Circulating Steroid Hormones and the Risk of Prostate Cancer

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Abstract

Epidemiologic studies have failed to support the hypothesis that circulating androgens are positively associated with prostate cancer risk and some recent studies have even suggested that high testosterone levels might be protective particularly against aggressive cancer. We tested this hypothesis by measuring total testosterone, androstanediol glucuronide, androstenedione, DHEA sulfate, estradiol, and sex hormone–binding globulin in plasma collected at baseline in a prospective cohort study of 17,049 men. We used a case-cohort design, including 524 cases diagnosed during a mean 8.7 years follow-up and a randomly sampled subcohort of 1,859 men. The association between each hormone level and prostate cancer risk was tested using Cox models adjusted for country of birth. The risk of prostate cancer was ~30% lower for a doubling of the concentration of estradiol but the evidence was weak (P_{trend} = 0.07). None of the other hormones was associated with overall prostate cancer ($P_{trend} \geq 0.3$). None of the hormones was associated with nonaggressive prostate cancer (all $P_{trend} \geq 0.2$). The hazard ratio [HR; 95% confidence interval (95% CI)] for aggressive cancer almost halved for a doubling of the concentration of testosterone (HR, 0.55; 95% CI, 0.32-0.95) and androstenedione (HR, 0.51; 95% CI, 0.31-0.83), and was 37% lower for a doubling of the concentration of DHEA sulfate (HR, 0.63; 95% CI, 0.46-0.87). Similar negative but nonsignificant linear trends in risk for aggressive cancer were obtained for free testosterone, estradiol, and sex hormone–binding globulin (P_{trend} = 0.06, 0.2, and 0.1, respectively). High levels of testosterone and adrenal androgens are thus associated with reduced risk of aggressive prostate cancer but not with nonaggressive disease. (Cancer Epidemiol Biomarkers Prev 2006;15(1):86–91)

Introduction

Although it is established that sex steroid hormones, particularly androgens, are essential to the growth, development, and maintenance of healthy prostate epithelium, and to the progression of prostate cancer, epidemiologic studies have thus far failed to show that high levels of circulating androgens increase the risk of developing prostate cancer—the ''androgen hypothesis.'' A review of 10 prospective epidemiologic studies where blood had been sampled before diagnosis of prostate cancer (1) found that there was no evidence that serum levels of endogenous sex hormones and their binding protein [sex hormone–binding globulin (SHBG)] were associated with the risk of developing prostate cancer. There was only a slightly increased risk associated with high levels of androstanediol glucuronide that was of marginal statistical significance. Of the 10 studies reviewed, only one (2) reported positive associations between androgen concentrations (testosterone and androstanediol glucuronide) and the risk of prostate cancer and, incidentally, inverse associations with estradiol and SHBG levels. Many reasons for the lack of evidence to support the ''androgen hypothesis'' have been offered in explanation, including laboratory measurement error in hormone assays and the heterogeneity of prostate cancer phenotypes, a problem that has been compounded in recent years by prostate-specific antigen (PSA) testing (3).

Some of these issues have been addressed in most of the five recent prospective studies that were published after Eaton's review (4-8). Interestingly, in three of these studies (6-8), the risk of prostate cancer was reduced in men with higher levels of testosterone although none of the estimates were statistically significant. Suggestive evidence from two studies (6, 7) led us to hypothesize that high testosterone levels decrease the risk of aggressive prostate cancer. We also hypothesized that estrogens and adrenal androgens might be inversely associated with aggressive prostate cancer.

We tested these hypotheses by analyzing a number of steroid hormones and related molecules measured in blood samples taken at baseline from men enrolled in the Melbourne Collaborative Cohort Study.

Materials and Methods

Subjects and Case-Cohort Design. The Melbourne Collaborative Cohort Study is a prospective cohort study of 41,528 people (17,049 men) ages between 27 and 75 years at baseline (99.3% of whom were ages 40-69 years). Recruitment occurred between 1990 and 1994 in the Melbourne metropolitan area. Details of the study have been published elsewhere (9, 10). The Human Research Ethics Committee of the Cancer Council Victoria approved the study protocol. Subjects gave written consent to participate and for the investigators to obtain access to their medical records.

A case-cohort design was used for studies that included the analysis of plasma. All men first diagnosed with prostate cancer between baseline and June 30, 2002, were eligible, as was a random sample (hereafter called the subcohort) of 2,167 men from the cohort. The study was designed to have the same

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power as a nested case-control study with two controls per case; preliminary analysis based on a method by Wacholder (11) suggested the subcohort needed to have 3.6 times as many members as there were cases of prostate cancer. For this analysis, men were excluded if they had a confirmed diagnosis of prostate cancer before baseline (n = 106 in the full cohort and 9 in the subcohort).

Case Ascertainment. Addresses and vital status of the subjects were determined by record linkage to Electoral Rolls, Victorian death records, the National Death Index, from electronic phone books, and from responses to mailed questionnaires and newsletters. Cases were ascertained by record linkage to the Victorian Cancer Registry, the population registry that covers the region in which the cohort resides. Between baseline attendance and June 30, 2002, 279 men in the full cohort had left Victoria and 1,257 had died.

A total of 614 men were diagnosed with prostate cancer over an average of 8.7 person-years of follow-up between 1990 and mid-2002. Seventy-five of these cases were members of the subcohort. Classification of cases as aggressive and nonaggressive was made on the basis that only cases with a distant-stage or poorly differentiated tumor have excess mortality compared with the general population (12). Prostate cancer was, therefore, defined as ''aggressive'' if the Gleason score was higher than 7 or if it was classified as poorly differentiated. Cases with stage T_4 or N_+ (positive lymph nodes) or M_+ (distant metastases) were classified as aggressive irrespective of the Gleason score or grade of tumor differentiation. Nine cases had no blood collected at baseline and were therefore excluded, leaving 605 cases eligible for this study.

Assessment of Circulating Levels of Steroid Hormones. To study the relationship between disease and steroid sex hormones and other biological markers, each participant had blood collected at baseline of which 2 mL plasma was stored in liquid nitrogen. Hormone measurements were not made for 367 men (81 cases) because they had insufficient plasma left, a few samples were contaminated, and one batch of samples was not retrieved from storage. Therefore, hormone measurements and the statistical analysis were made for only 1,859 members of the subcohort (86%) and 524 case subjects (85%). There were no statistically significant differences in either demographics (age at baseline, year of attendance, country of birth, education, and smoking and alcohol consumption) or tumor characteristics (stage and Gleason score) between the men who had their hormones measured and those who did not.

Plasma samples were retrieved from storage, aliquoted into 450 μL amounts, and shipped on dry ice in batches of ~80 samples each to the laboratory of one of us (H.A. Morris), where SHBG, testosterone, estradiol, androstanediol glucuronide, androstenedione, and DHEA sulfate (DHEAS) were to be measured. Assignment to batches was done randomly and the proportions of cases and subcohort members were approximately equal for all batches. Ten percent of the samples in each batch were aliquots from pooled plasma that had been stored with the samples from participants. The laboratory was blind to status of the samples. One scientist did all measurements.

Samples were thawed in a warm water bath, vortexed rapidly for a few seconds, and centrifuged at 2,000 rpm (210 × g) for 10 minutes. Total PSA was measured by microparticle enzyme immunoassay (AXSYM analyzer, Abbott Laboratories, Abbott Park, IL) with an interassay coefficient of variation (CV) at 0.4 ng/mL of 9.5%. DHEAS was measured by competitive immunoassay (IMMULITE analyzer, DPC, Los Angeles, CA) with a CV at 2.1 μmol/L of 12.4%. Testosterone followed by estradiol was measured by electrochemiluminescence immunoassay (Elecsys 2010 analyzer, Roche Diagnostics GmbH, Mannheim, Germany) with a CV for testosterone at 36 nmol/L of 1.6% and estradiol at 93 pmol/L of 11.1%. SHBG was measured by immunometric assay (IMMULITE analyzer, DPC) with a CV at 26 nmol/L of 6%. Androstenedione and androstanediol glucuronide were analyzed by RIA (DSL-4200 and DSL-6000, respectively; TX) with a CV for androstenedione at 3.3 nmol/L of 10.7% and androstanediol glucuronide at 21.1 nmol/L of 4.3%.

Before the study began, a reliability study was done. Plasma samples from 44 men who had given blood twice ~1 year apart were each divided into two aliquots. The two aliquots were measured in separate batches a week apart. As a measure of reliability, we used the intraclass correlation, which is the proportion of the total variance due to variation between persons, where the total variance included components due to between persons, between-sampling occasions, and between-laboratory runs.

Statistical Analysis. Steroid hormone levels were categorized into quartiles according to the distribution of the values for the subcohort. Quartiles were assigned within each laboratory batch to adjust for any variation between batches. Tests for linear trend were based on pseudocontinuous variables under the assumption that all subjects within each quartile had the same concentrations equal to the within-quartile median. The pseudocontinuous variables were $\log_2$ transformed before inclusion in the models so that the hazard ratio (HR) would represent the relative difference in risk associated with a doubling of the concentration.

Free testosterone and free estradiol were calculated from the total concentration and from the concentration of SHBG using the law of mass action (13) under the assumption of a fixed albumin concentration of 40 g/L. For this calculation, we used the association constants of de Ronde et al. (14). The ratio estradiol/testosterone was calculated as an indicator of feminization.

Cox proportional hazards regression models, with age as the time axis (15), were used to estimate HR values and 95% confidence intervals (95% CI). We used the Prentice method to take the case-cohort sampling into account and the robust method was used to calculate the variance-covariance matrix (16, 17). Follow-up for a subcohort member began at baseline and ended at diagnosis of prostate cancer or cancer of unknown primary site, death, the date last known to be in Victoria, or June 30, 2002, whichever came first. To estimate HR values for nonaggressive and aggressive cases and to test their difference, we fitted stratified Cox models based on competing risks using a data duplication method (18).

Tests based on Schoenfeld residuals (19) showed no evidence that proportional hazard assumptions were violated for any of the hormones. In a sensitivity analysis to investigate the possible effect of prevalent prostate cancers, we tested whether the HR values differed before and after the first 2 years of follow-up (15).

The Melbourne Collaborative Cohort Study was specifically designed to be a multiethnic cohort and the Italian and Greek communities were ''oversampled'' to obtain a wider range of exposures. Analyses were, therefore, adjusted for country of birth (Australia/New Zealand, United Kingdom, Italy, and Greece). Adjustments for smoking status, alcohol consumption, education, body mass index, and energy intake did not appreciably change the HR values, so these variables were not included in final analyses. To study the influence of simultaneous adjustment for all measured hormones, we included them in a single model. Free testosterone and estradiol and the ratio estradiol/testosterone were not included simultaneously with total testosterone and estradiol because of their high correlations.

Statistical analyses were done using Stata/SE 8.2 (Stata Corporation, College Station, TX). Because the robust method was used to calculate the variance-covariance matrix, the Wald test, not the likelihood ratio test, was used to test hypotheses. All P values were two-sided and $P < 0.05$ was considered as statistically significant.

Results

For the case subjects included in the analysis, the mean age at diagnosis was 67 years (range, 47-80 years) and 88 (17%) had aggressive cancer (i.e., had Gleason score >7 or had extraprostatic invasion: T_4 or N_+ or M_+). Table 1 shows baseline characteristics of the cases and the subcohort. About 72% of men in the subcohort were born in Australia, New Zealand, or the United Kingdom and 27% in Italy or Greece.

Table 2 shows HR values for prostate cancer by quartiles and the test for linear trend across the quartiles for each hormone, adjusted for country of birth. There was little evidence that levels of androgens influenced overall risk of prostate cancer. The risk of prostate cancer was ~30% lower for a doubling of the concentration of estradiol (HR, 0.71; 95% CI, 0.50-1.03) but the evidence was weak ($P_{trend} = 0.07$). The HR values for quartiles II to IV relative to the first quartile of estradiol were all between 0.68 and 0.73 and significantly less than unity.

Competing risk analyses showed that the linear trends in the HR values for testosterone, DHEAS, androstenedione, and free testosterone differed significantly between aggressive and nonaggressive cancers ($P = 0.005$ for androstenedione, 0.007 for DHEAS, 0.01 for testosterone, and 0.03 for free testosterone; Table 3). Although there was virtually no relationship between the incidence of nonaggressive prostate cancer and hormone levels, the risk of aggressive prostate cancer significantly decreased with increasing levels of testosterone, DHEAS and androstenedione (P_{trend} between 0.005 and 0.03). For example, the risk almost halved with a doubling of the concentration of testosterone (HR, 0.55; 95% CI, 0.32-0.95) and androstenedione (HR, 0.51; 95% CI, 0.31-0.83), and was 37% lower with a doubling of the concentration of DHEAS (HR, 0.63; 95% CI, 0.46-0.87). The dose-response relationship for free testosterone was virtually identical to that observed for testosterone, the HR for a doubling of the concentration being 0.54 (95% CI, 0.29-1.01, $P_{trend} = 0.06$). Similar, but not statistically significant, negative linear trends in risk for aggressive prostate cancer were observed for estradiol and SHBG ($P_{trend} = 0.2$ and 0.1, respectively). The HR values did not change appreciably after removing the adjustment for country of birth or after further adjustment for baseline PSA values. The HR values relative to the first 2 years of follow-up did not significantly differ from the estimates relative to the rest of the follow-up (data not shown).

The inclusion of all measured hormones in a single model did not appreciably change the HR values for overall prostate cancer (all $P_{trend} \geq 0.09$, data not shown). The inclusion of all measured hormones in the competing risk model widened 95% CI values and increased HR values associated with a doubling of the concentrations of testosterone, DHEAS, and androstenedione. The HR values for aggressive prostate

Table 1. Demographic characteristics and hormone levels of subjects (cases and subcohort)

	Prostate cancer cases		Subcohort (n = 1,859)
	Aggressive* (n = 88)	Nonaggressive (n = 430)	
Age at baseline (y)			
<50	2 (2%)	18 (4%)	585 (31%)
50-59	14 (16%)	120 (28%)	577 (31%)
60+	72 (82%)	292 (68%)	697 (37%)
Country of birth			
Australia/New Zealand/United Kingdom	72 (82%)	342 (80%)	1,345 (72%)
Italy	13 (15%)	49 (11%)	263 (14%)
Greece	3 (3%)	39 (9%)	251 (14%)
Smoking			
Never	33 (38%)	176 (41%)	735 (40%)
Former	49 (56%)	209 (49%)	830 (45%)
Current	6 (7%)	45 (11%)	294 (16%)
Alcohol (g/d)			
No alcohol	25 (28%)	88 (21%)	341 (18%)
1-39	49 (56%)	275 (64%)	1,195 (64%)
40+	14 (16%)	66 (15%)	321 (17%)
BMI			
Mean (SD)	27.9 (3.8)	27.1 (3.5)	27.2 (3.6)
Hormone and PSA levels†, median (IQ range)			
PSA (ng/mL)	4.6 (2.1-18.4)	3.4 (1.9-6.9)	0.8 (0.5-1.5)
T (nmol/L)	15.0 (11.8-17.7)	15.6 (13-19.3)	15.7 (12.3-19.5)
DHEAS (μmol/L)	2.0 (1.1-3.1)	2.8 (1.7-4.1)	3.4 (2.2-5.1)
SHBG (nmol/L)	39.0 (29.4-46.1)	38.2 (31.1-46.6)	35.8 (27.8-44.9)
A (nmol/L)	3.1 (2.3-4.2)	3.7 (2.7-4.6)	3.7 (2.8-4.8)
E2 (pmol/L)	101 (80-124)	105 (88-126)	105 (89-125)
AG (ng/mL)	14.0 (8.1-20.5)	13.4 (9.7-18.9)	14.7 (10.2-20.1)
E2/T ($\times 10^{-3}$)	6.9 (5.5-9.0)	6.7 (5.5-8.2)	6.6 (5.4-8.5)
Free T‡ (nmol/L)	0.35 (0.30-0.39)	0.36 (0.31-0.42)	0.37 (0.31-0.45)
Free E2‡ (pmol/L)	2.9 (2.4-3.4)	3.0 (2.5-3.6)	3.1 (2.5-3.6)

Abbreviations: T, total testosterone; A, androstenedione; E2, total estradiol; AG, androstanediol glucuronide; BMI, body mass index.

*A tumor was classified as aggressive if Gleason score was higher than 7 or if stage was advanced (T_4 or N_+ or M_+). We were not able to define aggressiveness for six cases because Gleason score and tumor stage were not available (clinical diagnoses only).

†The number of missing measures were 25 for PSA, 24 for total testosterone, 47 for DHEAS, 2 for SHBG, 8 for androstenedione, 81 for total estradiol, and 200 for androstanediol glucuronide.

‡Derived from the total concentration (bound + free), concentration of SHBG using the law of mass action under the assumption of a fixed albumin concentration of 40 g/L (5.77×10^{-4} mol/L), and the association constants of de Ronde et al. (14).

Table 2. Relative risk of prostate cancer by quartile of hormone levels

	Quartile I*	Quartile II	Quartile III	Quartile IV	P_{trend} †
		HR‡ (95% CI)	HR (95% CI)	HR (95% CI)	
T	Reference	1.30 (0.97-1.75)	1.09 (0.80-1.48)	1.09 (0.80-1.47)	0.9
DHEAS	Reference	0.84 (0.64-1.10)	0.95 (0.71-1.26)	0.82 (0.58-1.15)	0.3
SHBG	Reference	1.14 (0.83-1.57)	1.00 (0.72-1.38)	0.92 (0.67-1.26)	0.3
A	Reference	0.92 (0.69-1.22)	0.88 (0.66-1.17)	0.92 (0.69-1.24)	0.5
E2	Reference	0.68 (0.50-0.93)	0.68 (0.50-0.92)	0.73 (0.55-0.98)	0.07
AG	Reference	0.91 (0.68-1.22)	0.83 (0.61-1.14)	0.87 (0.64-1.18)	0.3
E2/T	Reference	1.13 (0.83-1.54)	1.01 (0.75-1.37)	0.93 (0.69-1.27)	0.5
Free T	Reference	1.35 (1.02-1.79)	1.20 (0.89-1.61)	1.01 (0.74-1.38)	0.9
Free E2	Reference	0.95 (0.71-1.27)	0.83 (0.61-1.12)	0.90 (0.67-1.20)	0.4

*The quartiles were assigned within each laboratory batch to adjust for any variation between batches.
†HR values from Cox regression models adjusted for country of birth (Australia/New Zealand, United Kingdom, Italy, and Greece). The Prentice method has been used to take into account the case-cohort sampling (see Materials and Methods).
‡The hypothesis of a linear trend in the HR was tested including in the model a pseudocontinuous variable computed assigning the median level of the specific hormone for each quartile.

cancer, however, remained well below unity: 0.75 (95% CI, 0.33-1.67) for testosterone, 0.70 (95% CI, 0.48-1.02) for DHEAS, and 0.67 (95% CI, 0.36-1.25) for androstenedione. The HR associated with a doubling of the concentration of estradiol increased to 0.99 (95% CI, 0.35-2.81). No HR for any other hormone was statistically significant after simultaneous adjustment.

Reliability and Quality Control. From the reliability study, the intraclass correlation for testosterone was 0.73 (95% CI, 0.60-0.86), for DHEAS 0.91 (95% CI, 0.86-0.95), for SHBG 0.88 (95% CI, 0.82-0.94), for androstenedione 0.46 (95% CI, 0.25-0.68), for estradiol 0.65 (95% CI, 0.41-0.88), for androstanediol glucuronide 0.84 (95% CI, 0.75-0.92), and for PSA 0.56 (95% CI, 0.35-0.76). For the pooled plasma samples, the overall CV was 7% for testosterone (4% within batches and 5% between batches), 10% for DHEAS (9% and 6%), 7% for SHBG (6% and 4%), 15% for androstenedione (11% and 9%), 10% for estradiol (8% and 6%), 10% for androstanediol glucuronide (9% and 5%), and 12% for PSA (8% and 10%).

Discussion

We found that prediagnostic circulating levels of testosterone and other androgens were associated with a reduced risk of aggressive, but not localized, prostate cancer. Further, levels of a major estrogen, estradiol, also seemed to be protective against aggressive disease. Our findings do not support the long prevailing ''androgen hypothesis'' that high levels of circulating androgens increase the risk of prostate cancer (3).

The main strengths of our study are its large size, high level of follow-up, and a large number of aggressive cases relative to other studies. To increase phenotype specificity compared with the other two studies that considered tumor aggressiveness (6, 7), we did not include T_3 cases with Gleason scores of 7 or lower with the aggressive cases. Another strength is the quality of our hormone measurement as evidenced by high intraclass correlations and low CV values for pooled plasma samples. One weakness is the lack of information on family history of prostate cancer.

Table 3. Relative risk of prostate cancer by quartile of hormone levels and by tumor aggressiveness

	Quartile I*	Quartile II	Quartile III	Quartile IV	P_{trend} †	P ‡
		HR§ (95% CI)	HR (95% CI)	HR (95% CI)		
Nonaggressive cases						
T	Reference	1.40 (1.01-1.93)	1.18 (0.85-1.64)	1.25 (0.90-1.72)	0.4	—
DHEAS	Reference	0.92 (0.69-1.23)	1.08 (0.79-1.47)	0.96 (0.67-1.38)	0.9	—
SHBG	Reference	1.25 (0.89-1.77)	0.98 (0.69-1.40)	1.01 (0.72-1.42)	0.6	—
A	Reference	1.04 (0.76-1.42)	1.04 (0.76-1.41)	1.09 (0.79-1.49)	0.6	—
E2	Reference	0.78 (0.56-1.08)	0.82 (0.60-1.13)	0.76 (0.55-1.05)	0.2	—
AG	Reference	0.94 (0.69-1.29)	0.81 (0.58-1.13)	0.90 (0.65-1.25)	0.4	—
E2/T	Reference	1.14 (0.83-1.59)	1.03 (0.75-1.43)	0.84 (0.60-1.17)	0.2	—
Free T	Reference	1.45 (1.07-1.96)	1.29 (0.93-1.78)	1.16 (0.83-1.63)	0.4	—
Free E2	Reference	1.01 (0.74-1.38)	0.87 (0.63-1.21)	0.94 (0.69-1.30)	0.6	—
Aggressive cases						
T	Reference	0.96 (0.54-1.70)	0.67 (0.36-1.25)	0.53 (0.28-1.03)	0.03	0.01
DHEAS	Reference	0.53 (0.31-0.92)	0.54 (0.29-1.02)	0.38 (0.15-0.95)	0.005	0.007
SHBG	Reference	0.71 (0.36-1.39)	0.90 (0.48-1.70)	0.54 (0.28-1.04)	0.1	0.2
A	Reference	0.56 (0.31-1.00)	0.49 (0.27-0.88)	0.46 (0.24-0.88)	0.007	0.005
E2	Reference	0.40 (0.21-0.74)	0.24 (0.12-0.50)	0.63 (0.37-1.09)	0.2	0.5
AG	Reference	0.71 (0.38-1.36)	1.04 (0.56-1.91)	0.80 (0.41-1.55)	0.7	0.9
E2/T	Reference	0.93 (0.46-1.88)	0.91 (0.47-1.79)	1.47 (0.79-2.74)	0.2	0.06
Free T	Reference	1.00 (0.57-1.73)	0.81 (0.44-1.48)	0.50 (0.24-1.04)	0.06	0.03
Free E2	Reference	0.75 (0.41-1.38)	0.73 (0.39-1.37)	0.73 (0.39-1.36)	0.3	0.5

NOTE: A tumor was classified as aggressive if Gleason score was higher than 7 or stage was advanced (T_4 or N_+ or M_+). We were not able to define aggressiveness for six cases because Gleason score and tumor stage were not available (clinical diagnoses only).
*The quartiles were assigned within each laboratory batch to adjust for any variation between batches.
†The hypothesis of a linear trend in the HR was tested including in the model a pseudocontinuous variable computed assigning the median level of the specific hormone for each quartile.
‡Test for difference in the estimates for the pseudocontinuous variables (i.e., linear trend) between aggressive and nonaggressive cases.
§HR values from Cox regression model fitted using competing risk methods to obtain separate estimates for aggressive and nonaggressive tumors.

Historically, the lack of epidemiologic evidence for any association between circulating androgens and prostate cancer may have been due to grouping all prostate cancers as a single entity. This interpretation is supported by the lack of associations when all prostate cancers were analyzed together (see Table 1). Older epidemiologic studies may also have had problems with measuring various hormone levels adequately, which would have tended to attenuate risk estimates. However, none of the five cohort studies of hormones and prostate cancer risk published since Eaton's review (1) found evidence to support the androgen hypothesis (4-8). A nested case-control analysis of 300 cancers and 300 controls from the CARET study (6) showed that higher serum testosterone concentrations were not associated with increased risk [odds ratio (OR), 0.72; 95% CI, 0.45-1.14] and that OR values for higher concentrations of androstenedione, DHEAS, and androstanediol glucuronide were not significantly different from unity. A nested case-control study of 166 cases and 332 controls within a Finnish cohort study (5) showed no association between testosterone, SHBG, and androstenedione and prostate cancer risk: the relative risk comparing the highest and lowest quintiles of testosterone being 1.27 (95% CI, 0.67-2.37). The Massachusetts Male Aging Study measured serum levels of 17 hormones (4) and, comparing 70 cases of prostate cancer with the remaining 1,576 members, reported only one significant finding—a nonlinear association with androstanediol glucuronide levels.

Our findings are consistent with some recent well-conducted prospective studies that show that higher circulating levels of testosterone are associated not with an increased but with a decreased risk of prostate cancer (7, 8). In a case-control study of 708 cases and 2,242 controls nested in three Scandinavian cohort studies, men in the highest quintiles of serum levels of testosterone had a 20% lower risk of prostate cancer than men in the lowest quintile but the result was not statistically significant (OR, 0.80; 95% CI, 0.59-1.06; ref. 8). The most recent analysis, from the Health Professionals Follow-up Study (7), included 460 prostate cancer cases diagnosed in the PSA era (only 40 of which were regionally invasive or metastatic) and 460 age-matched controls that were PSA screened after their blood draw. Based on Gleason score, they categorized cases into low grade (<7) and high grade (≥7). Although no association was observed between plasma testosterone, DHT, androstanediol glucuronide, estradiol, or SHBG and total prostate cancer, a positive association with testosterone (top compared with bottom quartile) was observed for low-grade disease (OR, 1.91; 95% CI, 0.89-4.07; P_{trend} = 0.02) and an inverse association was observed for high-grade disease (OR, 0.26; 95% CI, 0.10-0.66; P_{trend} = 0.01). For high-grade disease, SHBG was positively associated with risk (OR, 2.72; 95% CI, 1.02-7.24) as was estradiol/testosterone (OR, 3.02; 95% CI, 1.29-7.04). An analysis of regionally invasive or metastatic disease, comparing top and bottom quartiles of testosterone, gave an OR of 0.48 (95% CI, 0.06-3.69).

Our finding that both DHEAS and androstenedione had similar associations to testosterone with the risk of aggressive prostate cancer is noteworthy as both of these weaker androgens can be converted by 17-β-hydroxysteroid dehydrogenase to testosterone (20). Further, androstenedione can be converted to DHT by first being converted by 5α reductase type 2 to androstanedione and then by 17-β-hydroxysteroid dehydrogenase to DHT (20). Interestingly, androstanediol glucuronide levels, which are supposed to reflect the conversion of testosterone to DHT (21), were not associated with the risk of either local or aggressive disease.

As discussed in a recent review, there are additional complexities that need to be considered to clarify the effect of hormones on prostate cancer risk (3); for example, the relevance of hormone measurements that are usually made on a single blood sample drawn in middle age, the relevance of circulating levels of hormones *vis a vis* intraprostatic levels, and the possibility that the hormonal milieu *in utero* and during puberty might be important to carcinogenic processes much later in life.

In conclusion, the results of our study contribute to the gathering evidence that the longstanding ''androgen hypothesis'' of increasing risk with increasing androgen levels can be rejected, suggesting instead that high levels within the reference range of androgens, estrogens, and adrenal androgens decrease aggressive prostate cancer risk. This evidence is consistent with the role that testosterone plays in the proper differentiation of prostatic epithelium and the rising incidence of prostate cancer with the androcline—as testosterone levels decline with increasing age, their control over differentiation similarly declines. A recent report on the Prostate Cancer Prevention Trial, an intervention trial to prevent prostate cancer using finasteride, a 5α reductase inhibitor, showed a decrease in the risk of all prostate cancer in the intervention arm together with an excess of high-grade cancers (22). Although the increased incidence of high-grade tumors in the group treated with finasteride might be due to a pathologic artifact (22), our results suggest that the drug, lowering androgens levels, might favor the development of aggressive prostate cancer.

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