting authentic A, T, and E_2 (Sigma, St. Louis, MO) for construction of a standard curve. The standards and sample eluates were assayed by RIA using a commercial kit from DSL for A, tracer and precipitating reagent from DSL for T, and a Pantex kit (Santa Monica, CA) for E_2. Fitzgerald (Concord, MA) Rabbit anti-Testosterone 19 Antibody was used for T to increase specificity. The appropriate factor was used to correct results for procedural loss. The intra- and interassay percentage coefficient of variations were 5–9% for A, 1–12% for T, and 5–13% for E_2. The lower limit of detection was 0.07 nmol/liter for A, 0.17 nmol/liter for T, and 9.25 pmol/liter for E_2. There was insufficient quantity of serum from 1 case for the analyses of A, T, and E2, and insufficient quantity of serum from a second participant with prostate cancer to repeat T and E2 analyses when the initial assays did not yield a satisfactory recovery ($>$70%) of internal standard after column chromatography.

We calculated free T and percentage of free T using concentrations of SHBG, T, and albumin; and calculated free E2 and percentage of free E2 using concentrations of total E2, SHBG, and albumin according to the method of Sodergard *et al.* (18).

We measured dehydroepiandrosterone sulfate (DHEAS) using DSL DHEAS Double Antibody kits. The minimum de-

tectable level was 68 nmol/liter. The intra- and interassay percentage coefficient of variations were 0.8–1.8% and 1.8–1.9%, respectively. Linearity was verified between 542 and 4607 nmol/liter.

We measured 3α-diol G with DSL 3α-Diol Glucuronide Double Antibody kits. The minimum detectable level was 0.3 nmol/liter. The intra- and interassay percentage coefficient of variations were 1.5–2.8% and 3.4–4.8%, respectively. Linearity was verified between 1 and 170 nmol/liter.

Statistical Analyses. To compare hormone levels in cases *versus* controls, we conducted two-sample *t* tests on the log-transformed hormone concentrations. Bivariate Pearson and Spearman correlation coefficients for A, total T, SHBG, 3 α Diol, E2, and DHEAS were estimated using the control data. To assess whether there was a trend for higher or lower hormone concentrations to be associated with prostate cancer risk, we first computed the quartiles for hormone concentrations among controls. Then, for each hormone, we estimated the odds ratio associated with the second through fourth quartiles, *i.e.,* the odds of a prostate cancer diagnosis given hormone concentration in that quartile relative to the odds of a prostate cancer diagnosis given hormone concentration in the lowest quartile. The odds ratios were calculated via logistic regression analysis, adjusted for age and race, but not for the other hormone concentrations. The variables representing hormone quartiles were entered as dummy variables in the logistic regressions. To test for a trend in risk across hormone quartiles, we entered the quartile of hormone concentration as an ordinal variable, taking values from 1 through 4, and tested whether the coefficient of this variable in the regression equation was significantly different from zero. In what follows we refer to the exponential value of this coefficient as the "interquartile relative risk" because it may be interpreted as the relative risk associated with hormone concentrations in one quartile as compared with the previous (next lower) quartile. We conducted similar logistic regression analyses adjusting for concentrations of the other hormones in Table 5. These analyses were performed for the entire group of cases and controls, as well as for subgroups of cases defined by disease aggressiveness and date of diagnosis. We also conducted analyses stratified by age and smoking status (former *versus* current smokers). All of the analyses were performed using the SAS system, version 8e.

Table 1 Selected baseline characteristics of CARET[a] participants who did (cases) and did not (controls) go on to be diagnosed with prostate cancer during follow-up

Characteristics	Cases (n = 300)			Controls (n = 300)		
	Mean (SD)	No.	%	Mean (SD)	No.	%
Age (yrs)						
45–49		6	2.0		6	2.0
50–59		112	37.3		111	37.0
60–69		170	56.7		172	57.3
70–79		12	4.0		11	3.7
Race						
White		281	93.7		281	93.7
Non-white		8	2.7		8	2.7
Other		11	3.7		11	3.7
Height (cm)	174.8 (7.3)			174.9 (7.6)		
Weight (kg)	86.0 (13.7)			84.9 (14.9)		
Body mass index (Kg/m^2)	28.2 (4.1)			27.7 (4.5)		
Married		247	82.3		256	85.9
>High school education		122	55.2		121	55.3
Smoking history at baseline						
Former smokers		149	49.7		134	44.7
Current smokers		142	47.3		150	50.0
Intervention arm						
Placebo		149	49.7		142	47.3
β-Carotene/retinyl palmitate		151	50.3		158	52.7
Time of blood draw						
AM		164	54.7		162	54.0
PM		136	45.3		138	46.0
Stage (pathologic when available, otherwise clinical)						
Localized (stage 0–2)		148	49.3			
Regional (stage 3)		48	16.0			
Distant (stage 4)		20	6.7			
Histologic grade						
Well differentiated (Gleason 2–4)		30	10			
Moderately differentiated (Gleason 5–6)		135	45			
Poorly differentiated (Gleason 7–10)		111	37			
Could not be assessed		24	8			
Years from blood draw to diagnosis						
<1		34	11			
1–<2		74	25			
2–<3		60	20			
3–<4		47	16			
4–<5		34	11			
5+		51	17			

[a] CARET, β Carotene and Retinol Efficacy Trial.

Results

Approximately 94% of cases and controls were Caucasians. At the time of enrollment in CARET, cases and controls were similar in height, weight, body mass index, marital status, education level, and smoking history (Table 1). The respective median and mean age at first visit was 62 and 61.2 for cases and 61 and 60.8 for controls. The majority of cases had localized and moderately-to-poorly differentiated tumors.

Mean and median serum concentrations of hormones, 3α-diol G, SHBG, and T:SHBG ratio are shown in Table 2. Total T levels were strongly correlated with those of SHBG (r = 0.692), and to a lesser extent with levels of E2 (r = 0.305) and (r = 0.320) (Table 3). Androstenedione levels were correlated with those of DHEAS (r = 0.408).

Table 4 shows the age- and race-adjusted odds ratios and 95% confidence intervals (CIs) for prostate cancer in relation to hormone concentrations by control quartiles of hormone concentrations. We did not observe a positive association between total T, free T, percentage of free T, or the ratio of T:SHBG with prostate cancer risk. If anything, risk appeared to fall with increasing concentration. Risks among men in the upper fourth of the distribution, relative to those among men in the lowest fourth, were 0.82 for total T (95% CI, 0.52–1.29), 0.72 for free T (95% CI, 0.45–1.14), 0.74 for percentage of free T (95% CI, 0.46–1.19), and 0.52 for T:SHBG (95% CI, 0.32–0.83). Serum levels of 3α-diol G tended to be somewhat higher in cases than controls, and the risk of prostate cancer among men in the upper fourth of distribution of 3α-diol G levels was 1.27 times that of men whose levels were in the lowest fourth (95% CI, 0.80–2.06). There was only a suggestion that increased concentrations of A and DHEAS were associated with increased risk. We observed a reduction in risk associated with increasing serum concentrations of total E2, and an even more pronounced reduction for levels of free E2. For example, the risk of prostate cancer of men in the upper fourth of the distribution of free E2 was 52% that of men on the lowest fourth (95% CI, 0.33–0.82).

Possible variation in the size of the association between prostate cancer and endogenous hormone levels was assessed by comparing the size of the interquartile OR within categories of tumor aggressiveness, recency of blood draw, smoking status, and age, with simultaneous adjustment for several other hormones (Table 5). In no subgroup was there an appreciable association between serum levels of total or free T and risk of prostate cancer. The associations between prostate cancer and serum levels of 3α-diol G (positive) and E2 (negative) virtually disappeared when the analysis was restricted to cases (and their controls) in whom ≥3 years had gone by between blood draw and date of diagnosis.

Table 2 Concentrations of serum analytes in cases and controls

Endogenous hormones	Cases[a]		Controls[b]		p^c
	Mean	SD	Mean	SD	
A (nmol/L)	2.87	1.10	2.80	1.04	0.45
Total T (nmol/L)[d]	14.5	5.0	15.1	5.64	0.21
Free T (nmol/L)	0.33	0.10	0.35	0.11	0.03
% free T	2.36	0.31	2.40	0.33	0.20
SHBG (nmol/L)	30	13	30	13	0.72
Total T/SHBG	0.52	0.17	0.54	0.16	0.04
3α-diol G (nmol/L)	15.08	8.35	13.80	7.84	0.06
Total E2 (nmol/L)	0.19	0.04	0.20	0.04	0.05
Free E2 (pmol/L)	5.03	1.02	5.24	1.10	0.02
% Free E2	2.64	0.26	2.67	0.30	0.25
DHEAS (nmol/L)	2545	1634	2339	1447	0.16

[a] All analyte values were based on 300 cases.
[b] Analyte values for A, total T, free T, % free T, total E2, free E2, and % free E2 were based on 298 controls; values for SHBG, 3α-diol G, and DHEAS were based on 300 controls.
[c] Student's t for paired log-transformed values.
[d] T, testosterone; E2, estradiol; A, androstene dione; 3α-diol G, 3α-androstanediol glucuronide; DHEAS, dehydroepiandrosterone sulfate.

Table 3 Bivariate Pearson correlations among controls between concentrations of serum hormones and sex-hormone binding globulin[a]

	A[b]	Total T	SHBG	3α-Diol G	E2	DHEAS
A	1.000					
Total T	0.320	1.000				
SHBG	0.156	0.692	1.000			
3α-diol G	0.105	0.074	0.012	1.000		
Total E2	0.150	0.305	0.150	0.117	1.000	
DHEAS	0.408	0.032	−0.032	0.178	0.004	1.000

[a] Analyte concentrations for A, total T, free T, % free T, total E2, free E2, and % free E2 were based on 298 controls; concentrations for SHBG, 3α-diol G, and DHEAS were based on 300 controls.
[b] A, androstenedione; T, testosterone; 3α-diol G, 3α-androstanediol glucuronide; DHEAS, dehydroepiandrosterone sulfate; E2, estradiol; SHBG, sex hormone binding globulin.

Discussion

Testing the hypothesis that endogenous hormones are associated with prostate cancer risk has been greatly facilitated by the availability of stored serum specimens from large prospective studies like CARET. Nested case-control studies can then be conducted on sera from men who went on to develop prostate cancer and from a sample of men who remained free of cancer. Compared to case-control studies using blood from men already diagnosed with prostate cancer and controls, these studies are less likely to be biased as a result of the effect of the cancer and/or its treatment on hormone levels. However, even prospective studies have limitations. First, results are based on a single serum sample (although there is evidence that sex steroid hormone levels in men are relatively stable over time; Refs. 19, 20). Second, the serum sample is obtained relatively late in life; it is possible that serum concentrations earlier in life are more relevant to cancer risk. Third, prostate cancer was no doubt present in a preclinical stage in many of the cases when the blood specimen was drawn, even in some of the cases in whom >3 years had elapsed between blood draw and cancer diagnosis. Finally, there is some imprecision in measuring the levels of the hormones themselves.

None of the prospective studies reported that serum total T concentration was appreciably related to prostate cancer risk. The results of Gann *et al.* (6) raised the hypothesis that the concentration of bioavailable T is positively related to risk. They reported that after adjustment for levels of SHBG, E2, dihydrotestosterone (DHT), and 3α-diol G, men in the upper fourth of the distribution had 2.6 times the risk of men in the lowest fourth (95% CI, 1.34–5.02). This hypothesis has not been supported by results of our study or any of the others, whether the means of assessing bioavailable T was through calculation of levels of free T, through measurement of non-SHBG-bound T (8, 9, 11, 13), or as in Gann *et al.* (6), through adjustment of total T concentration for levels of SHBG and other hormones (12). In most studies (including this report) there has been little or no relation of SHBG levels to risk of prostate cancer, and, thus, adjustment for this variable has had little or no potential to confound the association between levels of T and prostate cancer.

Table 4 Risk of prostate cancer in relation to hormone concentrations in serum, adjusted for age and race

Hormone	Quartiles[a]				*P* for trend
	1 (low)	2	3	4 (high)	
A (nmol/L)[b]	1.0	0.89 (0.56–1.41)	1.07 (0.68–1.69)	1.20 (0.76–1.89)	0.32
	[74, 75][c]	[64, 74]	[78, 75]	[84, 74]	
	0.85–2.04[d]	2.04–2.59	2.59–3.32	3.32–7.48	
SHBG (nmol/L)	1.0	0.82 (0.52–1.31)	0.98 (0.62–1.54)	1.04 (0.66–1.64)	0.69
	[77, 75]	[64, 75]	[78, 76]	[81, 74]	
	8–21	21–27	27–36	36–81	
Total T (nmol/L)	1.0	0.84 (0.53–1.32)	0.87 (0.56–1.37)	0.82 (0.52–1.29)	0.43
	[85, 75]	[71, 74]	[75, 75]	[69, 74]	
	4.0–11.3	11.3–14.2	14.2–17.8	17.8–39.4	
Free T[e] (pmol/L)	1.0	1.07 (0.69–1.67)	0.8 (0.51–1.26)	0.72 (0.45–1.14)	0.09
	[84, 75]	[89, 74]	[68, 75]	[59, 74]	
	89–274	274–344	344–412	412–723	
% Free T	1.0	1.21 (0.78–1.87)	0.83 (0.52–1.30)	0.74 (0.46–1.19)	0.10
	[80, 75]	[98, 76]	[67, 77]	[55, 70]	
	1.41–2.16	2.16–2.39	2.39–2.63	2.63–3.31	
Total T/SHBG	1.0	0.72 (0.46–1.13)	0.93 (0.60–1.43)	0.52 (0.32–0.83)	0.03
	[96, 75]	[68, 74]	[87, 74]	[49, 75]	
	0.10–0.43	0.43–0.51	0.51–0.64	0.64–1.11	
3α-diol G (nmol/L)	1.0	0.90 (0.56–1.45)	1.48 (0.95–2.33)	1.27 (0.80–2.00)	0.10
	[67, 77]	[58, 74]	[94, 74]	[81, 75]	
	1.83–8.65	8.65–12.27	12.27–18.06	18.06–81.96	
Total E2 (nmol/L)	1.0	0.96 (0.62–1.50)	0.90 (0.57–1.40)	0.71 (0.42–1.13)	0.15
	[84, 75]	[80, 74]	[76, 75]	[60, 74]	
	0.13–0.17	0.17–0.19	0.19–0.22	0.22–0.36	
Free E2[e] (pmol/L)	1.0	0.65 (0.41–1.01)	0.71 (0.46–1.10)	0.52 (0.33–0.82)	0.01
	[104, 74]	[67, 74]	[75, 75]	[54, 74]	
	2.38–4.52	4.52–5.15	5.15–5.90	5.90–9.64	
% Free E2	1.0	1.05 (0.68–1.62)	1.07 (0.69–1.67)	0.65 (0.4–1.05)	0.14
	[81, 76]	[87, 78]	[82, 72]	[50, 72]	
	1.81–2.47	2.47–2.66	2.66–2.88	2.88–3.50	
DHEAS (nmol/L)	1.0	0.99 (0.62–1.58)	1.25 (0.79–1.99)	1.38 (0.86–2.21)	0.11
	[68, 75]	[65, 75]	[81, 75]	[86, 75]	
	87–1249	1249–1949	1949–3079	3079–7965	

[a] Odds ratio (95% confidence interval) by control quartiles of serum levels.
[b] A, androstenedione; T, testosterone; 3α-diol G, 3α-androstanediol glucuronide; DHEAS, dehydroepi androsterone sulfate; E2, estradiol; SHBG, sex hormone binding globulin.
[c] [Case N, control N].
[d] Range of analyte values within each control quartile.
[e] Calculated value—see text.

DHT is the active metabolite of T and is the major source of androgenic activity in the prostate. The conversion of T to DHT in the prostate is catalyzed by the enzyme 5α-reductase type II. The blood level of a distal DHT metabolite, 3α-diol G, is believed to correlate well with levels of activity of this enzyme. Most studies, including ours, have observed a weak positive association between serum level of 3α-diol G and risk of prostate cancer, with an interquartile OR of ~1.1 (8–11, 13). Lacking a more precise means of gauging intraprostatic DHT activity, at present it is not possible to clearly interpret this association. Both type I and type II 5α-reductase catalyze the conversion of T to DHT, with the subsequent conversion of DHT to 3α-diol G. The 5α-reductase type I enzyme is found predominantly in the skin, whereas type II is found predominantly in the prostate and genital skin (21), with secretory epithelium expressing only the type I enzyme and the basal layer, and stroma expressing both type I and type II (22, 23). There is no published literature to indicate what fraction of 3α-diol G in the circulation is prostatic in origin. However, the evidence that 3α-diol G in blood results from extrasplanchnic events (21), and that administration of finasteride, a 5α-reductase type II inhibitor, leads to a 60–70% reduction of circulating DHT and 3α-diol G (24, 25), suggest that a large portion of the circulating 3α-diol G is prostatic in origin.

There are reasons to believe that the risk of prostate cancer could rise with increasing levels of circulating estrogens, and also reasons to believe that the reverse could be true. Serum levels of estrone were observed to be higher in healthy African-American men, a group at relatively high risk of prostate cancer, than in Caucasian American men (26). The incidence of advanced benign prostatic hypertrophy is increased in men with relatively high blood levels of E2 (27). Also, administration of estrogens to some strains of rats or mice produces proliferation of prostate cells and/or cancer (1, 28–32). On the other hand, hyperestrogenic states (such as cirrhosis of the liver) are associated with a reduced risk of prostate cancer (33), and estrogen therapy appears to retard tumor progression in many men with prostate cancer (34). There has been no consistent pattern in the five prior studies of prediagnostic serum estrogens and prostate cancer risk. No association was seen in the studies of Hsing and Comstock (98 cases; Ref. 5) and Dorgan *et al.* (109 cases; Ref. 11), and Barrett-Connor *et al.* (57 cases; Ref. 4) observed a suggestion of an increasing risk with increasing levels. However, Nomura *et al.* (98 cases; Ref. 7) observed a decrease in risk of ~40% in men in the upper third of the distribution in blood estrone. In the largest study (222 cases; Ref. 6) before the present one, men in the upper three-fourths of the distribution of plasma E2 had about half the risk of other men, but with no

Table 5 Interquartile relative risk[a] of prostate cancer in relation to level of serum hormones, by tumor aggressiveness, interval between blood draw and diagnosis, smoking history, and age

Hormone	Odds ratio	95% CI[b]
Total T[c]		
All men	0.89	0.73–1.08
"Aggressive" tumors	0.84	0.66–1.08
Nonaggressive tumors	1.04	0.79–1.38
Diagnosis ≥ 3 yrs after blood draw	0.89	0.67–1.19
Diagnosis < 3 yrs after blood draw	0.88	0.67–1.17
Former smokers	0.69	0.51–0.92
Current smokers	1.11	0.83–1.48
Age ≤ 61	0.84	0.63–1.10
Age > 61	0.95	0.71–1.23
Free T[d]		
All men	0.89	0.77–1.03
"Aggressive" tumors	0.87	0.72–1.05
Nonaggressive tumors	0.97	0.79–1.20
Diagnosis ≥ 3 yrs after blood draw	0.87	0.70–1.07
Diagnosis < 3 yrs after blood draw	0.91	0.74–1.12
Former smokers	0.67	0.53–0.83
Current smokers	1.11	0.89–1.38
Age ≤ 61	0.86	0.71–1.06
Age > 61	0.94	0.76–1.18
3 α-diol G[e]		
All men	1.12	0.97–1.30
"Aggressive" tumors	1.21	1.00–1.45
Nonaggressive tumors	1.00	0.82–1.23
Diagnosis ≥ 3 yrs after blood draw	1.04	0.84–1.29
Diagnosis < 3 yrs after blood draw	1.20	0.98–1.46
Former smokers	1.03	0.83–1.27
Current smokers	1.20	0.96–1.50
Age ≤ 61	1.20	1.00–1.18
Age > 61	1.03	0.83–1.27
Total E2[f]		
All men	0.90	0.77–1.05
"Aggressive" tumors	0.91	0.75–1.11
Nonaggressive tumors	0.75	0.60–0.93
Diagnosis ≥ 3 yrs after blood draw	0.95	0.76–1.20
Diagnosis < 3 yrs after blood draw	0.85	0.68–1.05
Former smokers	0.99	0.79–1.24
Current smokers	0.84	0.67–1.05
Age ≤ 61	1.07	0.86–1.33
Age > 61	0.75	0.60–0.94
Free E2[g]		
All men	0.83	0.72–0.96
"Aggressive" tumors	0.83	0.69–1.00
Nonaggressive tumors	0.70	0.56–0.86
Diagnosis ≥ 3 yrs after blood draw	0.95	0.76–1.18
Diagnosis < 3 yrs after blood draw	0.74	0.61–0.90
Former smokers	1.03	0.83–1.27
Current smokers	0.68	0.55–0.85
Age ≤ 61	0.96	0.78–1.18
Age > 61	0.71	0.58–0.88

[a] As estimated by the odds ratio. All of the analyses were adjusted for age at first visit (except for analysis of age subgroups), race, and levels of other hormones. The overall analysis is based on 300 cases and 298 controls for each hormone. The numbers of cases/controls used in the subgroup analyses are as follows: aggressive tumors (see text for definition) 139/298 (included all controls), nonaggressive tumors 101/298 (all controls); diagnosis ≥ 3 yrs 132/131 (matched controls only), diagnosis < 3 yrs 168/167 (matched controls); former smokers 149/147 (matched controls), current smokers 142/142 (matched controls); and age ≤ 61 146/155 (controls ≤ 61 years of age), age > 61 154/143 (controls > 61 years of age).
[b] T, testosterone; 3α-diol G, 3α-androstanediol glucuronide; E2, estradiol; SHBG, sex hormone binding globulin.
[c] Adjusted for SHBG, 3α-diol G, and total E2.
[d] Adjusted for 3α-diol G and free E2.
[e] Adjusted for free T and free E2.
[f] Adjusted for total T, SHBG, and 3α-diol G.
[g] Adjusted for free T and 3α-diol G.

additional decrease in risk with additional increases in E2 levels. We also observed the risk of prostate cancer to be relatively low in men with relatively high levels of E2 in serum. However, the association was considerably attenuated when the analysis was restricted to cases and controls in whom at least 3 years had elapsed between the date of blood draw and the date the case's diagnosis was made (*i.e.*, cases in whom there was the least possibility of the presence of the cancer having an influence on plasma hormone levels). Thus, although some of the evidence supports an inverse relation between circulating levels of estrogens and the incidence of prostate cancer, it would be premature to draw a firm conclusion.

The response of human prostate cells to estrogen and antiestrogen depends on the estrogen receptor subtypes expressed in the cells (35). There is also evidence that E2 regulates the expression of insulin-like growth factor-I and insulin-like growth factor-I receptor (36), and that E2 and insulin-like growth factor-I act through a complex cross-talk mechanism to stimulate cellular proliferation in a number of tissues (37, 38). Future investigations of the causes of prostate cancer may benefit from examination of the role of estrogen receptors, and of a potential interaction between estrogens and other hormones and growth factors.

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