American Journal of Epidemiology
Vol. 168, No. 12
DOI: 10.1093/aje/kwn272
Advance Access publication October 21, 2008

Original Contribution

Serum Steroid and Sex Hormone-Binding Globulin Concentrations and the Risk of Incident Benign Prostatic Hyperplasia: Results From the Prostate Cancer Prevention Trial

Alan R. Kristal, Jeannette M. Schenk, YoonJu Song, Kathryn B. Arnold, Marian L. Neuhouser, Phyllis J. Goodman, Daniel W. Lin, Frank Z. Stanczyk, and Ian M. Thompson

Initially submitted February 15, 2008; accepted for publication July 29, 2008.

The authors conducted a nested case-control study of serum steroid concentrations and risk of benign prostatic hyperplasia (BPH), using data from the placebo arm of the Prostate Cancer Prevention Trial (1993–2003). Incident BPH over 7 years ($n = 708$) was defined as receipt of treatment, a report of 2 International Prostate Symptom Score (IPSS) values greater than 14, or 2 increases of 5 or more from baseline IPSS values with at least 1 value greater than or equal to 12. Controls ($n = 709$) were selected from men who reported no BPH treatment or any IPSS greater than 7. Baseline serum was analyzed for testosterone, estradiol, estrone, 5α-androstane-3α, 17β-diol-glucuronide, and sex hormone-binding globulin. Covariate-adjusted odds ratios contrasting the highest quartiles with the lowest quartiles of testosterone, estradiol, and testosterone:17β-diol-glucuronide ratio were 0.64 (95% confidence interval (CI): 0.43, 0.95; $P_{trend} = 0.04$), 0.72 (95% CI: 0.53, 0.98; $P_{trend} = 0.09$), and 0.64 (95% CI: 0.46, 0.89; $P_{trend} = 0.004$), respectively. Findings did not differ by age, body mass index, time to BPH endpoint, or type of BPH endpoint. High testosterone levels, estradiol levels, and testosterone:17β-diol-glucuronide ratio are associated with reduced BPH risk, which may reflect decreased activity of 5-α-reductase. Genetic or environmental factors that affect the activity of 5-α-reductase may be important in the development of symptomatic BPH.

gonadal steroid hormones; prostatic hyperplasia

Abbreviations: BPH, benign prostatic hyperplasia; CI, confidence interval; IPSS, International Prostate Symptom Score; OR, odds ratio; SHBG, sex hormone-binding globulin.

Benign prostatic hyperplasia (BPH) is one of the most common medical conditions in older men (1, 2). Although the pathogenesis of BPH is not well understood, it is probably linked to age-related changes in hormonal and other growth-regulatory factors that affect prostate growth (3). Testosterone and estrogens play important roles in prostate growth and function, and many scientists have hypothesized that the slow decline in serum testosterone levels or the decreasing ratio of testosterone to estrogen that begins in midlife are factors in BPH pathogenesis. It is also possible that the activity of 5-α-reductase, which reduces testosterone to its highly active metabolite dihydrotestosterone in prostate tissues, plays a role in BPH pathogenesis, given that 5-α-reductase inhibitors are effective for both the treatment and prevention of symptomatic BPH (4, 5). Studies of serum steroids and BPH risk have yielded inconsistent results, which is not surprising given that most studies have been cross-sectional and very small and have not controlled for critical covariates such as age.

Here we give results of a nested case-control study examining the risk of incident, symptomatic BPH among men participating in the Prostate Cancer Prevention Trial. Data from the Prostate Cancer Prevention Trial include rigorous assessment of both the symptoms and treatment of BPH; extensive information on anthropometric, dietary, and other factors that may affect BPH risk; and assays of serum testosterone, estrone, estradiol, 5-α-androstane-3α, 17β-diol-glucuronide (an indirect measure of dihydrotestosterone

Correspondence to Dr. Alan R. Kristal, Cancer Prevention Program, Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue North, M4-B402, P.O. Box 19024, Seattle, WA 98109-1024 (e-mail: akristal@fhcrc.org).

formation), and sex hormone-binding globulin (SHBG). In this report, we examine whether concentrations of these steroids and SHBG affect the risk of incident, symptomatic BPH in a population of healthy men aged 55 years or older.

MATERIALS AND METHODS

Data were from the Prostate Cancer Prevention Trial, a US randomized, placebo-controlled trial (1993–2003) testing whether finasteride, a 5-α-reductase inhibitor, could reduce the 7-year period prevalence of prostate cancer. The study design and participant characteristics have been described in detail previously (4). Briefly, 18,880 men aged 55 years or older with a normal digital rectal examination and a prostate-specific antigen level of 3 ng/mL or below, as well as no history of prostate cancer or other clinically significant coexisting conditions and no severe BPH symptoms (defined as an International Prostate Symptom Score (IPSS) (6) of 20 or higher), were randomized to receive finasteride (5 mg/day) or placebo.

Participants for this nested case-control study were drawn from the 9,457 men randomized to the placebo arm of the Prostate Cancer Prevention Trial. Exclusion criteria for these analyses included men who, at baseline, had undergone medical or surgical treatment for BPH ($n = 701$), had a self-reported history of BPH ($n = 1{,}904$), or had an IPSS greater than 7 ($n = 1{,}820$). In addition, men using steroid hormones at any time during the study ($n = 61$) were excluded. This left 4,971 men eligible for the study.

Extensive medical data, including physician diagnosis of and treatment for BPH, prostatitis, diabetes, cardiovascular disease, and cancer, were collected at the baseline clinic visit, at each annual and 6-month clinic visit, and at every 3- and 9-month phone contact between scheduled clinic visits. At recruitment, randomization, and each annual follow-up clinic visit, participants completed the 7-item IPSS questionnaire as a self-administered questionnaire. Clinic staff measured height and weight at recruitment. Data on age, race/ethnicity, education, physical activity, alcohol consumption, and history of smoking were collected at baseline using self-administered questionnaires.

Table 1. Demographic and Lifestyle Characteristics of Cases With Benign Prostatic Hyperplasia and Controls, Prostate Cancer Prevention Trial, 1993–2003

	Cases ($n = 708$)		Controls ($n = 709$)		*P* Value[a]
	No.	%	No.	%	
Age, years					
55–<60	215	30.4	213	30.0	0.99
60–<65	199	28.1	198	27.9	
65–<70	188	26.6	189	26.7	
≥70	106	15.0	109	15.4	
Race/ethnicity					
White	656	92.7	658	92.8	0.88
African-American	26	3.7	23	3.2	
Other	26	3.7	28	4.0	
Body mass index[b]					
Normal (<25)	165	23.7	200	28.4	0.12
Overweight (25–29)	368	52.9	356	50.6	
Obese (≥30)	163	23.4	148	21.0	
Alcohol consumption					
<1 drink/month	204	28.9	155	21.9	0.006
1–3 drinks/month	95	13.4	129	18.2	
1–6 drinks/week	245	34.7	236	33.3	
7–13 drinks/week	100	14.1	110	15.5	
≥14 drinks/week	63	8.9	79	11.1	
Smoking status					
Current smoker	55	7.8	41	5.8	0.14
Not a current smoker	652	92.2	667	94.2	
Baseline International Prostate Symptom Score					
1–3	226	40.6	412	75.0	<0.0001
4–5	222	39.9	120	21.9	
6–7	109	19.6	17	3.1	

[a] *P* value from chi-square test.
[b] Weight (kg)/height $(m)^2$.

Definition of BPH cases and controls

Incident BPH was defined either as a report of treatment or the development of significant lower urinary tract symptoms. Treatments included use of α-blockers, finasteride, or any surgical intervention (transurethral prostatectomy, balloon dilation, or laser prostatectomy). Development of significant symptoms was defined as either 1) 2 IPSS values greater than 14 or 2) 2 IPSS values at least 5 units higher than baseline with at least 1 score greater than or equal to 12. The latter definition is a more conservative version of the definition of BPH progression used in the Medical Therapy of Prostatic Symptoms Trial (7), in which a single increase of 4 was used to define significant, clinical progression. There were 727 incident BPH cases in total, of which 322 were due to medical or surgical treatment, 105 were due to 2 IPSS values greater than 14, and 300 were due to a substantial increase in symptoms from baseline. Men who reported transient elevations in IPSS or a physician diagnosis of BPH in the absence of symptoms or treatment were not included as cases.

Controls were drawn from the 1,497 men who, during 7 years of observation, had no more than 2 missing IPSS values, no single IPSS greater than 7, no surgical or medical treatment for BPH, and no report of a physician diagnosis of BPH. These criteria deliberately excluded men with mild-to-moderate symptoms and yielded a control group with a definitive absence of BPH symptoms. From this sample, we selected all men aged 70 years or over and all non-Caucasian men. The remaining controls were randomly selected in order to obtain a total control sample ($n = 727$) that was frequency-matched to the age distribution (in 5-year age groups) of cases.

Table 2. Mean Values and Percentile Cutpoints for Serum Steroid Hormone Levels in Cases With Benign Prostatic Hyperplasia and Controls, Prostate Cancer Prevention Trial, 1993–2003

	Cases (n = 708)				Controls (n = 709)				P Value
	Mean (SD)	25th Percentile	50th Percentile	75th Percentile	Mean (SD)	25th Percentile	50th Percentile	75th Percentile	
Testosterone, ng/dL	492.5 (185.9)	364.4	463.7	593.7	521.1 (92.6)	374.6	490.7	638.9	0.005
Estrone, pg/mL	37.8 (11.7)	28.5	36.3	45.5	38.5 (12.0)	29.3	36.6	45.1	0.277
Estradiol, pg/mL	30.42 (8.91)	24.0	29.7	35.7	31.5 (9.4)	25.0	30.0	36.7	0.035
Sex hormone-binding globulin, nmol/L	36.7 (13.4)	27.0	35.0	44.8	37.3 (13.6)	27.5	35.0	46.3	0.419
3-α-diol glucaronide, ng/mL	6.7 (3.7)	4.2	6.0	8.1	6.4 (3.3)	4.0	5.8	7.8	0.040
Free estradiol, pg/mL	0.80 (0.2)	0.6	0.8	0.9	0.8 (0.2)	0.7	0.8	1.0	0.073
Bioavailable testosterone, ng/dL	289.2 (94.0)	223.2	280.0	338.9	305.0 (98.5)	234.4	287.4	364.0	0.002
Testosterone:estradiol ratio	16.9 (6.5)	12.4	15.9	20.4	17.4 (6.8)	12.3	16.4	21.2	0.144
Testosterone:3-α-diol glucaronide ratio	95.7 (70.4)	52.5	78.1	117.7	103.5 (69.4)	59.4	87.8	126.9	0.036

Abbreviation: SD, standard deviation.

Blood collection, processing, and laboratory analysis

Blood samples were drawn at screening (approximately 3 months prior to randomization) into a 7-mL ethylenediaminetetraacetic acid Vacutainer tube (Becton, Dickinson and Company, Franklin Lakes, New Jersey) and shipped via overnight express to a central storage facility, where they were centrifuged, aliquoted, and stored at −70°C until they were analyzed in the laboratories of Dr. Frank Stanzyk at the University of Southern California (Los Angeles, California). Levels of serum testosterone, estrone, and estradiol were determined by radioimmunoassay after organic extraction and chromatography on Celite (Celite Corporation, Santa Barbara, California) columns. Serum albumin concentration was measured using a Roche Cobas Centrifugal Chemistry Analyzer (Hoffman-La Roche, Indianapolis, Indiana). SHBG was measured with a competitive radioimmunoassay using DSL double antibody kits (Diagnostic Systems Laboratories, Inc., Webster, Texas), and 17β-diol-glucuronide was measured with a competitive radioimmunoassay using DSL double antibody kits. Bioavailable testosterone and estradiol were calculated using concentrations of albumin and SHBG according to the methods of Södergard et al. (8). Serum was not available from 37 men. This left data from 708 cases and 709 controls available for analysis.

Statistical methods

Descriptive statistics were used to characterize the study sample and generate distributions of steroid hormone concentrations in cases and controls. In addition, we examined the ratio of testosterone to estradiol as an indicator of the relative amounts of these hormones, which both affect prostate growth, and the ratio of testosterone to 17β-diol-glucuronide as an indicator of the relative amount of conversion of testosterone to dihydrotestosterone. Serum concentrations of steroids were categorized into quartiles based on the distribution in the controls, and unconditional logistic regression was used to calculate odds ratios and their 95% confidence intervals for risk of BPH. Logistic regression models were adjusted for cigarette smoking (current smoker vs. not a current smoker) plus covariates associated with BPH risk in this cohort, including age at baseline (continuous), body mass index (weight (kg)/height $(m)^2$; continuous), race (African-American, white, other), alcoholic beverage consumption (drinks per month: <1, 1–3, 4–27, 28–55, or ≥56), and level of insulin-like growth factor binding protein 3 (model 1). Model 2 also controlled for SHBG, and model 3 also controlled for SHBG plus other steroids. Tests for linear trend across the quartiles were performed by using an ordinal variable corresponding to rank from the lowest category to the highest, as described by Breslow and Day (9). All P values were 2-sided and were considered statistically significant at $P < 0.05$.

RESULTS

Distributions of age, race, body mass index, and smoking were similar in cases and controls (Table 1). Participants were mostly white, overweight or obese, and nonsmokers. Mean age was 63.4 years (standard deviation, 5.5), and mean body mass index was 27.6 (standard deviation, 4.1). Baseline IPSS was significantly higher and alcoholic beverage consumption was significantly lower in cases than in controls.

Table 2 gives mean values and quartile distributions of steroid concentrations in cases and controls. With the exception of 17β-diol-glucuronide, all mean steroid concentrations were lower in cases. Differences between cases and controls were statistically significant for testosterone (−5.3%), bioavailable testosterone (−5.2%), estradiol (−3.3%), and 17β-diol-glucuronide (+6.0%). The mean

testosterone:17β-diol-glucuronide ratio was also significantly lower (−7.5%) in cases than in controls. There were no differences in bioavailable estradiol, SHBG, or testosterone:estradiol between cases and controls.

Table 3 gives adjusted odds ratios for risk of BPH associated with serum steroid concentrations. In models controlled for covariates (model 1) and covariates plus SHBG (model 2), men in the highest quartile of serum testosterone level had a significantly reduced risk of BPH, with no evidence of a dose-response association. In a post-hoc analysis using quartiles 1–3 as the contrast group, high testosterone controlled for SHBG (model 2) was associated with a 37% (95% confidence interval (CI): 54, 15; $P = 0.003$) reduced BPH risk. Results were similar though not as strong for bioavailable testosterone. Men with estradiol levels above the lowest quartile had a significantly reduced risk of BPH, with no evidence of a dose-response association. In post-hoc analyses contrasting quartile 1 with quartiles 2–4, high estradiol controlled for SHBG (model 2) was associated with a 26% (95% CI: 43, 7; $P = 0.02$) lower BPH risk. Results for bioavailable estradiol were similar but not as strong. Mutual adjustment of testosterone and estradiol had little effect on these results (model 3). There were no associations of estrone or SHBG with risk; however, when results were controlled for testosterone (model 3), there was a linear and positive association of 17β-diol-glucuronide with BPH risk. The linear and inverse association of testosterone:17β-diol-glucuronide ratio with BPH risk was strong and highly statistically significant ($P < 0.002$); on average, risk of BPH decreased 14% (95% CI: 23, 5; $P = 0.004$) for each quartile of increasing testosterone:17β-diol-glucuronide ratio.

Multiple dietary factors, including percentages of energy derived from protein and fat and servings per week of vegetables and red meat, were associated with BPH risk in this cohort (10). We therefore completed analyses in the subset of 612 cases and 638 controls with dietary data. Associations of steroids with BPH were generally stronger in this subset of men, most notably for 17β-diol-glucuronide controlled for testosterone (model 3) (odds ratio (OR) = 1.55, 95% CI: 1.11, 2.16; $P_{trend} = 0.01$) and for testosterone:17β-diol-glucuronide ratio controlled for SHBG (model 2) (OR = 0.56, 95% CI: 0.39, 0.79; $P_{trend} = 0.0004$). However, control for dietary factors associated with BPH risk had no effect on these associations.

We conducted several analyses to explore possible effect modification. Associations of steroids with BPH risk differed very little across strata defined by time from baseline to BPH (<4 years vs. ≥4 years), type of BPH endpoint (treatment or multiple IPSS values >14 vs. an increase of ≥5 from baseline IPSS), age (<65 years vs. ≥65 years), and body mass index (<25, 25–29, or ≥30). Excluding the 8 men with diabetes at baseline (6 cases and 2 controls) did not affect study results. Control for baseline IPSS had only modest effects on the magnitude of associations between steroid hormones and BPH risk—for example, the contrasts of quartile 4 with quartile 1 for testosterone controlled for SHBG (model 2: OR = 0.61, 95% CI: 0.40, 0.94; $P_{trend} = 0.07$) and estradiol controlled for SHBG (model 2: OR = 0.67, 95% CI: 0.48, 0.94; $P_{trend} = 0.07$). For 17β-diol-glucuronide controlled for testosterone (model 3), the contrast of quartile 4 with quartile 1 was weaker than in Table 3 (OR = 1.25, 95% CI: 0.90, 1.74; $P_{trend} = 0.13$), but for testosterone:17β-diol-glucuronide ratio controlled for SHBG (model 2), the association was almost identical (OR = 0.66, 95% CI: 0.46, 0.93; $P_{trend} = 0.05$).

DISCUSSION

In this prospective study, we found several significant associations between serum steroid concentrations and incident BPH. The most consistent findings were linear dose-response associations between higher 17β-diol-glucuronide levels controlled for testosterone and higher testosterone:17β-diol-glucuronide ratio and reduced BPH risk. High serum testosterone and estradiol levels were also associated with lower BPH risk; however, there was no dose-response. In analyses based on quartiles (quartiles 1–4) of testosterone, BPH risk was lower only in quartile 4 (vs. quartiles 1–3); in analyses based on quartiles of estradiol, BPH risk was lower in quartiles 2–4 (vs. quartile 1). Associations of testosterone, estradiol, and testosterone:17β-diol-glucuronide ratio with BPH risk did not differ by age, body mass index, definition of BPH endpoint, or time between baseline and BPH endpoint.

The hypothesis that serum steroids, particularly high levels of testosterone and/or estradiol, are associated with BPH risk has been studied for over 30 years. The study designs have included cross-sectional studies contrasting men undergoing surgery for BPH with hospital controls (11–24); cross-sectional studies contrasting men with and without clinically defined BPH (25, 26); cross-sectional studies correlating lower urinary tract symptom severity or prostate size with steroid concentrations (27–34); and a single prospective study on the risk for BPH surgery (35). The majority of these studies were only marginally informative about whether or not steroid concentrations affect BPH risk, either because the sample sizes were very small (e.g., <75 BPH cases) (11–23, 33) or because analyses were not adequately controlled for age (13–20, 23, 32, 33). We therefore focus the discussion below on studies that we believe contribute reliable information on steroids and BPH risk.

In our study, both high serum testosterone levels and the ratio of testosterone to 17β-diol-glucuronide were associated with reduced BPH risk, which is consistent with much of the published literature. Two studies have found that high testosterone is associated with reduced lower urinary tract symptoms (27, 31), and none have found high testosterone to be associated with increased risk. All studies that have examined the ratio of testosterone to 17β-diol-glucuronide (or dihydrotestosterone) have found that high levels of testosterone relative to 17β-diol-glucuronide are significantly associated with reduced risks of clinical BPH (25, 26), lower urinary tract symptoms (29), or surgical BPH treatment (21, 24) or with smaller prostate size (34). While confirmation in additional larger studies is warranted, the consistency of these findings makes it reasonable to assume that high levels of testosterone relative to 17β-diol-glucuronide are predictive of reduced BPH risk.

We propose 2 alternative interpretations of our findings on testosterone and the testosterone:17β-diol-glucuronide

Table 3. Associations of Steroid Hormone Concentrations With Risk of Symptomatic Benign Prostatic Hyperplasia, Prostate Cancer Prevention Trial, 1993–2003

	Model 1[a]		Model 2[b]		Model 3[c]	
	OR	95% CI	OR	95% CI	OR	95% CI
Testosterone						
Q1 (referent)	1.00		1.00		1.00	
Q2	1.09	0.81, 1.46	1.07	0.79, 1.45	1.10	0.81, 1.50
Q3	1.01	0.75, 1.37	0.99	0.71, 1.37	1.03	0.74, 1.45
Q4	0.67	0.48, 0.93	0.64	0.43, 0.95	0.69	0.46, 1.05
P for trend		0.002		0.04		0.13
Bioavailable testosterone						
Q1 (referent)	1.00		1.00		1.00	
Q2	0.92	0.68, 1.25	0.93	0.69, 1.26	0.96	0.71, 1.30
Q3	0.97	0.72, 1.31	1.00	0.73, 1.35	1.05	0.77, 1.43
Q4	0.72	0.53, 0.99	0.74	0.54, 1.04	0.81	0.57, 1.14
P for trend		0.08	0.14			0.36
Estradiol						
Q1 (referent)	1.00		1.00		1.00	
Q2	0.68	0.50, 0.91	0.68	0.51, 0.92	0.70	0.52, 0.95
Q3	0.82	0.61, 1.10	0.83	0.62, 1.12	0.88	0.65, 1.18
Q4	0.70	0.52, 0.95	0.72	0.53, 0.98	0.79	0.57, 1.10
P for trend		0.06		0.09		0.33
Free estradiol						
Q1 (referent)	1.00		1.00		1.00	
Q2	0.82	0.61, 1.11	0.82	0.60, 1.10	0.86	0.63, 1.16
Q3	0.80	0.59, 1.08	0.79	0.59, 1.07	0.85	0.62, 1.16
Q4	0.81	0.60, 1.10	0.80	0.59, 1.09	0.89	0.64, 1.22
P for trend		0.18		0.15		0.47
Estrone						
Q1 (referent)	1.00		1.00		1.00	
Q2	0.79	0.58, 1.07	0.79	0.58, 1.07	0.82	0.61, 1.12
Q3	0.84	0.62, 1.13	0.85	0.62, 1.14	0.89	0.66, 1.21
Q4	0.89	0.62, 1.21	0.91	0.67, 1.23	0.98	0.72, 1.33
P for trend		0.54		0.31		0.99

Table continues

ratio: 1) these steroids are mediating factors that explain associations of genetic or environmental characteristics with BPH risk or 2) these steroids are serving as nonspecific measures of aging. A strong argument can be made for the mediating factor hypothesis, specifically that the effects of reduced 5-α-reductase activity are mediated through decreased tissue dihydrotestosterone and reflected by an increase in the serum testosterone:17β-diol-glucuronide ratio. This is supported by the well-established effects of 5-α-reductase inhibitors, which include reduced prostate tissue dihydrotestosterone (36), serum dihydrotestosterone (37), and 17β-diol-glucuronide (38) levels and prostate size (4) and increased levels of serum testosterone (37) and estradiol (unpublished observation from the Prostate Cancer Prevention Trial). Thus, factors that reduce the activity of 5-α-reductase could be important determinants of BPH risk. There are known polymorphisms in the steroid 5-α-reductase type 2 (*SRD5A2*) gene, the gene that codes for the predominant 5-α-reductase in prostate, which affect enzyme activity (39). Few investigators have examined variants in *SRD5A2* and BPH risk; in 2 studies, researchers found no associations (40, 41), in 1 they reported that the LL genotype of the *V89L* variant was associated with increased BPH risk in Hispanic men (42), and in 1 they reported an association of the same genotype with increased prostate volume (43). Whether behavioral or environmental factors affect 5-α-reductase is unknown; however, the prospect is reasonable (44). An equally strong argument can be made for an alternative interpretation

Table 3. Continued

	Model 1[a]		Model 2[b]		Model 3[c]	
	OR	95% CI	OR	95% CI	OR	95% CI
3-α-diol glucaronide						
Q1 (referent)	1.00		1.00		1.00	
Q2	0.98	0.80, 1.56	0.98	0.72, 1.34	1.01	0.74, 1.38
Q3	1.03	0.76, 1.49	1.04	0.76, 1.42	1.10	0.80, 1.50
Q4	1.26	1.00, 1.92	1.27	0.94, 1.72	1.37	1.00, 1.86
P for trend		0.12		0.11		0.04
Sex hormone-binding globulin						
Q1 (referent)	1.00					
Q2	0.93	0.68, 1.26				
Q3	1.06	0.78, 1.44				
Q4	0.78	0.56, 1.09				
P for trend		0.27				
Testosterone:estradiol ratio						
Q1 (referent)	1.00		1.00			
Q2	1.21	0.89, 1.63	1.24	0.91, 1.68		
Q3	1.09	0.80, 1.48	1.14	0.82, 1.57		
Q4	0.91	0.65, 1.27	0.97	0.68, 1.38		
P for trend		0.46		0.74		
Testosterone:3-α-diol glucaronide ratio						
Q1 (referent)	1.00		1.00			
Q2	0.75	0.56, 1.00	0.75	0.56, 1.01		
Q3	0.63	0.47, 0.86	0.64	0.47, 0.87		
Q4	0.63	0.46, 0.87	0.64	0.46, 0.89		
P for trend		0.002		0.004		

Abbreviations: CI, confidence interval; OR, odds ratio; Q, quartile.

[a] Results were adjusted for age at baseline, race, body mass index, smoking status, alcohol consumption, and insulin-like growth factor binding protein 3.

[b] Results were additionally adjusted for sex hormone-binding globulin.

[c] Results were additionally adjusted for estradiol (for testosterone) or testosterone (for estrone, estradiol, and 3-α-diol glucaronide).

that lower testosterone is simply a nonspecific indicator of aging. In our study sample of men without BPH at baseline, testosterone levels did not differ by age, which is in contrast both to what we have observed in the Prostate Cancer Prevention Trial population overall and to what we would expect based on the well-established decline in serum testosterone associated with aging (45). There is some evidence that declines in serum testosterone are greater in men who develop clinical conditions associated with aging, such as diabetes and obesity (46). Thus, older men without BPH may be men who are less affected by aging generally, which is reflected by a sustained serum testosterone level. Further research to identify genetic and environmental factors that affect both BPH and 5-α-reductase activity would be helpful in determining whether testosterone and the testosterone: 17β-diol-glucuronide ratio are mediators or noncausal consequences of factors that affect BPH pathogenesis.

Whether or not there is a relation of estrogens with BPH risk remains uncertain. Gann et al. (35) reported a positive association between estradiol and incident BPH surgery but only among men with low testosterone and only after controlling for estrone, which suggests that the strong collinearity of estrone and estradiol may have yielded an unstable statistical model. Rohrmann et al. (29) reported a positive association of estradiol with lower urinary tract symptoms. In contrast, both we and others (26, 27) found that estradiol was significantly and negatively associated with BPH. The prostate expresses both estrogen receptor β (in epithelium, where it inhibits growth) and estrogen receptor α (in stroma, where it promotes growth). There is little evidence that estrogen receptor α has a role in BPH (47), and thus a growth-inhibitory effect of estradiol could be consistent with reduced BPH risk. It is also possible that a high estradiol level simply reflects a high testosterone level, because

estradiol in older men is almost exclusively formed by aromatization of testosterone. This picture is further complicated by observations in the Prostate Cancer Prevention Trial (48) and other studies (49–51) that abdominal obesity is a risk factor for BPH, because abdominal obesity also increases estradiol levels. Additional studies of the effects of estradiol on BPH pathogenesis are needed.

We examined whether insulin-like growth factors were mediators or confounders of the associations between steroids and BPH risk. This is because insulin-like growth factor binding protein 3 has been found to be associated with reduced BPH risk (52) and because there are complex, although not entirely consistent, associations among insulin-like growth factors, insulin, testosterone, and androgen receptor activation (53–56). Nevertheless, the associations of insulin-like growth factor binding protein 3, insulin-like growth factor 1, and steroids with BPH risk were unchanged when all of them were included in statistical models. We also examined whether associations of dietary patterns and obesity with BPH risk were mediated by serum steroids. This is because the diet-related measures associated with increased risk of BPH, including total fat, red meat, and obesity (10, 48), are also associated with increased levels of estradiol and decreased levels of testosterone (57, 58). For reasons that are not clear, associations of steroids were somewhat stronger in the subset of men with dietary data; however, associations of dietary factors and steroids with BPH risk were unchanged when all of them were included in statistical models. On the basis of these results, our observed associations of steroids with BPH risk are independent of diet, obesity, insulin-like growth factor 1, and insulin-like growth factor binding protein 3.

The unique strength of this analysis is that it was prospective. Men with a history of BPH were excluded and, at the time of the baseline blood drawing, men were free of BPH symptoms. Cross-sectional or case-control studies of BPH and steroid hormones are problematic, because it is plausible that differences in serum steroids, especially higher levels of 17β-diol-glucuronide, are due to and not the cause of prostate enlargement. In addition, this study reflects both lower urinary tract symptoms and all current BPH treatments; many previous studies used BPH surgery alone as an endpoint, which may have biased the results, because this definition cannot separate factors associated with surgical treatment (e.g., health insurance, age, comorbidity) from those that predict severe BPH.

This study had several important limitations. The definition of BPH by severe lower urinary tract symptoms is not specific, as symptoms such as urgency can be related to bladder conditions and not prostate enlargement (59). There is little agreement about the best definition of BPH for large epidemiologic studies, and we chose a definition based on treatment or symptoms collected by standardized and validated questionnaire. Our definition did not include clinical measures of BPH—for example, prostate size or urinary flow. We did have measures of prostate size based on ultrasonography, but only in the subset of 260 cases and 214 controls who completed end-of-study biopsies. In these men, mean prostate size in cases was significantly larger than that in controls (37.5 cm^3 vs. 33.6 cm^3; $P < 0.0001$). We limited our study to men with no significant lower urinary tract symptoms at baseline, and we considered as controls only men who were free of significant lower urinary tract symptoms through the 7-year trial, because we judged that this would yield the clearest contrast between BPH cases and noncases. We could have included as an additional study group men who developed intermediate lower urinary tract symptoms that were not sufficiently severe to meet the BPH definition; however, we felt this was not justified considering the fact that the Prostate Cancer Prevention Trial biorepository is a limited resource with many competing demands for baseline blood samples and the cost of serum steroid assays was high. Our study sample was not a representative sample of older men; they were all participants in a clinical trial, mostly Caucasian, and free of significant lower urinary tract symptoms at baseline. Finally, blood samples were nonfasting and were drawn at all times during the day, which would have introduced variability in steroid concentrations. However, this is unlikely to have introduced bias, because we have no reason to believe that the distribution of times of blood drawing differed markedly between cases and controls.

In summary, we found no evidence that higher levels of testosterone or estradiol increase the risk of symptomatic BPH. To the contrary, we found that higher concentrations of testosterone relative to 17β-diol-glucuronide (measuring indirectly the conversion of testosterone to dihydrotestosterone in the prostate), as well as testosterone and estradiol, were associated with reduced risk. The consistency of findings on both 17β-diol-glucuronide controlled for testosterone and testosterone:17β-diol-glucuronide ratio with the published literature and the known effects of 5-α-reductase inhibitors suggests that either genetic or environmental factors that reduce the conversion of testosterone to dihydrotestosterone may reduce the risk of symptomatic BPH.

ACKNOWLEDGMENTS

Author affiliations: Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, Washington (Alan R. Kristal, Jeannette M. Schenk, YoonJu Song, Kathryn B. Arnold, Marian L. Neuhouser, Phyllis Goodman, Daniel W. Lin); Department of Epidemiology, School of Public Health and Community Medicine, University of Washington, Seattle, Washington (Alan R. Kristal, Jeannette M. Schenk); School of Public Health, Kunming Medical College, Kunming, China (YoonJu Song); Department of Urology, School of Medicine, University of Washington, Seattle, Washington (Daniel W. Lin); Department of Obstetrics and Gynecology, Keck School of Medicine, University of Southern California, Los Angeles, California (Frank Z. Stanczyk); and Department of Urology, The University of Texas Health Sciences Center at San Antonio, San Antonio, Texas (Ian M. Thompson).

This work was supported by National Institutes of Health grants DK63303, CA37429, CA18964, and CA108964 and by the Korean Research Foundation (grant KRF-2006-214-C00100), which is funded by the South Korean Ministry

of Education and Human Resources Development (Basic Research Promotion Fund).

Conflict of interest: none declared.

REFERENCES

1. Kirby RS. The natural history of benign prostatic hyperplasia: what have we learned in the last decade? *Urology.* 2000; 56(5 suppl 1):3–6.
2. Platz EA, Smit E, Curhan GC, et al. Prevalence of and racial/ethnic variation in lower urinary tract symptoms and non-cancer prostate surgery in U.S. men. *Urology.* 2002;59(6): 877–883.
3. Foster CS. Pathology of benign prostatic hyperplasia. *Prostate Suppl*. 2000;9:4–14.
4. Thompson IM, Goodman PJ, Tangen CM, et al. The influence of finasteride on the development of prostate cancer. *N Engl J Med.* 2003;349(3):215–224.
5. McConnell J, Roehrborn C, Bautista OM, et al. The long-term effect of doxazosin, finasteride, and combination therapy on the clinical progression of benign prostatic hyperplasia. *N Engl J Med.* 2003;349(25):2387–2398.
6. Barry MJ, Fowler FJ Jr, O'Leary MP, et al. The American Urological Association symptom index for benign prostatic hyperplasia. The Measurement Committee of the American Urological Association. *J Urol.* 1992;148(5):1549–1557.
7. Bautista OM, Kusek JW, Nyberg LM, et al. Study design of the Medical Therapy of Prostatic Symptoms (MTOPS) trial. *Control Clin Trials*. 2003;24(2):224–243.
8. Södergård R, Bäckström T, Shanbhag V, et al. Calculation of free and bound fractions of testosterone and estradiol-17β to human plasma proteins at body temperature. *J Steroid Biochem.* 1982;16(6):801–810.
9. Breslow NE, Day NE. Statistical methods in cancer research. Vol 1. The analysis of case-control studies. *IARC Sci Publ.* 1980;(32):5–338.
10. Kristal AR, Arnold KB, Schenk JM, et al. Dietary patterns, supplement use, and the risk of symptomatic benign prostatic hyperplasia: results from the Prostate Cancer Prevention Trial. *Am J Epidemiol.* 2008;167(8):925–934.
11. Lagiou P, Mantzoros CS, Tzonou A, et al. Serum steroids in relation to benign prostatic hyperplasia. *Oncology.* 1997; 54(6):497–501.
12. Tan MO, Karabiyik I, Uygur MC, et al. Serum concentrations of sex hormones in men with severe lower urinary tract symptoms and benign prostatic hyperplasia. *Int Urol Nephrol.* 2003;35(3):357–363.
13. Hulka BS, Hammond JE, DiFerdinando G, et al. Serum hormone levels among patients with prostatic carcinoma or benign prostatic hyperplasia and clinic controls. *Prostate*. 1987; 11(2):171–182.
14. Brochu M, Belganger A. Comparative study of plasma steroid and steroid glucuronide levels in normal men and in men with benign prostatic hyperplasia. *Prostate*. 1987;11(1): 33–40.
15. Stege R, Carlstrom K. Testicular and adrenocortical function in healthy men and in men with benign prostatic hyperplasia. *J Steroid Biochem Mol Biol.* 1992;42(3–4):357–362.
16. Ortega E, Ruiz E, Mendoza MC, et al. Plasma steroid and protein hormone concentrations in patients with benign prostatic hypertrophy and in normal men. *Experientia*. 1979; 35(6):844–845.
17. Sköldefors H, Blomstedt B, Carlström K. Serum hormone levels in benign prostatic hyperplasia. *Scand J Urol Nephrol.* 1978;12(2):111–114.
18. Horton R, Hsieh P, Barberia J, et al. Altered blood androgens in elderly men with prostate hyperplasia. *J Clin Endocrinol Metab.* 1975;41(4):793–796.
19. Rannikko S, Adlercreutz H. Plasma estradiol, free testosterone, sex hormone binding globulin binding capacity, and prolactin in benign prostatic hyperplasia and prostatic cancer. *Prostate*. 1983;4(3):223–229.
20. Hammond GL, Kontturi M, Vihko P, et al. Serum steroids in normal males and patients with prostatic diseases. *Clin Endoncrinol (Oxf).* 1978;9(2):113–121.
21. Vermeulen A, De Sy W. Androgens in patients with benign prostatic hyperplasia before and after prostatectomy. *J Clin Endocrinol Metab.* 1976;43(6):1250–1254.
22. Carter H, Pearson J, Metter EJ, et al. Longitudinal evaluation of serum androgen levels in men with and without prostate cancer. *Prostate*. 1995;27(1):25–31.
23. Drafta D, Proca E, Zamfir V, et al. Plasma steroids in benign prostatic hypertrophy and carcinoma of the prostate. *J Steroid Biochem.* 1982;17(6):689–693.
24. de Jong FH, Oishi K, Hayes RB, et al. Peripheral hormone levels in controls and patients with prostatic cancer or benign prostatic hyperplasia: results from the Dutch-Japanese Case-Control Study. *Cancer Res.* 1991;51(13):3445–3450.
25. Meigs JB, Mohr B, Barry MJ, et al. Risk factors for clinical benign prostatic hyperplasia in a community-based population of healthy aging men. *J Clin Epidemiol.* 2001;54(9):935–944.
26. Platz EA, Kawachi I, Rimm EB, et al. Plasma steroid hormones, surgery for benign prostatic hyperplasia, and severe lower urinary tract symptoms. *Prostate Cancer Prostatic Dis.* 1999;2(5/6):285–289.
27. Roberts RO, Jacobson DJ, Rhodes T, et al. Serum sex hormones and measures of benign prostatic hyperplasia. *Prostate*. 2004;61(2):124–131.
28. Joseph MA, Wei JT, Harlow SD, et al. Relationship of serum sex-steroid hormones and prostate volume in African American men. *Prostate*. 2002;53(4):322–329.
29. Rohrmann S, Nelson WG, Rifai N, et al. Serum sex steroid hormones and lower urinary tract symptoms in Third National Health and Nutrition Examination Survey (NHANES III). *Urology.* 2007;69(4):708–713.
30. Liu C, Huang SP, Li WM, et al. Relationship between serum testosterone and measures of benign prostatic hyperplasia in aging men. *Urology.* 2007;70(4):677–680.
31. Litman HJ, Bhasin S, O'Leary MP, et al. An investigation of the relationship between sex-steroid levels and urological symptoms: results from the Boston Area Community Health Survey. *BJU Int.* 2007;100(2):321–326.
32. Schatzl G, Brössner C, Schmid S, et al. Endocrine status in elderly men with lower urinary tract symptoms: correlation of age, hormonal status, and lower urinary tract function. The Prostate Study Group of the Austrian Society of Urology. *Urology.* 2000;55(3):397–402.
33. Suzuki K, Ito K, Ichinose T, et al. Endocrine environment of benign prostatic hyperplasia: prostate size and volume are correlated with serum estrogen concentration. *Scand J Urol Nephrol.* 1995;29(1):65–68.
34. Bartsch W, Becker H, Pinkenburg F-A, et al. Hormone blood levels and their inter-relationships in normal men and men with benign prostatic hyperplasia (BPH). *Acta Endocrinol (Copenh).* 1979;90(4):727–736.
35. Gann PH, Hennekens CH, Longcope C, et al. A prospective study of plasma hormone levels, nonhormonal factors and

development of benign prostatic hyperplasia. *Prostate*. 1995; 26(1):40–49.
36. Geller J. Effect of finasteride, a 5α-reductase inhibitor on prostate tissue androgens and prostate-specific antigen. *J Clin Endocrinol Metab.* 1990;71(6):1552–1555.
37. Matthiesson KL, Amory JK, Berger R, et al. Novel male hormonal contraceptive combinations: the hormonal and spermatogenic effects of testosterone and levonorgestrel combined with a 5α-reductase inhibitor or gonadotropin-releasing hormone antagonist. *J Clin Endocrinol Metab.* 2005; 90(1):91–97.
38. Fruzzetti F, de Lorenzo D, Parrini D, et al. Effects of finasteride, a 5α-reductase inhibitor, on circulating androgens and gonadotropin secretion in hirsute women. *J Clin Endocrinol Metab.* 1994;79(3):831–835.
39. Makridakis NM, di Salle E, Reichardt JK. Biochemical and pharmacogenetic dissection of human steroid 5α-reductase type II. *Pharmacogenetics*. 2000;10(5):407–413.
40. Klotsman M, Weinberg CR, Davis K, et al. A case-based evaluation of *SRD5A1*, *SRD5A2*, *AR*, and *ADRA1A* as candidate genes for severity of BPH. *Pharmacogenomics J*. 2004; 4(4):251–259.
41. Li Z, Habuchi T, Mitsumori K, et al. Association of V89L *SRD5A2* polymorphism with prostate cancer development in a Japanese population. *J Urol.* 2003;169:2378–2381.
42. Salam MT, Ursin G, Skinner EC, et al. Associations between polymorphisms in the steroid 5-α reductase type II (*SRD5A2*) gene and benign prostatic hyperplasia and prostate cancer. *Urol Oncol.* 2005;23(4):246–253.
43. Roberts RO, Bergstralh EJ, Farmer SA, et al. Polymorphisms in the 5α reductase type 2 gene and urologic measures of BPH. *Prostate*. 2005;62(4):380–387.
44. Kaput J, Rodriguez RL. Nutritional genomics: the next frontier in the postgenomic era. *Physiol Genomics*. 2004;16(2): 166–177.
45. Kaufman JM, Vermeulen A. The decline of androgen levels in elderly men and its clinical and therapeutic implications. *Endocr Rev.* 2005;26(6):833–876.
46. Travison TG, Araujo AB, Kupelian V, et al. The relative contributions of aging, health, and lifestyle factors to serum testosterone decline in men. *J Clin Endocrinol Metab.* 2007; 92(2):549–555.
47. Ellem SJ, Risbridger GP. Treating prostate cancer: a rationale for targeting local oestrogens. *Nat Rev Cancer.* 2007;7(8): 621–627.
48. Kristal AR, Arnold KB, Schenk JM, et al. Race/ethnicity, obesity, health related behaviors and the risk of symptomatic benign prostatic hyperplasia: results from the Prostate Cancer Prevention Trial. *J Urol.* 2007;177(4):1395–1400.
49. Giovannucci E, Rimm EB, Chute CG, et al. Obesity and benign prostatic hyperplasia. *Am J Epidemiol.* 1994;140(11): 989–1002.
50. Chokkalingam AP, Gao YT, Deng J, et al. Insulin-like growth factors and risk of benign prostatic hyperplasia. *Prostate*. 2002;52(2):98–105.
51. Dahle SE, Chokkalingam AP, Gao YT, et al. Body size and serum levels of insulin and leptin in relation to the risk of benign prostatic hyperplasia. *J Urol.* 2002;168(2):599–604.
52. Neuhouser ML, Schenk J, Song YJ, et al. Insulin-like growth factor-I, insulin-like growth factor binding protein-3 and risk of benign prostate hyperplasia in the Prostate Cancer Prevention Trial. *Prostate*. 2008;68(13):1477–1486.
53. Orio F, Térouanne B, Georget V, et al. Potential action of IGF-1 and EGF on androgen receptor nuclear transfer and transactivation in normal and cancer human prostate cell lines. *Mol Cell Endocrinol.* 2002;198(1–2):105–114.
54. Horton R, Pasupuletti V, Antonipillai I. Androgen induction of steroid 5α-reductase may be mediated via insulin-like growth factor-I. *Endocrinology.* 1993;133(2):447–451.
55. Hill M, Bílek R, Safarík L, et al. Analysis of the relations between serum levels of epitestosterone, estradiol, testosterone, IGF-1 and prostatic specific antigen in men with benign prostatic hyperplasia and carcinoma of the prostate. *Physiol Res.* 2000;49(suppl 1):S113–S118.
56. Kaaks R, Lukanova A, Rinaldi S, et al. Interrelationships between plasma testosterone, SHBG, IGF-I, insulin and leptin in prostate cancer cases and controls. *Eur J Cancer Prev.* 2003; 12(4):309–315.
57. Dorgan JF, Judd JT, Longcope C, et al. Effects of dietary fat and fiber on plasma and urine androgens and estrogens in men: a controlled feeding study. *Am J Clin Nutr.* 1996;64(6): 850–855.
58. Gordon GG, Altman K, Southren AL, et al. Effect of alcohol (ethanol) administration on sex-hormone metabolism in normal men. *N Engl J Med.* 1976;295(15):793–797.
59. Abrams P, D'Ancona C, Griffiths D, et al. Lower urinary tract symptoms: etiology, patient assessment and predicting outcome from therapy. In: McConnell J, Abrams P, Denis L, et al, eds. *Male Lower Urinary Tract Dysfunction—Evaluation and Management*. Paris, France: Health Publications; 2005:69–142.