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ORIGINAL ARTICLE

Association between serum 25-hydroxyvitamin D and serum sex steroid hormones among men in NHANES

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Summary

Background Recent literature suggests that high circulating vitamin D may increase prostate cancer risk. Although the mechanism through which vitamin D may increase risk is unknown, vitamin D concentration could influence circulating sex steroid hormones that may be associated with prostate cancer; an alternate explanation is that it could be associated with prostate-specific antigen (PSA) concentration causing detection bias.

Objective We examined whether serum vitamin D concentration was associated with sex steroid hormone and PSA concentrations in a cross-sectional analysis of men in the National Health and Nutrition Examination Surveys (NHANES).

Design Testosterone, oestradiol, sex hormone-binding globulin (SHBG), androstanediol glucuronide, and 25-hydroxyvitamin D (25(OH)D) were measured in serum from men aged 20 and older participating in NHANES III (n = 1315) and NHANES 2001–2004 (n = 318). Hormone concentrations were compared across 25(OH)D quintiles, adjusting for age, race/ethnicity, body fat percentage, and smoking. PSA concentration was estimated by 25(OH)D quintile in 4013 men from NHANES 2001–2006.

Results In NHANES III, higher testosterone (quintile (Q) 1 = 17·2, 95% confidence interval (CI) = 16·1–18·6; Q5 = 19·6, 95% CI = 18·7–20·6 nmol/l, P-trend = 0·0002) and SHBG (Q1 = 33·8, 95% CI = 30·8–37·0; Q5 = 38·4, 95% CI = 35·8–41·2 nmol/l, P-trend = 0·0005) were observed with increasing 25(OH)D. Similar results were observed in NHANES 2001–2004. PSA concentration was not associated with serum 25(OH)D (P-trend = 0·34).

Conclusion Results from these nationally representative studies support a positive association between serum 25(OH)D and testosterone and SHBG. The findings support an indirect mechanism through which vitamin D may increase prostate cancer risk, and suggest the link to prostate cancer is not due to PSA-detection bias.

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Introduction

25-Hydroxyvitamin D (25(OH)D) is the primary form of circulating vitamin D and considered the best measure of vitamin D status as it integrates vitamin D obtained from diet, supplement use, and sun exposure.[1] Although higher circulating vitamin D concentrations have been hypothesized to be associated with a significantly lower risk of some cancers[2,3] including prostate cancer,[4,5] a recent meta-analysis of 21 studies (including those conducted in the PSA era) indicated that higher 25(OH)D levels (the lower limit of the highest quantile ranged from 28·8 to 48 ng/ml across studies) are associated with a 17% increase in risk of overall prostate cancer.[6]

One hypothesized mechanism through which vitamin D may increase the risk of developing prostate cancer is by influencing the concentration of circulating sex steroid hormones, particularly testosterone. Although observational studies have not found an association between circulating levels of testosterone and prostate cancer risk,[7] androgen deprivation therapy is an effective treatment for prostate cancer,[8] supporting a role for androgens in prostate cancer growth and progression. Further, in the Prostate Cancer Prevention Trial (PCPT), men who were randomized to receive finasteride, which inhibits the conversion of testosterone to the more biologically active form, dihydrotestosterone (DHT),

had a significantly lower period prevalence of low-grade prostate cancer compared to the placebo group.[9] A similar reduction in prostate cancer incidence was observed in the Reduction by Dutasteride of Prostate Cancer Events (REDUCE) study for men taking dutasteride compared with men taking a placebo.[10] However, both these drugs were associated with the development of high-grade prostate cancer.[9,10]

Some prior studies found a positive association between serum vitamin D and circulating testosterone. Positive associations between serum vitamin D and testosterone were observed at baseline in the Health Professionals Follow-Up Study[11] and the Ludwigshafen Risk and Cardiovascular Health study.[12] Similarly, a small controlled trial observed an increase in serum testosterone among 31 men who received daily vitamin D supplementation (3333 IU) for a year.[13] However, no associations were seen between serum vitamin D and sex steroid hormones in the European Male Ageing Study,[14] or in smaller studies of young- and middle-aged men from the USA[15] and Germany.[16] Thus, the relationship between circulating 25(OH)D and circulating sex steroid hormones is still unclear and has not been evaluated in a sample representative of the general US male population.

We evaluated the association between circulating concentrations of 25(OH)D and testosterone and other sex steroid hormones among men who participated in the National Health and Nutrition Examination Surveys (NHANES). Further, we evaluated the association between circulating concentrations of 25 (OH)D and PSA to determine whether detection bias is a possible explanation for the positive association between circulating vitamin D and risk of prostate cancer now observed in the literature.[6] Detection bias would occur if circulating vitamin D was associated with higher PSA concentration independent of any aetiologic relationship with prostate cancer; in this case, men with higher vitamin D who were undergoing PSA screening would be more likely to be diagnosed with prostate cancer. To our knowledge, no previous studies have examined the association between 25(OH)D and PSA concentrations.

Subjects and methods

Study population

We studied men who participated in NHANES III and continuous NHANES. NHANES is a series of cross-sectional studies conducted by the National Center for Health Statistics (NCHS). They are nationally representative of the US civilian, noninstitutionalized population aged 2 months and older. To more precisely calculate estimates for certain population subgroups, Mexican Americans, non-Hispanic blacks, and the elderly were oversampled, using a stratified multistage probability design.

NHANES III was conducted in two phases (1988–1991 and 1991–1994). A total of 33 944 individuals were interviewed, 30 818 of whom gave blood samples and underwent physical examinations and 47% of whom were male. Surplus serum, which had been aliquoted and stored at −70 °C since the interview, was assayed for sex steroid hormone concentrations for 1637 males aged 12 and older. Hormones were only measured for participants examined in the morning session to minimize measurement error due to diurnal variation in hormone levels. We excluded men <20 years old (10·2%), previously diagnosed with prostate cancer (0·7%), missing information on body fat percentage or waist circumference (8·6%), and for whom serum 25(OH)D concentration was not available (0·01%). After exclusions, 1315 men remained in this analysis. Protocols for NHANES III were approved by the Institutional Review Board of the NCHS, Centers for Disease Control and Prevention (CDC), and all participants provided informed consent. The Institutional Review Boards at the Johns Hopkins Bloomberg School of Public Health, the NCHS, and CDC, approved the assay of stored serum specimens for the Hormone Demonstration Program.

To determine the consistency of the findings, we also examined the association between circulating concentrations of vitamin D and sex steroid hormones using 2001–2004 data from continuous NHANES. These analyses included 318 men, after excluding men under age 20 (32·1%), men with prostate cancer (1·0%), men missing body fat or waist circumference data (36·8%), and those missing serum vitamin D data (0·2%).

Further, we evaluated the association between vitamin D and prostate-specific antigen (PSA) in 4013 men from NHANES 2001–2006 to determine whether the positive association between vitamin D and prostate cancer could be explained by detection bias resulting from a link between vitamin D and PSA concentrations. If vitamin D is linked with higher PSA, which prompts prostate biopsies, then vitamin D would appear to be positively associated with prostate cancer risk without a causal influence on prostate cancer aetiology. The association between sex steroid hormones and PSA concentration (including a positive association between testosterone and PSA concentration) in continuous NHANES has been reported previously.[17]

Laboratory measurements

For both NHANES III and NHANES 2001–2004, participants had blood drawn after an overnight fast. Serum concentrations of total testosterone, total oestradiol, and sex hormone-binding globulin (SHBG) were measured using a competitive electrochemiluminescence immunoassay on the 2010 Elecsys autoanalyser (Roche Diagnostics, Indianapolis, IN, USA). Androstanediol glucuronide (3α-diol-G), a surrogate measure of the conversion of testosterone to dihydrotestosterone, was measured using enzyme immunoassays (NHANES III: DSL-10–9200 ACTIVE® Androstanediol Glucuronide EIA kit; Diagnostic Systems Laboratories, Webster, TX, USA; NHANES 2001–2004: Direct 3α Diol-G ELISA kit; ALPCO Diagnostics, Salem, NH, USA). The immunoassay kits used to measure steroid hormone levels were standardized to isotope dilution gas chromatography–mass spectrometry. Hormone concentrations were tested in surplus serum samples which underwent at least two freeze–thaw cycles prior to being shipped to the biorepository. Samples in the hormone analysis must have been in unopened vials as indicated on the

label for at least 12 months. Once opened, the samples were used within 14 days or aliquoted and stored frozen. Samples tested for testosterone, SHBG, and oestrogen were not eligible if frozen more than once after storage in the biorepository. All samples were analysed at Children's Hospital, Boston, and arranged in random order for testing.

The lowest detectable limits were 0·06 nmol/l for testosterone, 18·4 pmol/l for oestradiol, 3 nmol/l for SHBG, and 1·05 nmol/l for 3α-diol-G. Coefficients of variation (CV) for the quality control samples in NHANES III were as follows: testosterone 5·9% and 5·8% at 8·0 and 17·5 nmol/l, respectively; oestradiol 6·5% and 6·7% at 377·0 and 1740·4 pmol/l, respectively; SHBG 5·3% and 5·9% at 5·3 and 16·6 nmol/l, respectively; and 3α-diol-G 9·5% and 5·0% at 9·2 and 32·1 nmol/l, respectively. In addition, when NHANES III quality control samples were run with the mean typical male oestradiol concentration of 144·6 pmol/l, the intra-assay CV was 5·2% and the interassay CV was 2·5%. Similarly, the CV for samples assayed in duplicate in NHANES 2001–2004 was as follows: testosterone 4·8%, 3α-diol-G 9·7%, oestradiol 21·4%, and SHBG 5·6%. Free testosterone[18] and free oestradiol[19] were estimated from total testosterone and oestradiol, SHBG, and albumin concentrations, using mass action equations.

Serum 25(OH)D was assayed in NHANES III and NHANES 2001–2004 with a radioimmunoassay (RIA) kit (DiaSorin, Stillwater, MN, USA) at the National Center for Environmental Health, CDC, Atlanta, GA, USA. The CV for serum 25(OH)D was 10–25% (average 17·6%) for lower 25(OH)D values (20–62·5 nmol/l) and 12–18% (average 15%) for higher values (85–147·5 nmol/l).[20] In NHANES 2001–2004, CVs ranged from 10–13%.[21] We adjusted the serum 25(OH)D concentrations in NHANES III to make them comparable to NHANES 2000–2006 using the adjustment equation provided in the NHANES documentation.[22]

Serum total PSA concentration was measured as a component of NHANES 2001–2006 using the Hybritech method on the Beckman Access Immunoassay System (Beckman Coulter, Fullerton, CA, USA). The CV for total PSA was 4·6% with a range of 0·17 to 22·32 μg/l.[23] Men with a history of any of the following which are known to increase PSA concentration were excluded from the PSA measurement: current prostate infection, rectal examination in the past week, prostate biopsy or cystoscopy in the past month, and history of prostate cancer.

Covariate assessment

Information on physical activity, alcohol intake, cigarette smoking, and frequency and duration of vitamin D supplement use was collected during in-person interviews. Trained personnel measured participants' height, weight, and waist circumference. Body fat percentage was estimated with a prediction formula that used bioelectrical impedance analysis data, measured height and weight, and age.[24] Serum total cholesterol and serum cotinine, an indicator of recent exposure to cigarette smoke, were measured for all participants.[25]

Statistical analysis

Analyses were conducted using SUDAAN 9.0 (Durham, NC, USA) implemented in SAS 9.3 (Cary, NC, USA). Sampling weights were used to account for the NHANES complex survey design. To account for seasonal variation of serum 25(OH)D, season-specific quintile cut-points of serum 25(OH)D were created by season of blood collection (winter/spring: January–June or summer/fall: July–December) and then merged into one variable. Hormone concentrations were natural-log-transformed to normalize their distribution. Age-adjusted means or percentages of participant characteristics by quintile of serum 25(OH)D were calculated by directly standardizing to the US population age distribution in the 2000 Census. Linear regression was used to calculate geometric mean concentrations, and corresponding 95% confidence intervals (CIs) were calculated for each hormone by quintile of serum 25(OH)D and by commonly used *a priori* cut-points of 25(OH)D (<50, 50 to <75, ≥75 nmol/l).[26] The change in serum hormone concentration per 25 nmol/l change in serum 25(OH)D concentration was estimated using linear regression. To test for linear trend, a variable for quintiles of serum 25(OH)D was entered into the model as a continuous ordinal term.

All models adjusted for age (continuous) and race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, other). Factors associated with hormone concentrations in previous NHANES analyses were considered as potential confounders: per cent body fat (quintiles), waist circumference (quintiles), total serum cholesterol (quintiles), moderate or vigorous physical activity (quintiles of times/week), cigarette smoking (never, current, former), alcohol intake (nondrinker, >0 to <1 drink/week, 1 drink/week to <1 drink/day, ≥1 drink/day), and vitamin D supplement use (yes/no). Body fat percentage and smoking status were the only factors to alter crude effect estimates; therefore, multivariable models included age, race/ethnicity, body fat percentage, and cigarette smoking. Because bioavailable testosterone and oestradiol are dependent on SHBG concentration, additional models for total testosterone and total oestradiol were run with adjustment for SHBG.

To identify factors that potentially modify the association between serum 25(OH)D and sex hormones in NHANES III, for hormones for which we observed a main effect association with 25(OH)D, we conducted analyses stratified by age (20–39, 40–59, ≥60 years), race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican American), cigarette smoking (never, current, former), serum cotinine (unexposed, passively exposed, actively exposed), serum total cholesterol (<200, 200–<240, ≥240 mg/dl), body fat percentage (tertiles), and alcohol intake (nondrinker, <1, ≥1 drink/day). The sample size in NHANES 2001–2004 was insufficient to conduct stratified analyses. To test for interaction, a cross-product term with an ordinal term for the 25(OH)D quintiles and the potential effect modifier was entered into a model with the main effect terms, and the Wald statistic was used to determine its statistical significance.

To evaluate whether serum 25(OH)D concentration is associated with PSA, geometric mean and 95% CI of PSA concentration were calculated by quintile of serum 25(OH)D using linear regression and adjusting for age and race/ethnicity.

Results

25(OH)D and sex steroid hormones in NHANES III

Of the 1315 male participants with measured hormones in NHANES III, serum 25(OH)D concentrations were <50 nmol/l for 34·0% (n = 447), 50 to <75 nmol/l for 40·8% (n = 537), and ≥75 nmol/l for 25·2% (n = 331). The race/ethnicity distribution of participants was 46·6% non-Hispanic white (n = 613), 23·8% non-Hispanic black (n = 313), 25·4% Mexican American (n = 334), and 4·2% other (n = 55). Participant characteristics by quintile of serum 25(OH)D are presented in Table 1. After adjusting for age and race, men with higher serum 25(OH)D were more likely to be non-Hispanic white, have their blood collected during the summer, have lower BMI, body fat percentage, and waist circumference, and engage in more frequent physical activity.

Mean serum 25(OH)D concentration varied by season at blood draw. As expected, mean 25(OH)D concentration was higher in men with blood drawn in the summer (July–September: 76·4 nmol/l) and fall (October–December: 73·3 nmol/l) and lower in men with blood drawn in the winter (January–March: 66·6 nmol/l) and spring (April–June: 63·3 nmol/l). No seasonal variation was observed for testosterone, oestradiol, SHBG, or 3α-diol-G (data not shown).

In multivariable models, serum 25(OH)D was statistically significantly positively associated with total testosterone and SHBG (Table 2). There was also a suggestion that serum 25(OH)D was positively associated with free testosterone. No association was observed between serum 25(OH)D and total or free oestradiol or 3α-diol-G. Further adjusting for SHBG did not change the null association with total oestradiol (data not shown). Mutual adjustment of total testosterone and SHBG somewhat attenuated the associations with each, although both associations remained

Table 1. Age* and race-adjusted weighted characteristics by quintile of serum 25-hydroxyvitamin D for adult men in NHANES III 1988–1991

	Serum 25-hydroxyvitamin D (nmol/l)†					
	Q1	Q2	Q3	Q4	Q5	*P*-value
N (unweighted)	265	264	261	256	269	
Age (years), mean (SE)	43·4 (1·5)	43·5 (1·3)	38·9 (1·3)	37·9 (0·9)	35·4 (1·3)	<0·0001
Race/ethnicity (%)						
Non-Hispanic white	47·4	68·9	81·1	87·3	94·9	<0·0001
Non-Hispanic black	31·6	13·9	5·9	2·5	1·0	
Mexican American	5·0	6·8	6·1	3·7	1·8	
Other race/ethnicity	16·0	10·4	6·8	6·5	3·3	
Season of blood collection (%)						
Spring	30·2	27·6	27·2	32·2	34·1	<0·0001
Summer	19·8	24·0	26·0	21·7	37·7	
Fall	27·6	18·2	22·5	23·1	8·8	
Winter	22·5	30·3	24·3	23·0	19·5	
Body mass index (kg/m^2), mean (SE)	27·6 (0·5)	27·1 (0·7)	26·4 (0·6)	26·1 (0·4)	24·5 (0·5)	0·002
Body fat (%), mean (SE)	27·6 (0·6)	27·0 (0·5)	26·0 (0·6)	25·6 (0·6)	24·1 (0·7)	0·0004
Waist circumference (cm), mean (SE)	98·3 (1·4)	95·9 (1·5)	94·6 (1·6)	93·1 (1·0)	88·8 (1·1)	<0·0001
Cigarette smoking (%)						
Never	36·1	34·9	33·1	40·1	32·1	0·23
Former	27·7	24·8	34·0	30·0	32·1	
Current	36·3	40·4	33·0	29·9	35·8	
Cigarette smoke exposure (based on serum cotinine concentration) (%)						
Unexposed	10·1	19·3	15·1	17·3	16·2	0·68
Passively exposed	48·8	38·4	48·6	46·9	41·9	
Actively exposed	40·9	41·7	35·9	35·3	41·5	
Alcohol intake (%)						
Nondrinker	30·3	35·5	34·3	43·8	30·5	<0·0001
>0 to <1/week	15·6	12·1	10·9	7·5	15·1	
≥1/week to <1/day	32·8	39·4	38·0	33·8	34·7	
≥1/day	21·3	13·0	16·8	14·8	19·7	
Physical activity‡ (times/week), mean (SE)	5·1 (0·7)	5·8 (0·8)	6·0 (0·5)	7·8 (0·9)	8·0 (0·7)	0·004
Vitamin D supplement use (%)	13·9	19·9	15·7	19·3	26·0	0·12
Total serum cholesterol (mg/dL), mean (SE)	216·1 (5·6)	211·9 (3·7)	205·1 (2·6)	200·7 (2·0)	201·9 (2·9)	0·06

Q, quintile; SE, standard error.
*Adjusted for age in years (continuous) and race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, other).
†Season-specific cut-points (nmol/l): winter/spring months (January–June) – Q1: <43·7, Q2: 43·7–57·1, Q3: 57·2–68·9, Q4: 69·0–84·0, Q5 ≥ 84·1; summer/fall months (July–December) – Q1: <50·4, Q2: 50·4–63·5, Q3: 63·6–78·8, Q4: 78·9–97·5, Q5: ≥97·6.
‡Includes walking for a mile without stopping.

Table 2. Geometric mean (95% CI) serum sex hormone concentration by quintile of serum 25-hydroxyvitamin D in adult men in NHANES III (1988–1991) and continuous NHANES (2001–2004)

	NHANES III, 1988–1991 (N = 1315)*		Continuous NHANES, 2001–2004 (N = 318)†	
Serum vitamin D (nmol/l)	Age and race/ethnicity Adjusted	Multivariable adjusted‡	Age and race/ethnicity adjusted	Multivariable adjusted‡
Testosterone (nmol/l)				
Quintile 1	16·6 (15·3–18·0)	17·2 (16·1–18·3)	14·6 (12·7–16·9)	15·6 (13·7–18·1)
Quintile 2	15·5 (13·6–17·7)	15·6 (13·7–17·8)	16·5 (13·7–19·7)	16·9 (14·0–20·0)
Quintile 3	17·3 (16·5–18·1)	17·3 (16·6–18·1)	14·0 (12·7–15·6)	14·9 (13·4–16·2)
Quintile 4	17·7 (16·6–18·9)	17·9 (16·8–19·0)	16·2 (14·9–17·8)	16·2 (15·3–17·8)
Quintile 5	20·2 (19·2–21·2)	19·6 (18·7–20·6)	18·4 (16·9–20·4)	17·2 (15·6–18·8)
β§	0·07	0·05	0·22	0·08
P-trend	<0·0001	0·0002	0·05	0·65
Free testosterone (nmol/l)				
Quintile 1	0·34 (0·31–0·37)	0·34 (0·32–0·37)	0·39 (0·33–0·47)	0·41 (0·35–0·48)
Quintile 2	0·33 (0·28–0·37)	0·32 (0·28–0·37)	0·41 (0·34–0·50)	0·42 (0·35–0·50)
Quintile 3	0·36 (0·35–0·37)	0·36 (0·35–0·37)	0·36 (0·33–0·40)	0·38 (0·34–0·42)
Quintile 4	0·35 (0·33–0·38)	0·35 (0·33–0·38)	0·41 (0·38–0·44)	0·41 (0·37–0·44)
Quintile 5	0·37 (0·35–0·39)	0·37 (0·35–0·39)	0·41 (0·37–0·45)	0·39 (0·36–0·43)
β§	0·02	0·02	0·04	−0·04
P-trend	0·05	0·06	0·76	0·62
Oestradiol (pmol/l)				
Quintile 1	132 (124–142)	131 (123–142)	150 (115–198)	145 (115–183)
Quintile 2	130 (123–139)	128 (121–136)	129 (114–146)	130 (113–148)
Quintile 3	132 (127–138)	133 (128–138)	105 (95–118)	108 (98–119)
Quintile 4	134 (127–141)	135 (131–140)	113 (100–128)	113 (101–128)
Quintile 5	131 (123–139)	131 (123–139)	117 (103–133)	116 (102–131)
β§	−0·003	0·002	−0·07	−0·09
P-trend	0·93	0·79	0·08	0·05
Free Oestradiol (pmol/l)				
Quintile 1	3·5 (3·2–3·8)	3·4 (3·1–3·7)	4·3 (3·1–5·8)	4·0 (3·1–5·2)
Quintile 2	3·4 (3·2–3·7)	3·4 (3·2–3·6)	3·5 (3·1–3·9)	3·5 (3·1–4·0)
Quintile 3	3·5 (3·3–3·7)	3·5 (3·3–3·7)	2·9 (2·6–3·3)	3·0 (2·7–3·3)
Quintile 4	3·4 (3·2–3·6)	3·5 (3·3–3·6)	3·0 (2·7–3·5)	3·1 (2·7–3·5)
Quintile 5	3·2 (3·0–3·4)	3·2 (3·0–3·4)	3·0 (2·6–3·4)	3·0 (2·7–3·4)
β§	−0·03	−0·02	−0·17	−0·15
P-trend	0·06	0·22	0·01	0·01
3α-diol-G (nmolL)¶				
Quintile 1	37·5 (31·8–44·5)	37·2 (31·5–43·9)	20·5 (17·5–24·2)	21·0 (17·5–24·7)
Quintile 2	38·2 (33·4–43·2)	37·5 (32·8–42·9)	20·7 (18·0–24·2)	20·5 (17·7–23·5)
Quintile 3	39·1 (36·3–42·0)	38·8 (36·3–41·3)	19·0 (17·0–21·0)	18·7 (17·0–20·7)
Quintile 4	35·9 (32·8–39·4)	35·6 (32·8–39·1)	19·5 (17·7–21·2)	19·5 (17·7–21·2)
Quintile 5	37·8 (34·3–41·3)	38·5 (35·0–42·3)	18·5 (16·2–21·0)	18·7 (16·7–21·2)
β§	−0·02	−0·004	−0·14	−0·11
P-trend	0·79	0·88	0·22	0·35
SHBG (nmol/l)				
Quintile 1	32·3 (29·8–35·1)	33·8 (30·8–37·0)	21·6 (16·9–27·5)	22·7 (18·1–28·6)
Quintile 2	31·0 (29·1–32·9)	31·9 (30·3–33·7)	26·2 (23·2–29·7)	27·3 (24·6–30·4)
Quintile 3	31·5 (29·2–34·0)	31·7 (29·7–33·8)	24·2 (22·1–26·6)	25·0 (22·9–27·2)
Quintile 4	34·8 (33·1–36·6)	35·1 (33·4–36·8)	25·7 (22·6–29·2)	26·0 (23·0–29·4)
Quintile 5	40·1 (37·4–43·1)	38·4 (35·8–41·2)	34·4 (30·4–38·9)	31·5 (28·3–35·0)
β§	0·09	0·06	0·41	0·27
P-trend	<0·0001	0·0005	0·0001	0·01

*Season-specific quintile cut-points (nmol/l): winter/spring months (January–June) – Q1: <39·4. Q2: 39·4–50·7, Q3: 50·8–60·7, Q4: 60·8–73·4, Q5: ≥73·5; summer/fall months (July–December) – Q1: <45·1, Q2: 45·1–56·1, Q3: 56·2–69·0, Q4: 69·1–84·8, Q5: ≥84·9.

†Season-specific quintile cut-points (nmol/l): winter/spring months (November–April) – Q1: <32·4, Q2: 32·4–<48·7, Q3: 48·7–<54·9, Q4: 54·9–<64·9, Q5: ≥64·9; summer/fall months (May–October) – Q1: <37·4, Q2: 37·4–<49·9, Q3: 49·9–<59·9, Q4: 59·9–<77·4, Q5: ≥77·4.

‡Multivariable model adjusted for age in years (continuous), race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, other), per cent body fat (quintiles), and cigarette smoking (never, current, former).

§Beta for change in the natural logarithm of serum hormone concentration per 25 nmol/l increase in serum vitamin D.

¶NHANES III: DSL-10–9200 EIA kit, Diagnostic Systems Laboratories; NHANES 2001–2004: Direct 3α-diol-G ELISA kit, ALPCO Diagnostics.

statistically significant (data not shown). Results for total testosterone were essentially the same for the 249 men using (Q1 = 16·2, 95% CI = 14·0–18·8 and Q5 = 18·4, 95% CI = 17·2–19·4 nmol/l, *P*-trend = 0·02) and the 1066 men not using (Q1 = 15·6, 95% CI = 14·6–16·5 and Q5 = 17·8, 95% CI = 16·9–18·8 nmol/l, *P*-trend = 0·002) vitamin D supplements. Similarly, the association between serum 25(OH)D and total testosterone did not change in models that excluded 244 men with major comorbidities such as diabetes, stroke, myocardial infarction, and angina (data not shown). Age- and race-/ethnicity-adjusted results were similar using *a priori* cut-points for serum 25(OH)D (geometric mean, 95% CI; testosterone: <50 nmol/l = 14·0, 12·7–14·9 nmol/l, 50–<75 nmol/l = 14·9, 14·0–15·9 nmol/l, ≥75 nmol/l = 16·5, 15·3–17·5 nmol/l, *P*-trend = 0·0004; SHBG: <50 nmol/l 25(OH)D = 32·6, 30·5–34·8 nmol/l, 50–<75 nmol/l 25(OH)D = 34·5, 32·8–36·2 nmol/l, ≥75 nmol/l = 39·0, 37·1–41·0 nmol/l, *P*-trend ≤0·0001). Similarly, mean testosterone levels were similar for men with serum 25(OH)D < 30 nmol/l (14·6, 12·5–16·9 nmol/l testosterone) and serum 25(OH)D ≥ 30 nmol/l (15·1, 14·2–16·1 nmol/l testosterone) (*P*-value = 0·69).

Next, we performed stratified analyses of the age- and race-/ethnicity-adjusted association of 25(OH)D with total testosterone and SHBG. For total testosterone, the positive association with 25(OH)D was stronger in younger and older men than in middle-aged men (*P* for interaction = 0·008; Table 3). For SHBG, the positive association with 25(OH)D was present in younger and middle-aged men, but not in older men (*P* for interaction = 0·0002; Table 3). Race modified the association between serum 25(OH)D and total testosterone (*P* for interaction = 0·007; Table 4); positive associations were observed for non-Hispanic white and non-Hispanic black men (*P*-trend = 0·0005 and 0·002, respectively), but not Mexican American men (*P*-trend = 0·29). Possible differences by race were observed between serum 25(OH)D and SHBG (*P* for interaction = 0·16; Table 4): a positive association was observed for non-Hispanic white (*P*-trend ≤0·0001) and non-Hispanic black (*P*-trend = 0·0001) men, but not for Mexican American men (*P*-trend = 0·11). We did not observe any statistically significant interactions when the association of 25(OH)D with total testosterone and SHBG was stratified by cigarette smoking (*P* for interaction = 0·27 and 0·06), serum cotinine (*P* for interaction = 0·10 and 0·23), serum total cholesterol (*P* for interaction = 0·96 and 0·27), body fat percentage (*P* for interaction = 0·10 and 0·28), and alcohol intake (*P* for interaction = 0·92 and 0·08).

25(OH)D and sex steroid hormones in NHANES 2001–2004

Serum 25(OH)D concentrations were lower in NHANES 2001–2004 than in NHANES III. Of the 318 male participants included in the NHANES 2001–2004 analysis, serum 25(OH)D concentrations were <50 nmol/l for 39·9% (*n* = 127), 50 to <75 nmol/l for 42·5% (*n* = 135), and ≥75 nmol/l for 17·6% (*n* = 56). Similar to NHANES III, NHANES 2001–2004 was 44·7% non-Hispanic white (*n* = 142), 23·3% non-Hispanic black (*n* = 74), 24·2% Mexican American (*n* = 77), and 7·9%

Table 3. Geometric mean (95%CI) serum sex hormone concentration by quintile of serum 25-hydroxyvitamin D in adult men stratified by age, NHANES III (1988–1991)

	Age		
Serum 25-hydroxyvitamin D (nmol/l)*,†	20–39 years	40–59 years	≥60 years
Testosterone (nmol/l)			
Quintile 1	14·6 (13·0–16·2)	15·9 (13·7–18·8)	14·9 (12·7–17·5)
Quintile 2	16·2 (15·3–17·2)	12·1 (8·9–16·5)	14·3 (11·1–18·8)
Quintile 3	15·6 (13·7–17·2)	16·2 (15·3–17·2)	15·9 (13·4–19·4)
Quintile 4	16·5 (15·3–17·8)	16·2 (14·3–18·1)	15·3 (13·0–18·1)
Quintile 5	19·1 (17·5–21·0)	16·9 (15·6–18·1)	18·8 (15·6–22·9)
P-trend	<0·0001	0·05	0·002
P-interaction	0·008		
SHBG (nmol/l)			
Quintile 1	29·0 (25·8–32·5)	36·2 (32·9–39·8)	33·1 (27·0–40·6)
Quintile 2	29·3 (26·8–31·9)	30·2 (27·0–33·9)	38·5 (31·8–46·6)
Quintile 3	30·0 (25·7–35·1)	31·6 (29·4–34·0)	35·7 (29·7–42·8)
Quintile 4	34·2 (30·7–38·0)	34·3 (30·8–38·2)	38·3 (30·9–47·5)
Quintile 5	40·8 (36·9–45·0)	37·6 (33·2–42·5)	40·7 (33·0–50·3)
P-trend	<0·0001	0·06	0·13
P-interaction	0·0002		

SHBG, sex hormone-binding globulin.
*Season-specific quintile cut-points (nmol/l): inter/spring months (January–June) – Q1: <39·4. Q2: 39·4–50·7, Q3: 50·8–60·7, Q4: 60·8–73·4, Q5: ≥73·5; summer/fall months (July–December) – Q1: <45·1, Q2: 45·1–56·1, Q3: 56·2–69·0, Q4: 69·1–84·8, Q5: ≥84·9.
†Models adjusted for age in years (continuous) and race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, other).

Table 4. Geometric mean (95% CI) of serum sex hormone concentration by quintile of serum vitamin D in adult men stratified by race, NHANES III (1988–1991)

Serum 25-hydroxyvitamin D (nmol/l)*,†	Non-Hispanic White	Non-Hispanic Black	Mexican American
Testosterone (nmol/l)			
Quintile 1	15·3 (13·7–16·9)	15·9 (14·3–17·8)	15·3 (14·0–16·2)
Quintile 2	14·6 (12·7–17·2)	15·9 (14·9–17·2)	16·2 (14·9–17·8)
Quintile 3	15·9 (14·9–16·9)	17·2 (15·6–18·8)	15·9 (14·9–17·2)
Quintile 4	15·6 (14·6–16·9)	20·0 (18·1–22·3)	16·9 (15·9–18·4)
Quintile 5	18·4 (17·5–19·4)	21·0 (18·4–23·5)	16·2 (14·9–17·8)
P-trend	0·0005	0·002	0·29
P-interaction	0·007		
SHBG (nmol/l)			
Quintile 1	32·9 (29·0–37·3)	33·6 (31·6–35·7)	29·1 (27·2–31·0)
Quintile 2	30·9 (28·2–33·8)	36·1 (33·5–38·8)	33·4 (31·3–35·7)
Quintile 3	31·6 (28·8–34·8)	35·7 (33·0–38·6)	29·8 (27·6–32·2)
Quintile 4	34·3 (32·4–36·4)	37·7 (31·4–45·3)	36·7 (31·9–42·3)
Quintile 5	40·4 (37·6–43·5)	48·5 (42·5–55·3)	33·7 (29·1–39·0)
P-trend	<0·0001	0·0001	0·11
P-interaction	0·16		

SHBG, sex hormone-binding globulin.
*Season-specific quintile cut-points (nmol/l): winter/spring months (January–June) – Q1: <39·4. Q2: 39·4–50·7, Q3: 50·8–60·7, Q4: 60·8–73·4, Q5: ≥73·5; summer/fall months (July–December) – Q1: <45·1, Q2: 45·1–56·1, Q3: 56·2–69·0, Q4: 69·1–84·8, Q5: ≥84·9.
†Models adjusted for age in years (continuous).

other ($n = 25$). The patterns of association of serum 25(OH)D with testosterone and SHBG in NHANES 2001–2004 (Table 2) were generally similar to what we observed in NHANES III. As with NHANES III, mean testosterone levels were not significantly different for men with <30 nmol/l of serum 25(OH)D (16·2, 13·3–19·8 nmol/l testosterone) or ≥30 nmol/l of serum 25(OH)D (17·0, 16·0–18·1 nmol/l testosterone) ($P = 0·67$). 25(OH)D was possibly inversely associated with total and free oestradiol for NHANES 2001–2004 (Table 2), but not in NHANES III. 25(OH)D was not associated with 3α-diol-G in NHANES 2001–2004. It should be noted that the geometric means for 3α-diol-G were lower in NHANES 2001–2004, possibly due to the use of different assay kits for NHANES III and NHANES 2001–2004 for this hormone.

25(OH)D and PSA in NHANES 2001–2006

To further address why in the modern era, 25(OH)D concentration appears to be positively associated with prostate cancer risk, we evaluated whether serum 25(OH)D concentration was associated with PSA in NHANES 2001–2006. Among the 4013 men who had serum PSA and 25(OH)D data available, PSA levels were not significantly different across quintiles of serum 25(OH)D after adjustment for age and race/ethnicity (P-trend = 0·34) (Table 5).

Table 5. Geometric mean (95% CI) of prostate-specific antigen by quintile of serum vitamin D in adult men, NHANES 2001–2006

Serum vitamin D (nmol/l)	PSA (nmol/l)*
Quintile 1	3·3 (3·1–3·7)
Quintile 2	3·7 (3·4–4·0)
Quintile 3	3·3 (3·0–3·6)
Quintile 4	3·6 (3·3–3·8)
Quintile 5	3·7 (3·4–4·0)
P-trend	0·34

PSA, prostate-specific antigen.
*In men aged 50 years and older with no history of prostate cancer. Models adjusted for age and race/ethnicity.

Discussion

To our knowledge, this is the first study to examine associations between serum 25(OH)D concentration and sex steroid hormones in a sample of men representative of the general US population. After adjusting for age, race/ethnicity, and other potential confounders, higher serum 25(OH)D concentration was significantly associated with higher serum total testosterone and SHBG in NHANES III and continuous NHANES. No association was observed between serum 25(OH)D and PSA, suggesting the positive association between 25(OH)D and prostate cancer in the previous studies is not due to detection bias.

Our finding that serum vitamin D concentration is positively associated with serum testosterone concentration is consistent with the results from a small randomized controlled trial of vitamin D supplementation[13] and several observational studies.[11,12] In the European Male Ageing study, serum vitamin D was weakly associated with higher testosterone and lower oestradiol concentrations in men aged 40–79, but these associations did not remain significant after adjustment for confounding fac-

tors.[14] No association was observed between serum vitamin D and testosterone levels in a US study of 170 healthy men[15] or a German study of 200 middle-aged men.[16] For oestradiol, the Health Professionals Follow-up Study reported a positive association for serum 25(OH)D.[11] In NHANES III, we did not observe an association with total or free oestradiol concentration, but we did observe suggestive inverse associations in NHANES 2001–2004. In contrast to our finding of a positive association between serum 25(OH)D and SHBG, a German study observed that higher serum 25(OH)D was associated with significantly lower levels of SHBG.[12] The positive association we observed between serum 25(OH)D and SHBG appeared to be independent of testosterone concentration; however, the biological significance of this association is unclear.

PSA-associated detection bias is one possible explanation for the positive association between serum 25(OH)D and prostate cancer observed in contemporary observational studies (in the PSA era). If men with higher vitamin D also have higher PSA levels, they may be more likely to undergo a biopsy that detects prostate cancer. We did not observe an association between serum 25(OH)D and PSA concentration in NHANES surveys from 2001–2006. In a subset of these same data, we found a suggestion of a positive association between serum 25(OH)D and testosterone, supporting a possible role for testosterone, rather than detection bias, in the association between serum 25(OH)D and prostate cancer.

An interaction was observed with race, where the significant positive associations between serum 25(OH)D and total testosterone and SHBG were not observed in Mexican American men. The majority of studies that have examined 25(OH)D in relation to risk of prostate cancer have been conducted in white men, although a few studies have evaluated the association between serum 25(OH)D and prostate cancer risk in African American men[27–31] (with several also observing a positive association). However, the literature on 25(OH)D and prostate cancer in men of other race/ethnicities remains limited, and the findings from this study may be relevant as more studies compare the vitamin D–prostate cancer association across racial/ethnic groups.

The mechanisms through which vitamin D may influence circulating sex steroid hormones are unclear. The vitamin D receptor (VDR), which mediates the biologic effects of vitamin D, is expressed in tissue throughout the male reproductive tract,[32] including Leydig cells,[33] the primary source of testosterone production in the testes. Further supporting an influence of vitamin D on hormone synthesis, male VDR knockout mice have elevated serum luteinising hormone, the primary hormone responsible for initiating testosterone production, indicating hypogonadism in the presence of VDR inactivity.[34] In a study of prostate cancer cell lines treated with the active vitamin D metabolite 1α,25-dihydroxyvitamin D_3, a significant increase in testosterone production was observed after the cells were exposed to vitamin D.[35]

The strengths of these studies are the nationally representative data, the ability to address the consistency of associations using data from both NHANES III and continuous NHANES, and comprehensive assessment of potential confounding factors. However, the sample size was not large enough to perform stratified analyses in continuous NHANES or multivariable adjustments in stratified models in NHANES III. It is unlikely that confounding strongly influenced any associations observed in the stratified analyses given that there was little change in the effect estimates between age- and race-/ethnicity-adjusted and multivariable-adjusted models. Unlike most previous studies that were composed mostly of white men, this was the first study to also examine associations between serum 25(OH)D and hormones in Mexican American and non-Hispanic black men. Due to the broad age range in NHANES, we were also able to observe that the association between serum 25(OH)D and SHBG was age-dependent. A limitation is that due to the cross-sectional design, we cannot establish the temporality of the associations we observed. Also, the relatively high CV%s for serum 25(OH)D measurement may result in misclassification of vitamin D status, potentially underestimating the association between serum vitamin D and hormone concentrations.

In summary, our findings support positive associations between serum vitamin D and serum concentrations of testosterone and SHBG in men, and suggest a possible mechanism through which vitamin D may be related to increased prostate cancer risk. A lack of association between vitamin D and PSA suggests that the positive vitamin D–prostate cancer association reported in the literature is unlikely due to PSA-detection bias. However, the mechanism through which vitamin D is related to testosterone remains unknown and warrants further elucidation.

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