Short Communication

Serum Testosterone and the Risk of Prostate Cancer: Potential Implications for Testosterone Therapy

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Abstract

Objective: **A potential risk of testosterone replacement therapy is an increase in the incidence of prostate cancer, but it is unclear whether higher levels of serum testosterone are associated with a higher risk of prostate cancer. We prospectively evaluated serum androgen concentrations and prostate cancer risk.**

Method: **Included were 794 members of the Baltimore Longitudinal Study of Aging. We estimated the rate ratio (RR) of prostate cancer by entering serial measures of serum total testosterone, dehydroepiandrosterone sulfate, sex hormone binding globulin, calculated free testosterone, and free testosterone index (FTI) into a Cox proportional hazards regression model with simple updating.**

Results: **Higher calculated free testosterone was associated with an increased age-adjusted risk of prostate cancer {RRs by quartile: 1.00, 1.52 [95% confidence interval (95% CI), 0.93-2.50], 1.16 (95% CI, 0.61-2.20), 2.59 (95% CI, 1.28-5.25); P_{trend} = 0.03}, which persisted after excluding measures in men <45 years of age [RRs by quartile: 1.00, 1.33 (95% CI, 0.78-2.25), 1.26 (95% CI, 0.68-2.33), 1.89 (95% CI, 0.99-3.61); P_{trend} = 0.03]. Compared to men with eugonadal FTI (≥0.153), men with hypogonadal FTI had a decreased risk of prostate cancer (RR, 0.51; 95% CI, 0.31-0.82).**

Conclusion: **Higher levels of calculated serum free testosterone are associated with an increased risk of prostate cancer. These findings suggest that men receiving testosterone therapy should be regularly monitored for prostate cancer and underscore the need for prospective trials of testosterone therapy incorporating incidence of prostate cancer as a primary safety end point.** (Cancer Epidemiol Biomarkers Prev 2005;14(9):2257–60)

Introduction

Serum testosterone concentrations in men decline with age (1, 2). Although its clinical benefits remain unproven (3, 4), the popularity of testosterone replacement therapy for older men has grown substantially in recent years (3, 4).

A potential risk of testosterone therapy is an increase in the incidence of prostate cancer (3, 4). However, whereas androgen depletion hinders the development and clinical progression of prostate cancer (5, 6), and exogenous testosterone may stimulate growth of metastatic prostate cancer (7), it is unclear whether higher levels of serum testosterone are associated with a higher risk of prostate cancer (3, 4, 8, 9).

Therefore, we prospectively evaluated the association between serum androgen concentrations and the risk of prostate cancer in a cohort of community-dwelling men.

Materials and Methods

We identified a cohort of male participants in the Baltimore Longitudinal Study of Aging (BLSA) from whom serial serum hormone measures were obtained over a period of several decades. The BLSA is an ongoing cohort study, with continuous entry of new participants, of the physiology of aging directed by the U.S. National Institute of Aging and approved by the combined Institutional Review Board of the Johns Hopkins Medical Institutions and the Gerontology Research Center of the National Institute on Aging. The BLSA has been recruiting participants since 1958, who return approximately every 2 years for comprehensive physiologic and psychological testing (10).

Since 1958, prostate cancer diagnoses have been recorded as part of the study. In 1991, these diagnoses were confirmed by systematic review of all BLSA records and questionnaires mailed to BLSA participants. In addition, since 1991, male participants have undergone prostate cancer screening with digital rectal examination and serum prostate-specific antigen testing; since 1993, in accordance with standard clinical practice, male participants have undergone trans-rectal ultrasound-directed prostate biopsy for a prostate-specific antigen level of >4.0 ng/mL and/or a digital rectal examination suspicious for prostate cancer.

A total of 3,651 individual hormone measures, obtained from 1961 to 1998, were available on 901 male participants who were censored at date of last follow-up or at time of death. We also censored participants at the time of prostate cancer diagnosis (n = 88 observations for blood drawn after diagnosis), transurethral resection of the prostate (n = 469 observations), and first use of finasteride, a medication which alters the risk of prostate cancer (n = 136 observations; ref. 5). This left 794 male participants with 2,958 hormone measures, of whom 114 (14.4%) had a histologically confirmed diagnosis of prostate cancer. Participants who had incidental prostate cancer identified at autopsy (n = 14) were censored at date of death and were included

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in the analysis as noncases. Follow-up for person-time at risk began at date of first blood draw, which could have been at any point during the period over which samples were obtained, depending on when participants entered the study. All available samples for participants meeting study criteria were used in the analysis. Losses to follow-up were assumed to be noninformative. The follow-up period ended on March 14, 2003.

Samples were obtained between 7:00 a.m. and 9:30 a.m. after an overnight fast. Serum testosterone, sex hormone binding globulin (SHBG), and dehydroepiandrosterone sulfate (DHEAS) concentrations were measured as described previously (1). To account for the effect of storage time on apparent testosterone concentrations, date-adjusted estimates were developed and applied using mixed effects models as described previously (1). Free testosterone concentration was calculated with the mass action equation as described by Vermeulen et al. (11). Free testosterone index (FTI), another calculated indicator of free testosterone (11, 12), was calculated as the molar ratio of testosterone to SHBG.

We assessed differences between cases and noncases in age, hormone concentrations, height, and weight at time of initial evaluation and in the time elapsed from date of first evaluation to date of cancer diagnosis or censorship by two-tailed t test with $\alpha = 0.05$. We categorized tumor grade as low (Gleason sum < 7) or high (Gleason sum ≥ 7). We estimated the rate ratio (RR) of prostate cancer, high-grade tumor, and low-grade tumor by entering repeated measures of total testosterone, DHEAS, SHBG, calculated free testosterone, and FTI into a Cox proportional hazards regression model with simple updating; at each failure time, the most recent hormone measure for the case and the at-risk individuals was considered (13). We adjusted for age, body mass index, and muscle mass as determined by 24-hour urinary creatinine excretion.

Results

Mean (unadjusted) participant ages, anthropometric data, and sex steroid hormone concentrations are summarized in Table 1. Median follow-up for all participants was 18.5 years. The mean (SD) durations of follow-up for prostate cancer cases and noncases were 16.3 (8.8) and 20.0 (8.6) years, respectively ($P = 0.01$). The mean (SD) numbers of serum hormone measures for prostate cancer cases and noncases were 3.7 (1.8) and 3.8 (1.7), respectively ($P = 0.57$). Median age at cancer diagnosis was 73.1 years (range, 49-90 years). Associations of age with total testosterone, SHBG, and FTI did not differ from those previously described for this data set (data not shown; ref. 1). Mean times from date of final hormone measure to date of censoring for noncases and from date of final hormone measure to date of diagnosis for cases were 9.3 (5.0) and 4.5 (5.5) years, respectively ($P < 0.001$ by both Student's t test and Wilcoxon rank-sum test).

There were no significant age-adjusted associations of serum total testosterone, DHEAS, or SHBG with prostate cancer (Table 2). Calculated free testosterone and FTI were each associated with an increased risk of prostate cancer (Table 2). Because hormone measures in the fourth quartile were predominantly from young men with a lower risk of cancer, we were concerned about residual confounding by age. Therefore, we excluded observations in participants <45 years of age and generated new quartile cut points that showed similar patterns of risk (Table 2). Due to the relatively limited number of measures obtained from men <45 years of age, these data did not allow for an analysis of the risk difference between younger and older men.

Addition of SHBG to the models did not change the significance of the associations of calculated free testosterone and FTI with prostate cancer, suggesting that SHBG concentrations were not driving these associations. There was no association between hormone levels and tumor grade (data not shown).

Table 1. Mean (SD) sex steroid hormone concentrations and anthropometric data at study entry for a cohort of 794 men from the Baltimore Longitudinal Study of Aging, 1958 to 1998

	Prostate cancer cases	Noncases	P^*
n	114	680	
Age	57.0 (12.8)	50.9 (15.5)	<0.001
Testosterone (ng/dL)	449.4 (114.6)	461.5 (116.6)	0.31
DHEAS (ng/mL)	1,634.3 (1,118.1)	1,861.9 (1,108.9)	0.06
SHBG (ng/mL)	80.0 (25.4)	82.3 (26.6)	0.38
Calculated free testosterone (ng/dL)	5.02 (1.58)	5.23 (1.78)	0.22
FTI (nmol/L/nmol/L)	0.24 (0.10)	0.26 (0.12)	0.14
Height (cm)	176.4 (6.1)	177.1 (6.8)	0.25
Weight (kg)	78.0 (9.6)	80.9 (12.8)	0.02

NOTE: FTI = (testosterone / sex hormone binding globulin) × 100.
*For the hypothesis test of no difference in means (t test) between prostate cancer cases and noncases.

Because men in the top three quartiles had similar risks, we calculated the risk for prostate cancer of clinically hypogonadal men compared with eugonadal men using an FTI cutoff of 0.153, which represented the 2.5th percentile values of a prior BLSA study (1). The risk among hypogonadal men was significantly lower: 0.51 (95% CI, 0.31-0.82).

Discussion

In summary, in this cohort of aging men, higher levels of calculated serum free testosterone and FTI were associated with an increased risk of prostate cancer. Men with hypogonadal levels of free testosterone, as estimated by FTI, had a 49% decreased risk of prostate cancer compared with men with eugonadal levels, which suggests that clinically low levels of free testosterone may be protective against prostate cancer.

Previous epidemiologic evidence for an association between serum androgens and prostate cancer risk from 15 prospective studies has been inconclusive (8, 14-16). One prior study, a nested case-control analysis of participants in the U.S. Physicians' Health Study, observed positive associations of serum total testosterone and free testosterone index with prostate cancer risk. The associations were strongest among older men (17). Three recent nested case-control studies, however, found no association between serum androgens and prostate cancer (14-16). Another prior null study was a case-control analysis in a small sample of BLSA participants (18). The present, expanded analysis instead considered a much larger sample of BLSA participants and a substantially greater number of serum hormone measures in a survival model.

This study is unique in its utilization of serial serum androgen measures in a cohort of community-dwelling men over a period of nearly 40 years. In contrast, every prior prospective study of circulating androgens and prostate cancer, except for the previous BLSA study, used single serum measures. The median number of measures per participant was three (range of one to eight), and 85% of participants had at least two. The high number of repeat measures potentially provided a more comprehensive characterization of hormone levels and may have captured a more relevant period of exposure than prior studies (8). Also of note, all of the samples were drawn in the morning following an overnight fast, thus reducing intra- and interparticipant variation. These methods may have accounted, at least in part, for the differences between these results and those of the three recent null studies, which used single serum hormone measures drawn at varying times of the day.

Other strengths of the present study include the large number of individual observations (2,958 samples from 794 men); the use of samples drawn several years before diagnosis, which minimizes the potential for bias due to concomitant disease (8); and consideration of mediators that may potentially alter testosterone levels, testosterone bioavailability, or prostate cancer risk (8, 19, 20).

There is no consensus about the relative validity of serum free testosterone measures derived from calculated indicators. We used both free testosterone concentration, calculated by the mass action equation, and FTI. Although it highly correlates with dialysis-measured serum free testosterone, FTI is potentially less reliable for determining true levels among individuals (11, 12). Compared with equilibrium dialysis (the gold standard for measuring serum free testosterone), both the mass action equation and FTI have the potential for introducing error. However, we obtained the same inferences with both calculated indicators. Moreover, free testosterone measurement error would likely be nondifferential with respect to diagnosis of prostate cancer and thus would bias the association of prostate cancer with free testosterone toward the null (i.e., it would attenuate a true association, not cause the appearance of a nonexistent association).

It is possible that the use of simple updating in a Cox model could potentially introduce varying extents of imprecision due to differences in follow-up intervals between individual participants. This bias, however, would likely be nondifferential. It is also possible that because simple updating emphasizes the most recent hormone measures, the shorter follow-up time for cases may have potentially introduced bias due to undiagnosed cancers. However, it is unlikely that the presence of small, clinically insignificant cancers would have substantially altered free testosterone levels in these participants.

We did not observe an association between total testosterone and prostate cancer. However, ~40% of total testosterone is bound to SHBG and is unavailable to the prostate (4). Free testosterone, in contrast, is unbound and may diffuse into the prostate (4, 21). Diffusion of unbound testosterone into the prostate constitutes the primary source of androgens in prostate tissue; therefore, free testosterone, unlike total testosterone, reflects the amount of androgen to which prostate cells are actually exposed. This link to intraprostatic androgen levels potentially renders free testosterone a more relevant measure of prostate cancer risk because prostate androgens seem to play a more prominent role in the development of prostate cancer than serum androgens: selective pharmacologic reduction of prostate androgens with finasteride, an inhibitor of 5α-reductase, decreases the risk of prostate cancer without reductions in total serum testosterone (5, 22).

The findings of this study are relevant to testosterone replacement therapy in older men. The effect of testosterone therapy on prostate cancer incidence is not known. Prospective trials have not been sufficiently powered to adequately address prostate cancer risk as an end point (3, 4). Prospective cohort analyses of serum testosterone concentrations represent a potential means for estimating this risk and, in this respect, the BLSA is a particularly relevant study population, composed of community-dwelling, aging males representing the same individuals most likely to be candidates for testosterone therapy.

Because testosterone therapy produces stable and consistent increases in serum-free testosterone concentrations (23, 24), these findings suggest that older men receiving testosterone therapy should be carefully monitored for the development of clinically significant prostate cancer, especially because a substantial number with normal digital rectal examination and serum prostate-specific antigen level of <4.0 ng/mL may have occult but aggressive prostate cancer (25) that will not be

Table 2. Association of serum androgens and sex hormone binding globulin concentrations with risk of prostate cancer in a cohort of 794 men in the Baltimore Longitudinal Study of Aging, 1958 to 2003

	Quartile				P_{trend}*
	1	2	3	4	
Testosterone					
Range (ng/dL)	≤352	353-418	419-495	>495.0	
RR†	1.00	0.74	1.19	0.86	0.31
95% CI		0.44-1.24	0.73-1.96	0.43 to 1.73	
DHEAS					
Range (ng/dL)	≤754	755-1,250	1,251-1,940	>1,940	
RR†	1.00	1.06	0.67	1.65	0.36
95% CI		0.68-1.67	0.36-1.23	0.27-1.58	
SHBG					
Range (nmol/L)	≤63.1	63.2-79.1	79.2-98.7	>98.7	
RR†	1.00	1.06	0.86	0.70	0.42
95% CI		0.60-1.08	0.50-1.50	0.39-1.29	
Free testosterone (by mass action equation)					
Range (ng/dL)	≤3.60	3.61-4.50	4.51-5.7	>5.70	
RR†	1.00	1.52	1.16	2.59	0.03
95% CI		0.93-2.50	0.61-2.20	1.28-5.25	
Free testosterone (by mass action equation)‡					
Range (ng/dL)	≤3.40	3.41-4.23	4.24-5.2	>5.20	
RR†	1.00	1.33	1.26	1.89	0.03
95% CI		0.78-2.25	0.68-2.33	0.99-3.61	
Free testosterone index					
Range	≤0.148	0.149-0.212	0.213-0.285	>0.285	
RR†	1.00	1.86	1.90	1.34	0.05
95% CI		1.12-3.09	1.02-3.55	0.52-3.44	
Free testosterone index*					
Range	≤0.135	0.136-0.186	0.187-0.245	>0.245	
RR†	1.00	2.68	2.87	2.26	0.04
95% CI		1.57-4.56	1.55-5.29	1.05-4.87	

*By log likelihood ratio test.
†Adjusted for age.
‡Excluding measures in men <45 years of age.

detected with pretreatment screening. In addition, these findings underscore the need for long-term prospective trials of testosterone therapy that incorporate incidence of prostate cancer as a primary safety end point.

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