Prospective Study of Sex Hormone Levels and Risk of Prostate Cancer

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Background: **Sex steroids, particularly androgens, have been implicated in the pathogenesis of prostate cancer. Data from previous studies comparing circulating hormone levels in men with and without prostate cancer are difficult to interpret, since the studies were limited in size, hormone levels were analyzed in blood drawn after the diagnosis of cancer, nonrepresentative control subjects were used, and hormone and hormone-binding protein levels were not simultaneously adjusted.** ***Purpose:*** **We conducted a prospective, nested case–control study to investigate whether plasma hormone and sex hormone-binding globulin (SHBG) levels in healthy men were related to the subsequent development of prostate cancer.** ***Methods:*** **Among participants in the Physicians' Health Study who provided plasma samples in 1982, we identified 222 men who developed prostate cancer by March 1992. Three hundred ninety control subjects, matched to the case patients on the bases of age, smoking status, and length of follow-up, were also identified. Immunoassays were used to measure the levels of total testosterone, dihydrotestosterone (DHT), 3α-androstanediol glucuronide (AAG), estradiol, SHBG, and prolactin in the stored (at –82 °C) plasma samples. Correlations between individual hormone levels and between hormone levels and SHBG in the plasma of control subjects were assessed by use of Spearman correlation coefficients (*r*). Odds ratios (ORs) and 95% confidence intervals (CIs) specifying the prostate cancer risk associated with quartile levels of individual hormones, before and after adjustment for other hormones and SHBG, were calculated by use of conditional logistic regression modeling. Reported *P* values are two-sided.** ***Results:*** **No clear associations were found between the unadjusted levels of individual hormones or SHBG and the risk of prostate cancer. However, a strong correlation was observed between the levels of testosterone and SHBG (*r* = .55), and weaker correlations were detected between the levels of testosterone and the levels of both estradiol (*r* = .28) and DHT (*r* = .32) (all *P*<.001). When hormone and SHBG levels were adjusted simultaneously, a strong trend of increasing prostate cancer risk was observed with increasing levels of plasma testosterone (ORs by quartile = 1.00, 1.41, 1.98, and 2.60 [95% CI = 1.34-5.02]; *P* for trend = .004), an inverse trend in risk was seen with increasing levels of SHBG (ORs by quartile = 1.00, 0.93, 0.61, and 0.46 [95% CI = 0.24-0.89]; *P* for trend = .01), and a nonlinear inverse association was found with increasing levels of estradiol (ORs by quartile = 1.00, 0.53, 0.40, and 0.56 [95% CI = 0.32-0.98]; *P* for trend = .03). No associations were detected between the levels of DHT or prolactin and prostate cancer risk; for AAG, a marker of 5α-reductase activity, only suggestive evidence of a positive association was found. The results were essentially unchanged when case patients diagnosed within 4 years of plasma collection, case patients diagnosed with localized (i.e., nonaggressive) disease, or control subjects with elevated prostate serum antigen levels (>2.5 ng/mL) were excluded from the analyses.** ***Conclusions:*** **High levels of circulating testosterone and low levels of SHBG—both within normal endogenous ranges—are associated with increased risks of prostate cancer. Low levels of circulating estradiol may represent an additional risk factor. Circulating levels of DHT and AAG do not appear to be strongly related to prostate cancer risk. [J Natl Cancer Inst 1996;88:1118-26]**

A longstanding and diverse body of evidence suggests that sex steroids, particularly androgens, play a role in the etiology of prostate cancer. Androgens are essential for normal growth and maintenance of the prostate, stimulate the proliferation of human prostate cancer cells in vitro, and, when given in large amounts, can produce prostate cancer in rodents (*1,2*). In addition, eunuchs rarely develop prostate cancer, and androgen ablation frequently causes prostate tumors to regress (*3*). In contrast, a reduced risk of prostate cancer has been associated with certain hyperestrogenic states (*4*), and estrogen therapy has a palliative effect in advanced cases (*3*). These observations on extreme variations in sex-steroid exposure add credibility to the hypothesis that prostate cancer risk is also related to the smaller contrasts in levels of androgens and estrogens that are found within the normal endogenous range (*1*). However, studies com-

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See "Notes" section following "References."

paring circulating hormone levels in men with and without prostate cancer have produced widely varying results (*5*). In general, these studies have been limited by small sample sizes, analyses of hormone levels in blood collected after the diagnosis of cancer, and the use of control subjects not selected from the same source population as the case patients (*6*).

In this study, we investigated whether hormone levels in plasma, collected from generally healthy men who were 40-84 years of age, were related to the development of prostate cancer up to 10 years later. Our central focus was on testosterone and estradiol, the dominant contributors to androgenic and estrogenic activity in the circulation. We also evaluated two other forms of androgen: dihydrotestosterone (DHT) and 3α-androstanediol glucuronide (AAG). DHT, formed by the action of 5α-reductase on testosterone, is believed to be the principal intraprostatic androgen (*7*). AAG is a metabolic product of DHT and is considered to be a useful marker of global 5α-reductase activity (*8*). A large, ongoing trial is testing the effect of finasteride, an inhibitor of 5α-reductase, on prostate cancer incidence among healthy men (*9*). We also assayed sex hormone-binding globulin (SHBG) because it binds and sequesters testosterone and, to a lesser degree, estradiol, thus reducing the bioavailable amounts of these hormones. Furthermore, recent evidence suggests that SHBG can function as a hormone capable of direct interaction with prostatic cells (*10*).

Previous studies of hormone levels and prostate cancer risk have evaluated each hormone individually. However, the hormone levels under consideration are known to be correlated, which creates the possibility that the apparent association—or lack of association—of an individual hormone with risk could be due to confounding by other hormones. Inclusion of SHBG levels in this analysis allowed us to examine whether the associations observed for total testosterone or total estradiol were determined in part by the level of SHBG, as predicted by the hypothesis that bioavailable rather than total hormone levels are paramount. Disentangling the effects of SHBG and total testosterone is especially critical because we expect these levels to be substantially correlated in men (i.e., an increase in SHBG leads to a corresponding increase in testosterone secretion through the feedback effect of free testosterone on the hypothalamic–pituitary–gonadal axis). In previous studies, the true risks associated with total testosterone and SHBG could have been masked by the reciprocal relation of these compounds to bioavailable testosterone. Therefore, an important aim of our study was to estimate the impact of differences in total testosterone on risk at any given level of SHBG and, conversely, the impact of differences in SHBG at any given level of total testosterone.

Methods

The Study Population: the Physicians' Health Study

The Physicians' Health Study was a randomized, double-blind, placebo-controlled trial of aspirin and β-carotene among 22 071 U.S. male physicians, aged 40-84 years in 1982. Men were excluded if they reported a prior history of myocardial infarction, stroke, transient ischemic attacks, unstable angina, cancer (except for nonmelanoma skin cancer), current renal or liver disease, peptic ulcer or gout, contraindication to the use of aspirin, or current use of aspirin, other platelet-active agents, or vitamin A supplements. The aspirin component of the trial was terminated in January 1988, primarily because of a statistically extreme 44% reduction in risk of a first myocardial infarction among those in the aspirin group (*11*). The β-carotene component of the study was terminated in December 1995. Written informed consent was obtained from each participant, and the project has received ongoing approval from the Institutional Review Board at Brigham and Women's Hospital in accord with federal requirements.

Study participants completed two mailed questionnaires before being randomly assigned to exposure groups. Additional questionnaires were mailed at 6 months, 12 months, and annually thereafter. Before the randomization, we sent blood kits to all participants with instructions to have their blood drawn into vacutainer tubes containing EDTA. The participants were to fractionate the blood by centrifugation and to return (by overnight, prepaid courier) the plasma in polypropylene cryopreservation vials. Each kit included a cold pack to keep the specimens cool until they were received the following morning, when they were divided into aliquots and stored at –82 °C. During storage, precautions were taken so that no specimen was thawed or warmed substantially. We received specimens from 14 916 (68%) of the randomly assigned physicians; more than 70% of the specimens were received between September and November 1982.

Selection of Case Patients and Control Subjects

When participants report a diagnosis of cancer, we request medical records (including pathology reports) that are reviewed by study physicians from the End Points Committee. By March 1992, 520 cases of prostate cancer were confirmed among study participants. One hundred fifty individuals had not provided plasma specimens, and 148 others had given specimens with inadequate sample volumes, leaving 222 with plasma samples sufficient for analysis. The absence of plasma samples for some study participants is unlikely to have introduced selection bias, since it is implausible that physicians who did or did not provide a sample would differ significantly in terms of the potential relationship between hormone levels and the subsequent diagnosis of prostate cancer. Case patients with and without available plasma were not substantially different with respect to base-line lifestyle characteristics. For each case patient, two control subjects were selected who had provided plasma, who had not had either a total or a partial prostatectomy, and who had not reported a diagnosis of prostate cancer up to the time that the case patient's diagnosis was reported. Potential control subjects who had a history of partial prostatectomy were excluded because they might not have been fully at risk at the time the case patients were diagnosed. Analyses were repeated to exclude the two case patients who had partial prostatectomies prior to diagnosis. Control subjects were also matched on the bases of smoking status and age within 1 year, except for two case patients who were over 80 years of age for whom age was matched within 2 years. Fifty-four of the 444 chosen control subjects had insufficient plasma samples for hormone assays, leaving 390 subjects with plasma samples in the analysis. After 10 years of follow-up, more than 99% of surviving study participants were still reporting morbidity events; vital status was ascertained for 100%.

Laboratory Assays

Previously unthawed plasma samples for the case patients and the control subjects were shipped on dry ice to the laboratory of one of the investigators (C. Longcope) for hormone analyses. Each case sample and its matched control samples were assayed at the same time to minimize interference caused by interassay variability. Laboratory personnel were unable to distinguish case, control, or quality-control samples. Total testosterone was measured directly in the plasma by use of radioimmunoassay kits obtained from ICN Biomedicals, Inc. (Costa Mesa, CA). For DHT assays, 1-mL aliquots of plasma were first extracted with cyclohexane:ethyl acetate (1:2, vol/vol). After extraction, each organic phase was recovered, the solvent was removed by evaporation under a nitrogen atmosphere, and the residue was solubilized with 0.2 mL isooctane (three times) and subjected to chromatography on a celite 545 column (Fisher Scientific Co., Pittsburgh, PA). The appropriate fraction was then collected for analysis by radioimmunoassay (*12*). AAG was measured by use of radioimmunoassay kits obtained from Diagnostic Systems Laboratory (Webster, TX). Estradiol was measured by use of radioimmunoassay kits obtained from DPC (Los Angeles, CA). Prolactin was measured by means of an enzyme immunoassay (IMX, Abbott Laboratories, Abbott Park, IL). SHBG was measured by use of radioimmunometric assay kits (Orion Diagnostica, Oulu, Finland). Intra-assay

coefficients of variation for the various assays were as follows: estradiol, 6.8%; testosterone, 8.7%; DHT, 5.3%; SHBG, 8.9%; prolactin, 4.4%; and AAG, 7.6%.

Medical Record Review

A study physician (P. H. Gann), unaware of the hormone assay results, reviewed the medical records for each case patient to determine the tumor stage at diagnosis, the tumor grade, and the Gleason score (*13*). Stage was recorded according to the modified Whitmore–Jewett classification scheme (*14*). If multiple tissue samples were examined, the highest reported grade and Gleason score were recorded. Cases without pathologic staging were classified as indeterminate stage unless there was clinical evidence of distant metastases. Aggressive cases were defined as those diagnosed at either stage C or D (extraprostatic) plus those diagnosed at stage A, stage B, or indeterminate stage with either poor histologic differentiation or a Gleason score of 7 or greater. Among 222 total case patients analyzed, 103 were classified as having aggressive disease and 119 as having nonaggressive disease. Patients with localized prostate cancers having poor histologic features have increased mortality (*15*); thus, categorization of these cancers as aggressive is appropriate.

Data Analysis

We checked the univariate distributions of the plasma hormone concentrations to assess normality and determined that nonparametric techniques were preferable. To compare analyte levels in case patients versus control subjects, we computed Wilcoxon signed rank sum test statistics using the untransformed values. All *P* values were two-sided. We used the quartile cutpoints for the control subjects to assign each study participant to a quartile for each hormone. To estimate relative risks (RRs) by the level of plasma hormone, we computed odds ratios (ORs) and 95% confidence intervals (CIs) for each quartile, using the lowest hormone quartile as the reference group. To adjust simultaneously for the effects of potential confounders, we fit conditional logistic regression models using EGRET statistical software (SERC, Inc., Seattle, WA). Models were rerun excluding controls with elevated prostate-specific antigen (PSA) and including covariates, adjusting for the time of blood draw (which was known for 79% of the samples). Correlations between hormone levels were evaluated, using the control data only, by calculation of Spearman correlation coefficients. We assessed the variation in RR across subgroups in three ways: by fitting separate models within distinct subgroups (e.g., by age, tumor aggressiveness, or time to diagnosis), by assessing multiplicative interaction terms for continuous variables, and by constructing models with dummy variables to represent each combination of tertile categories for the two analytes (e.g., tertiles of testosterone and SHBG). We used tertiles rather than quartiles for these joint category analyses to avoid sparse cells. We tested for trend by including a continuous term or an ordinal term for hormone quartile in the conditional logistic regression models. We also computed four ratios that had previously been construed as indices of hormone balance or metabolism (estradiol [E2]/testosterone [T], E2/SHBG, T/DHT, and T/SHBG) and compared case patients with control subjects by use of both the Wilcoxon signed rank sum test and conditional logistic models with indicator variables for ratio quartiles.

Results

Selected characteristics of the case patients and control subjects are compared in Table 1. Median levels of plasma hormone concentrations and hormone ratios were not substantially different between these two groups.

Table 2 shows the Spearman correlation coefficients for the associations between any two hormone levels among the control subjects. There was a strong correlation between testosterone and SHBG ($r = .55$) and weaker but still substantial correlations between testosterone and both estradiol ($r = .28$) and DHT ($r = .32$). Estradiol was also correlated with AAG ($r = .34$), and DHT was correlated with SHBG ($r = .26$). Prolactin levels were not correlated with the levels of any other hormone. The correlation patterns were similar among the case patients (data not shown).

ORs for each quartile of plasma hormone level, as well as median hormone concentrations for each quartile, are shown in

Table 1. Selected characteristics of case patients with prostate cancer and control subjects at the start of follow-up: the Physicians' Health Study

	Case patients (n = 222)	Control subjects (n = 390)	*P* value*
Mean age, y	61.7	61.7	Matched
Mean follow-up y at risk	6.3	6.3	Matched
Stage at diagnosis, %			
Localized	57	—	—
Regional	21	—	—
Distant	15	—	—
Unknown	7	—	—
Vigorous exercise twice or more per wk, %	53.9	52.3	0.71
Cigarette smokers, %			
Current	9.0	8.5	Matched
Former†	48.2	48.5	Matched
Drink alcohol twice or more per wk, %	64.1	66.1	.62
Plasma hormones (median)			
Estradiol (E2), pg/mL	33	34	.23
Testosterone (T), ng/mL	4.81	4.68	.74
Dihydrotestosterone (DHT), ng/mL	0.34	0.36	.48
3α-Androstanediol glucuronide, ng/mL	6.8	6.5	.12
Sex hormone-binding globulin (SHBG), nmol/L	20.0	21.8	.16
Prolactin, ng/mL	7.2	6.9	.44
Hormone ratios			
E2/T	6.78	7.25	.19
E2/SHBG	1.62	1.56	.94
T/DHT	13.16	12.82	.29
T/SHBG	0.23	0.21	.13

**P* values were computed using the Wilcoxon signed rank sum test for continuous variables and the chi-squared test for categorized variables.
†"Former" refers to anyone who was a regular smoker at any time in the past but who was not smoking at base line.

Table 2. Bivariate associations between plasma hormone and sex hormone-binding globulin concentrations in control subjects (n = 390): Spearman correlation coefficients (and *P* values)*

	Testosterone	DHT	Prolactin	SHBG	AAG
Estradiol	0.28 (<.0001)	0.10 (.05)	0.05 (.32)	0.02 (.76)	0.34 (<.0001)
Testosterone	—	0.32 (<.0001)	–0.07 (.19)	0.55 (<.0001)	0.14 (.004)
DHT	—	—	–0.06 (.25)	0.26 (<.0001)	0.10 (.05)
Prolactin	—	—	—	–0.06 (.20)	0.07 (.17)
SHBG	—	—	—	—	–0.02 (.63)

*DHT = dihydrotestosterone; SHBG = sex hormone-binding globulin; AAG = 3α-androstanediol glucuronide.

Table 3. In these analyses, each hormone was considered individually, i.e., there was no adjustment for potential confounding by other hormone levels. No strong inverse or positive associations with prostate cancer risk were observed; however, weak positive associations for AAG and testosterone were detected, as well as weak inverse associations for both estradiol and SHBG. The inverse trend across quartiles of SHBG approached statistical significance ($P = .07$). Models with adjustment for body mass index, alcohol use, and exercise frequency—three factors previously postulated to be related to both hormone levels and prostate cancer risk—produced results nearly identical to those shown in Table 3.

The ORs for each quartile of selected hormone ratios, again considered without adjustment for additional hormone levels, are shown in Table 4. These results indicated weak positive associations between prostate cancer risk and the ratios of testosterone to DHT and testosterone to SHBG. A weak inverse association was observed between risk and the ratio of estradiol to testosterone. The results were not substantially altered by adjustment for body mass index, alcohol use, or exercise frequency.

Because estradiol and SHBG (hypothesized to have an inverse association with prostate cancer risk) were significantly correlated with testosterone levels (hypothesized to have a positive association with risk), the generally weak unadjusted associations shown in Table 3 could reflect the masking of stronger, independent hormone–cancer relationships. Therefore, we fit models in which the hormone levels were adjusted simultane-

Table 3. Unadjusted odds ratios (ORs)* for prostate cancer by control quartile of plasma hormone level (n = 222 case–control sets)

	Quartile					
Hormone†	1‡	2	3	4	Q4 95% CI§	*P* for trend
Estradiol (pg/mL)						
Median (range)	22 (2-26)	30 (27-33)	38 (34-42)	50 (43-110)		
OR	1.00	0.59‖	0.50‖	0.75	0.46-1.24	.24
Testosterone (ng/mL)						
Median (range)	2.99 (0.15-3.63)	4.18 (3.64-4.68)	5.19 (4.69-5.85)	7.02 (5.86-13.90)		
OR	1.00	1.26	1.27	1.30	0.79-2.16	.35
DHT (ng/mL)						
Median (range)	0.16 (0.07-0.22)	0.29 (0.23-0.36)	0.43 (0.37-0.52)	0.68 (0.53-1.52)		
OR	1.00	1.12	0.81	0.83	0.45-1.52	.41
AAG (ng/mL)						
Median (range)	3.6 (0.7-4.7)	5.7 (4.8-6.4)	7.3 (6.5-8.6)	10.8 (8.7-25.5)		
OR	1.00	1.35	1.54	1.47	0.90-2.39	.11
SHBG (nmol/L)						
Median (range)	11.0 (3.2-14.6)	17.6 (14.8-21.7)	25.2 (21.8-29.5)	39.0 (29.8-103.8)		
OR	1.00	1.05	0.71	0.69	0.41-1.16	.07
Prolactin (ng/mL)						
Median (range)	4.2 (0.2-5.2)	6.0 (5.3-6.8)	7.8 (6.9-9.1)	11.7 (9.3-118.4)		
OR	1.00	0.70	1.01	1.00	0.63-1.57	.64

*ORs are controlled for age and smoking status by matching. Models adjusting for body mass index, exercise frequency, and alcohol use yielded nearly identical results.

†DHT = dihydrotestosterone; AAG = 3α-androstanediol glucuronide; SHBG = sex hormone-binding globulin.

‡Quartile 1 is the reference.

§Q4 95% CI = 95% confidence intervals for quartile 4 ORs.

‖95% CIs exclude 1.00. The CIs for estradiol quartile 2 are 0.37-0.93; for estradiol quartile 3, they are 0.30-0.84.

Table 4. Unadjusted odds ratios (ORs)* for prostate cancer by control quartile of selected plasma hormone and SHBG ratios (n = 222 case–control sets)

Hormone ratio†	OR by quartile‡				Q4 95% CI§	*P* for trend
	1	2	3	4		
E2/T	1.00	0.72	0.51‖	0.77	0.47-1.27	.18
E2/SHBG	1.00	0.85	1.01	1.06	0.64-1.74	.72
T/DHT	1.00	1.68	1.37	2.35‖	1.22-4.53	.02
T/SHBG	1.00	1.18	1.35	1.59	0.92-2.74	.08

*ORs are controlled for age and smoking status by matching. The same results were obtained with additional adjustment for body mass index, exercise, and alcohol use.

†E2 = estradiol; T = testosterone; SHBG = sex hormone-binding globulin; DHT = dihydrotestosterone.

‡Lowest (quartile 1) to highest (quartile 4) plasma levels for each hormone, with quartile 1 levels as the reference.

§Q4 95% CI = 95% confidence intervals for quartile 4 ORs.

‖95% CIs exclude 1.00. The CIs for E2/T quartile 3 are 0.30-0.85.

ously to observe the separate impact of each hormone. The hormone–hormone correlations were not high enough to introduce collinearity problems in fitting these models. Prolactin was excluded from the final models because we found no evidence for an association between prolactin and the other hormones. The results of the model containing quartile indicator variables for the five remaining hormones are shown in Table 5. This analysis revealed a relatively strong, independent, positive association between testosterone level and prostate cancer risk, as well as substantial inverse associations for estradiol and SHBG. A weak, independent positive association for AAG was also observed. Most of the apparent confounding in the results for testosterone was due to SHBG, and vice versa, because a model adjusting for only these hormones gave results similar to those obtained from models with additional hormone covariates. Fig. 1 shows the ORs for testosterone and SHBG from the analysis adjusting only for those hormones plus estradiol. The trend across quartiles for estradiol was nonlinear (ORs for quartiles 1-4: 1:00, 0.56 [95% CI = 0.35-0.90], 0.43 [95% CI = 0.25-0.73], and 0.65 [95% CI = 0.39-1.09]; *P* for trend = .08), suggesting that elevated risk was confined to men with low plasma estradiol levels. We also assessed models for hormone ratios that adjusted for hormones not included in the ratios; these results were not materially different from those appearing in Table 4.

To reduce the potential impact of preclinical disease on hormone levels, we refit the five-hormone model after excluding 37 case patients (and their matched control subjects) with less than 4 years between the time of blood drawing and the diagnosis of prostate cancer. The results were essentially the same, except for a slightly stronger inverse association for SHBG (4th quartile OR = 0.35 [95% CI = 0.17-0.76]; *P* for trend = .004). We also refit the model using only 103 case patients (and matched control subjects) who were categorized as having aggressive disease at diagnosis. The positive association for testosterone was still present, and the inverse associations for estradiol and SHBG were slightly stronger (4th quartile ORs = 0.40 [95% CI = 0.17-0.95] and 0.38 [95% CI = 0.14-1.00], respectively). We then excluded 54 control subjects with plasma PSA levels exceeding 3.0 ng/mL (monoclonal immunoassay scale) from the analysis. In the five-hormone model, the previously observed patterns for testosterone, estradiol, and SHBG were the same or slightly stronger (4th quartile ORs = 2.78 [95% CI = 1.38-5.59], 0.49 [95% CI = 0.27-0.89], and 0.41 [95% CI = 0.20-0.84], respec-

Table 5. Odds ratios (ORs)* for prostate cancer by control quartile of plasma hormone level: simultaneous adjustment for five hormone levels (n = 222 case–control sets)

Hormone†	OR by quartile‡				Q4 95% CI§	*P* for trend
	1	2	3	4		
Estradiol	1.00	0.53‖	0.40‖	0.56‖	0.32-0.98	.03
Testosterone	1.00	1.41	1.98‖	2.60‖	1.34-5.02	.004
DHT	1.00	1.02	0.78	0.71	0.34-1.48	.30
AAG	1.00	1.44	1.58	1.60	0.93-2.76	.09
SHBG	1.00	0.93	0.61	0.46‖	0.24-0.89	.01

*ORs are adjusted for age and smoking status by matching and for all hormone levels shown by use of indicator variables for quartiles. The same results were obtained with additional adjustment for body mass index, exercise, and alcohol use.

†DHT = dihydrotestosterone; SHBG = sex hormone-binding globulin; AAG = 3α-androstanediol glucuronide.

‡Lowest (quartile 1) to highest (quartile 4) plasma hormone level, with quartile 1 as the reference.

§Q4 95% CI = 95% confidence intervals for quartile 4 ORs.

‖95% CIs exclude 1.00. The CIs for estradiol quartiles 2 and 3 are 0.33-0.85 and 0.23-0.70, respectively. The CI for testosterone quartile 3 is 1.09-3.58.

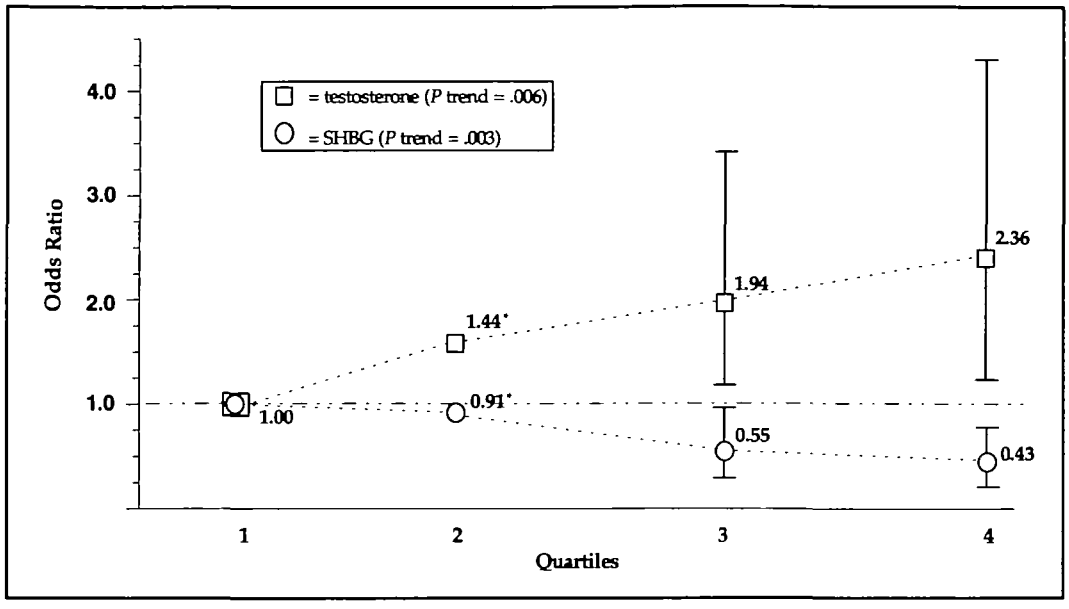

Fig. 1. Prostate cancer odds ratios (ORs) by quartile levels of plasma testosterone and sex hormone-binding globulin (SHBG): simultaneous adjustment for testosterone, SHBG, and estradiol. Quartile cutpoints were defined by use of control subject values, with quartile 1 values representing the lowest plasma levels and quartile 4 values representing the highest; the median quartile values and their ranges are presented in Table 3. ORs (95% confidence intervals [CIs]) for successive estradiol quartiles are as follows: 1.00, 0.56 (0.35-0.90), 0.43 (0.25-0.73), and 0.65 (0.39-1.09) (*P* for trend = .08). * = 95% CIs for testosterone quartile 2 and SHBG quartile 2 are not shown in the figure; these CIs are 0.85-2.43 and 0.54-1.51, respectively.

tively). Exclusion of 85 control subjects with a PSA of more than 2.5 ng/mL produced the same results. None of these exclusions had any effect on the results for hormone ratios.

To investigate whether the associations between hormone levels and prostate cancer risk varied between older and younger men, we repeated the multivariate analysis in subgroups over and under the median age for the study population, i.e., 61 years. The results, shown in Table 6, indicate that the association between plasma testosterone and prostate cancer risk was stronger in older men, whereas the inverse association between SHBG and risk was apparent mainly in the younger men. Among older men, there was also evidence suggestive of an inverse association with DHT level. There were 42 case–control sets among older men in which the case disease was classified as aggressive. The association between testosterone and prostate cancer (adjusted for estradiol and SHBG) was particularly strong in this subgroup, with the following ORs for successive testosterone quartiles: 1.00, 4.63 (95% CI = 0.74-29.17), 8.96 (95% CI = 1.70-47.13), and 10.61 (95% CI = 1.60-70.52) (*P* for trend = .009).

Study subjects were classified into nine groups according to joint tertiles (low, middle, and high) for testosterone and SHBG. The estimated risk of prostate cancer was four to five times higher in men who were in the middle or higher range for testosterone and also in the middle or lower range for SHBG, compared with men who were both in the low range for testosterone

Table 6. Odds ratios (ORs)* for prostate cancer by control quartile of plasma hormone level: results of separate multivariate models for men older and younger than the median age at base line

	OR by quartile‡					
Hormone†	1	2	3	4	Q4 95% CI§	*P* for trend
Age ≥62 y (n = 114 sets)						
Estradiol	1.00	0.38‖	0.39‖	0.49	0.21-1.13	.08
Testosterone	1.00	2.36	3.79‖	4.38‖	1.56-12.35	.005
DHT	1.00	0.91	0.35	0.35	0.09-1.32	.05
AAG	1.00	2.70‖	1.87	2.19	0.94-5.11	.15
SHBG	1.00	1.08	1.23	0.73	0.27-2.03	.73
Age ≤61 y (n = 108 sets)						
Estradiol	1.00	0.70	0.47	0.60	0.27-1.34	.20
Testosterone	1.00	1.05	1.37	1.75	0.65-4.69	.27
DHT	1.00	0.92	1.11	0.76	0.27-2.11	.74
AAG	1.00	0.93	1.43	1.49	0.69-3.23	.15
SHBG	1.00	0.74	0.32‖	0.32‖	0.12-0.86	.01

*ORs are derived from models simultaneously adjusting for five hormone levels by use of indicator variables for quartiles. Age and smoking status are controlled by matching.

†DHT = dihydrotestosterone; AAG = 3α-androstanediol glucuronide; SHBG = sex hormone-binding globulin.

‡Lowest (quartile 1) to highest (quartile 4) plasma hormone level, with quartile 1 as the reference.

§Q4 95% CI = 95% confidence intervals for quartile 4 ORs.

‖95% CIs exclude 1.00. The CIs for estradiol quartiles 2 and 3 in the 62 years or more age group are 0.19-0.78 and 0.18-0.86, respectively; the CI for testosterone quartile 3 in this age group is 1.48-9.72; and the CI for AAG quartile 2 in this age group is 1.23-5.92. The CI for SHBG quartile 3 in the 61 years or less age group is 0.13-0.78.

and in the high range for SHBG. We found no evidence for multiplicative risks that would suggest biological synergism between testosterone and SHBG.

Discussion

In this large prospective study, high levels of plasma testosterone compared with low levels—within the normal endogenous range—were associated with an approximately 2.5-fold increase in the risk of developing prostate cancer after adjustment for plasma SHBG and estradiol. Men with the highest levels of SHBG had approximately 50% lower risk than men with the lowest SHBG levels, independent of plasma testosterone. The testosterone association was stronger in older men, especially those with aggressive cancers, whereas the SHBG association was stronger in younger men. In addition, the risk appeared to be increased about twofold in men with low levels of estradiol relative to men with any higher levels, again independent of testosterone and SHBG. We observed weaker or nonexistent associations with the ratios of testosterone to estradiol and testosterone to SHBG, suggesting that absolute rather than relative levels of these compounds could be more important. Plasma prolactin levels were not associated with prostate cancer risk in any of our analyses.

The associations that we observed were not explained by confounding due to age, smoking, obesity, exercise frequency, or alcohol use. This was particularly of concern for SHBG because of the strong inverse relationship between SHBG and body mass index, which was evident in our data as well as in a previous study (*16*). It is thus conceivable that at least part of the association between obesity and prostate cancer seen in some studies could be mediated through an effect of obesity on SHBG production or clearance (*17*). It is unlikely that our findings can be explained by an effect of prevalent but undiagnosed cancer on hormone levels, because none of the important associations were weakened when case patients diagnosed within 4 years of the blood draw were excluded. Contamination of the control group with undiagnosed prostate cancer cases would be expected to dilute any true associations. We found, however, that exclusion from the analysis of control subjects with borderline or elevated PSA in the same base-line blood samples had little or no effect on the results. This is not surprising, since none of these control subjects with borderline or abnormal PSA values were diagnosed with prostate cancer during 10 years of follow-up.

Most previous studies of hormone levels and prostate cancer [reviewed in (*5,6*)] have been based on blood samples obtained from case patients after diagnosis. These studies, which have found both higher and lower testosterone, SHBG, and estradiol in case patients compared with control subjects, are potentially biased by the effects of clinical prostate cancer on hormone levels and by the difficulty of obtaining control subjects representative of the source population for case patients. The assertion that prostate cancer has a suppressive effect on androgen levels is supported by data indicating that men with prostate cancer have elevated rates of testosterone clearance (*18*) and by observations that androgen levels are positively associated with survival and inversely associated with tumor stage (*19*). Prospective analyses, including studies of case patients and control subjects nested within cohorts, such as the present one, avoid these biases. The three previous prospective analyses of blood hormone levels and prostate cancer included a combined total of only 253 cases (*20-22*). Two studies (*21,22*) reported positive associations for the T/DHT ratio, one reported a positive but nonsignificant association for testosterone (*22*), and one reported nonsignificant inverse associations for estradiol and DHT (*21*). These results are generally compatible with our observations when hormones are considered individually. In fact, the report of Hsing and Comstock (*22*) also concurs with our finding of a stronger association for testosterone among older men. None of the prospective studies reported correlations between hormone levels or RR estimates reflecting free hormone or simultaneous adjustment for multiple hormones. The one case–control study that measured free testosterone found higher levels of free testosterone (and lower levels of SHBG) in healthy control subjects compared with case patients (*23*). However, the case patients and control subjects were not age matched, and the observed differences—if not due to the effect of cancer on hormone levels—could have been because of the expected trend toward lower free testosterone and higher SHBG with aging.

The "free hormone hypothesis" for sex steroids, postulated soon after the discovery of SHBG in the 1960s, states that only hormone not tightly bound to protein is available to move through capillaries and into cells to initiate biological action (*24*). Of all circulating testosterone, approximately 2% is free and 44% is tightly bound to SHBG (*25*). The remainder is loosely bound to albumin and is thus considered to be available for biological action. Similar distributions apply for estradiol, although its binding to SHBG is considerably weaker. Increases in SHBG, independent of testosterone level, would be expected to produce a decrease in bioavailable hormone. In fact, in a large population of middle-aged men, SHBG was negatively correlated with free and albumin-bound testosterone (*26*). The free hormone concept provides the most parsimonious explanation of our results, which suggest that either an increase in total testosterone or a decrease in SHBG would independently increase prostate cancer risk. The free hormone mechanism would further predict that the combined influence of total testosterone and SHBG on risk would be additive, since both act through their relation to the amount of biologically active hormone. More complicated interpretations of our data are conceivable. Findings suggest that SHBG might be more than simply a carrier protein (*10*). A specific cell surface receptor for SHBG has been identified in prostate cells. Some investigators contend that signal transduction via this receptor can be activated or inhibited by different steroid–SHBG complexes and that the receptor could provide an alternative path of entry into the cell for steroids such as estradiol.

The ratio of testosterone to SHBG could be considered an index of the proportion of testosterone that is bioavailable (*27*). Indeed, we found that this ratio was slightly more strongly associated with prostate cancer than total testosterone alone. However, simultaneous adjustment for both testosterone and SHBG resulted in even stronger associations, suggesting that the absolute as well as the relative amounts of these compounds should be taken into account.

Prior evidence suggests that higher endogenous levels of estradiol, the most potent estrogen in the circulation, could be inversely related to prostate cancer risk. The inverse association that we observed for estradiol in the multivariate analysis was definitely nonlinear, in that risk appeared to be reduced to about the same degree in all three of the higher quartiles of plasma estradiol relative to the lowest quartile. Thus, there could be a threshold below which estradiol is no longer protective, although we are not aware of evidence to support such a conjecture about the dose–response relationship. In a previous study (*28*) of prediagnostic hormone levels and surgically treated benign prostatic hyperplasia (BPH), we found that higher estradiol levels were associated with a higher risk of advanced BPH. The lack of association between BPH and androgen or SHBG levels in that analysis means it is unlikely that BPH confounds the present results for prostate cancer. Opposite associations for estradiol in BPH and cancer raise the intriguing possibility that these diseases represent divergent responses to this hormone within prostatic tissue. There is evidence suggesting that exogenous estrogen causes extensive degeneration in the peripheral zone, where most cancers arise, but very little in the central zone, where most BPH occurs (*29*).

Testosterone and estradiol levels were correlated, as expected, because aromatization of androgens in peripheral tissues is a major source of estradiol in men. A relative imbalance of androgens and estrogens has been invoked as an explanation for various clinical phenomena, such as the development of female-sized breasts in individuals with severe androgen resistance (*30*). The ratio of estradiol to testosterone was not clearly associated with risk in our data, perhaps because once again ratios account for relative but not absolute hormone amounts. Changes in SHBG have a more profound effect on free testosterone than on free estradiol in the circulation, since the binding affinities are different for these hormones. Therefore, an increase in SHBG produces a greater fall in free testosterone than in free estradiol. This effect on the active estrogen/androgen balance is another potential mechanism for a protective effect of SHBG (*31*).

Although DHT is clearly important in regulating prostate cell growth, the absence of any strong association for this hormone in our data could be explained by a poor correlation between plasma DHT and the levels of DHT formed in the prostate. Indeed, much of the circulating DHT is produced in the skin, and DHT in the prostate is rapidly reduced to androstanediols. 3α-Androstanediol is a potent prostatic androgen in its own right, and its glucuronide conjugate (AAG) has been established as a useful plasma marker of androgen action in peripheral tissues (*8*). Ross et al. (*32*) compared AAG levels in age-matched groups of young Japanese, white, and African-American men and found significantly lower levels in the Japanese, who are at low risk for prostate cancer. Our results show a relatively weak independent association between plasma AAG and prostate cancer, especially when compared with the association for SHBG- and estradiol-adjusted testosterone. A small case–control study (*33*) previously found a nonsignificant elevation of plasma AAG in the case patients with prostate cancer. Further study of AAG is clearly warranted.

Our study was limited by the availability of only a single plasma sample. However, sex steroid levels in men are relatively stable over time, and a single value should adequately rank control subjects within a population (*34,35*). Small circadian and seasonal variations in testosterone level do exist. Although our case patients and control subjects were not matched for the time of blood drawing, 80% of the samples were collected during the morning. Furthermore, errors in hormone measurement that are randomly distributed between case patients and control subjects would attenuate the estimates of any real association between hormone levels and risk. In fact, when we adjusted for the time of blood draw using the 79% of study participants for whom this time was known, we found that the associations for testosterone and SHBG increased to a small degree (data not shown). We also observed a 20%-25% difference in the mean AAG level according to the time of day that blood was drawn, which is consistent with unpublished observations (Horton R: personal communication). Adjustment for the time of blood draw produced a small increase in the risk associated with AAG level, but these results must be interpreted cautiously because 21% of the study participants did not have a time recorded.

Although larger than previous epidemiologic studies addressing these questions, our study is still relatively limited in its power to detect varying effects among subgroups. In particular, the correlation between testosterone and SHBG reduces the number of men in the analysis whose levels of these hormones are discordant. More precise study of the combined effect of testosterone and SHBG is important because this could help determine whether both compounds act only through their relation to bioavailable testosterone or whether SHBG operates through an additional mechanism. Direct measurement or estimation of free and albumin-bound testosterone in future studies is important. Rather than measure each bioavailable hormone directly, we chose to measure SHBG and adjust for it in the analysis because this allowed more efficient use of the limited plasma volume available.

Our results support the view that long-term exposure to high levels of endogenous bioavailable testosterone—and perhaps to low levels of estradiol—promotes the development of prostate cancer. The interrelationships between these hormones and SHBG could have obscured similar findings in previous epidemiologic studies. Levels of testosterone, SHBG, and estradiol have overlapping but ultimately distinct sets of determinants. Confirmation that each compound has an independent effect on risk, as suggested by our data, should guide further research into ways to achieve sustained modification of these levels, with the ultimate goal of developing strategies for the primary prevention of prostate cancer.

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Notes

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