Published in final edited form as:
Int J Cancer. 2013 June 15; 132(12): 2940–2947. doi:10.1002/ijc.27969.

Circulating 25-Hydroxyvitamin D, Vitamin D Binding Protein, and Risk of Prostate Cancer

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Abstract

We recently reported a significant positive association between 25-hydroxyvitamin D [25(OH)D], the accepted biomarker of vitamin D status, and prostate cancer risk. To further elucidate this association, we examined the influence of vitamin D binding protein (DBP), the primary transporter of vitamin D compounds in the circulation. Prediagnostic serum concentrations of DBP were assayed for 950 cases and 964 matched controls with existing 25(OH)D measurements within the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study of Finnish men. Logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (CI), and statistical tests were two-sided. Serum DBP modified the association between serum 25(OH)D and prostate cancer, with higher risk for elevated 25(OH)D levels observed primarily among men having DBP concentrations above the median (OR=1.81, 95% CI 1.18–2.79 for highest vs. lowest quintile, p-trend = 0.001) compared to those with DBP below the median (OR=1.22, 95% CI 0.81–1.84, p-trend 0.97; p-interaction = 0.04). Serum DBP was not associated with prostate cancer risk overall (OR=0.96, 95% CI 0.70–1.33 for highest vs. lowest quintile); however, high serum DBP was associated with significantly decreased risk of prostate cancer in men with lower (<median) 25(OH)D concentrations (OR=0.59, 95% CI 0.38–0.90 for highest vs. lowest quintile, p-trend = 0.003) and increased risk in men with higher 25(OH)D concentrations (OR = 1.47, 95% CI 0.98–2.20, p-trend 0.10, p-interaction = 0.02). Our data suggest that the primary vitamin D carrier protein, DBP, modulates the impact of vitamin D status on prostate cancer.

Keywords

vitamin D binding protein; 25-hydroxyvitamin D; prostate cancer; serum biomarkers; prospective study

Higher vitamin D status is thought to reduce the risk of several malignancies, including prostate cancer, by inhibiting cellular proliferation and angiogenesis, reducing inflammation, and promoting differentiation and apoptosis.[1,2] The epidemiologic evidence does not, however, indicate a protective association between circulating 25-hydroxyvitamin D [25(OH)D], the accepted biomarker of vitamin D status, and prostate cancer risk. For example, three meta-analyses of up to 14 prospective studies found no relationship between circulating 25(OH)D and prostate cancer risk,[3–5] and a recent review concluded that the

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results were conflicting.[6] Yet several studies suggest that men with higher circulating 25(OH)D are at increased risk,[7] although individually most were not statistically significant.[3,4] Prostate cancer continues to be the most commonly diagnosed cancer in American men, with 241,700 new cases estimated for 2012, and is second only to lung cancer for male cancer mortality, with an estimated 28,200 deaths.[8]

We recently reported significantly elevated risk of prostate cancer among men with higher serum 25(OH)D concentrations in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study.[7] In an effort to further characterize and elucidate the underlying biology for the finding, we quantified serum vitamin D binding protein (DBP), the primary transport molecule of 25(OH)D and other vitamin D compounds in circulation.[9,10] The DBP - prostate cancer relation including the individual and interactive effects of DBP and 25(OH)D on prostate cancer risk was examined, as well as the association of the estimated unbound, free fraction of 25(OH)D.

Participants and Methods

Study population

ATBC was a controlled, primary prevention trial of α-tocopherol and β-carotene supplementation, which has been previously described.[11] Briefly, 29,133 Finnish male smokers, aged 50 – 69 years, were recruited between 1985 and 1988 for daily supplementation with α-tocopherol (50 mg dl-α-tocopheryl acetate/day), β-carotene (20 mg/day), both, or placebo. Supplementation continued for 5–8 years (median 6.1 years) until death or trial closure (April, 1993).[11] Participants are passively followed through linkage with the Finnish Cancer Registry, which provides nearly 100% case ascertainment.[12] The study was approved by the institutional review boards of the U.S. National Cancer Institute and the National Public Health Institute of Finland, and written informed consent was obtained from all participants.

Case identification and control selection

The present study is based on a prior nested case-control set[7] of 1,000 prostate cancer cases and 1,000 controls, matched on age (± 1 year) and date of serum collection (± 30 days), and assayed for 25(OH)D. Of these, 950 cases and 964 controls total (representing 948 matched sets) had available residual serum for the DBP assay. All cases were identified through the Finnish Cancer Registry, defined by International Classification of Diseases 9, code 185, and diagnosed through April 2005. Stage was based on the tumor-node-metastasis staging system of the American Joint Committee on Cancer[13] and 157, 180, 161, and 234 cases, respectively, were stage 1–4. Sixty cases were Gleason grade 2–4, 100 were grade 5–6, 78 were grade 7, and 80 were grades 8–10. Because of the design of the parent study, approximately 20% of cases were missing information on stage and 67% were missing information on Gleason grade. Aggressive cases (n=424) were defined as stage 3 or 4, or with a Gleason score of ≥8. Controls were alive and cancer free at the time of case diagnosis.

Data collection

Height and weight were measured at baseline, and participants completed questionnaires regarding general risk factors, smoking, medical history, family history of cancer, and vitamin supplement use[11] Food frequency questionnaires, intended to measure usual consumption over the previous 12 months, included both portion size and frequency of consumption for 276 food items and mixed dishes.[14] Nutrient intake was estimated using a national food composition database available from the National Public Health Institute of Finland.

Laboratory assays

Fasting serum samples were collected at baseline and stored at −70°C. DBP was measured using the Quantikine Human Vitamin D Binding Protein Immunoassay kit (Catalog number DVDBP0, R&D Systems, Inc, Minneapolis, MN) at the SAIC-Frederick, Frederick National Laboratory for Cancer Research facility in Frederick, MD. Matched case/control sets were assayed consecutively within each batch, along with approximately 10% masked quality control samples included in every batch. The quality control material was a serum pool from eight male smokers over 50 years of age. A nested components of variance analysis[15] was used to calculate interbatch and intrabatch coefficients of variation, of 10.8% and 15.2%, respectively. 25(OH)D was previously measured using a direct, competitive chemiluminescence immunoassay (DiaSorin Liaison 25(OH)D TOTAL assay) with interbatch and intrabatch coefficients of variation of 7.1% and 10.1%, respectively.[7,16] Serum α-tocopherol, β-carotene, cholesterol, and retinol were previously measured for all trial participants at baseline.[11]

Statistical analysis

Among controls, baseline descriptive data by quintile of vitamin D binding protein are presented as medians (continuous variables) or proportions (categorical variables), and tested for significance using the general linear models procedure. Odds ratios (OR) and 95% confidence intervals (CI) were determined using conditional logistic regression for the main models and unconditional logistic regression for the stratified models (the latter in order to retain subjects who were not in the same stratum as their matched case or control and to include unmatched subjects), although results were similar using either approach. DBP concentrations were categorized as quintiles, based on the control distribution, while 25(OH)D quintile categorizations were based on the control distribution separately by season (May-October and November-April) and then merged into one quintile variable. These variables were entered into the models as indicator variables and trend tests were obtained by assigning an ordinal value (1–5), treating this parameter as a continuous variable, and assessing its statistical significance using the Wald test. 25(OH)D was also modeled by 1) pre-defined cutpoints (<25, 25 to <37.5, 37.5 to <50, 50 to <75, and ≥ 75 nmol/L) based on clinical definitions in the literature,[7] and, 2) quintiles of season-standardized residuals from the regression of log transformed 25(OH)D on calendar week of blood collection using a locally weighted polynomial regression method, previously described.[16] In addition, 25(OH)D was residually-adjusted for DBP [17] and categorized into quintiles. DBP was also modeled as a continuous variable of 1000 nmol/L increments.

We calculated a “free 25(OH)D” using measured serum 25(OH)D, DBP, the affinity constants for albumin and DBP, and a fixed albumin value for every subject (0.6 mmol/L).[18,19] In addition, we estimated a “free 25(OH)D” without albumin because it was not measured (due to limited biospecimen availability), it has a minor vitamin D transport function compared to DBP, and as described previously, this approach provides a valid approximation comparable to the 25(OH)D:DBP molar ratio.[19] The latter also served as a proxy for free circulating 25(OH)D.[19,20]

Factors examined as potential confounding variables consisted of those used in the previous 25(OH)D analysis,[7] those associated with DBP in Table 1, those included in our previous DBP and pancreatic cancer analysis,[21] and other factors that have been associated with prostate cancer. These included age, body mass index (BMI, weight (kg)÷(height, m)^2), number of cigarettes smoked per day, years of smoking, education, physical activity; history of diabetes or benign prostatic hyperplasia; family history of prostate cancer; serum α-tocopherol, β-carotene, retinol, and cholesterol; intake of vitamin D and ethanol; and vitamin D supplement use. As none of the identified factors met the definition of a

confounder where the addition or removal of the factor would result in a >10% change in the DBP coefficients, models were adjusted only for the covariates included in the previous 25(OH)D/prostate analysis (age, family history of prostate cancer, and serum α-tocopherol).[7] In addition, we mutually adjusted 25(OH)D for DBP and vice versa.

To evaluate effect modification, we stratified 25(OH)D and the ratio of 25(OH)D:DBP by DBP. We also stratified DBP by 25(OH)D, using the season-specific ranking of 25(OH)D to calculate the median split, and we examined DBP stratified by age, BMI, number of cigarettes/day, years of smoking, physical activity, season of blood collection, α-tocopherol trial supplementation; serum α-tocopherol, retinol, and cholesterol; and intakes of fat (total, monounsaturated, saturated, and trans fat), vitamin D, calcium, and ethanol; disease aggressiveness; and follow-up time. Effect modification was tested by including the cross-product term of the main effect (DBP, 25(OH)D, or the 25(OH)D:DBP molar ratio) in quintiles and the effect modifier split at the median or as a 2-level category. Statistical analyses were performed using SAS software version 9.2 (SAS Institute, Inc., Cary, North Carolina) and all *P*-value were 2-sided.

Results

Case and control characteristics for this nested sample have been previously reported.[7] Cases had higher serum α-tocopherol and lower serum retinol, were more likely to have a family history of prostate cancer, but did not differ with respect to other factors.[7] Controls with higher circulating DBP had lower total vitamin D intake (from diet and supplements) and higher serum retinol compared to those in the lower DBP quintiles (Table 1). Men in the highest vs. lowest quintile had a 2.5 times greater median DBP level, and 2.5 times lower median 25(OH)D:DBP ratio (a proxy for free 25(OH)D). Among the controls, DBP concentrations ranged from 3414–8410 nmol/L (for 10^{th}–90^{th} percentiles) and did not vary by the season of blood collection.

Serum DBP was significantly inversely correlated with the molar ratio of 25(OH)D:DBP [Spearman correlation coefficient (r) = −0.46, p=<0.0001], but was only weakly correlated with serum 25(OH)D (r=0.06, p=0.08). DBP was not correlated with smoking characteristics (r=0.006 and −0.003 for cigarettes per day and years of smoking, respectively) or with serum α-tocopherol, β-carotene, or total or HDL cholesterol, but was weakly correlated with serum retinol (r=0.09, p=0.01). Serum DBP was also not correlated with age, height, weight, BMI, or intakes of vitamin D (diet or total), calcium, or alcohol, but was weakly correlated with energy (r=−0.06, p=0.05) and fat (r=−0.08, p=0.01) intake.

In line with our previously reported finding,[7] higher serum 25(OH)D was associated with significantly increased prostate cancer risk (OR= 1.45, 95% CI 1.06–1.99 for highest vs. lowest quintile, p-trend = 0.04, Table 2). The multivariate risk estimates did not differ with further adjustment for circulating DBP, and residual adjustment of 25OHD for DBP resulted in similar risk estimates (OR=1.59, 95% CI 1.13–2.24 for highest vs. lowest quintile; p-trend = 0.07).

DBP concentrations were not associated with prostate cancer risk (Table 2), and further adjustment for 25(OH)D did not alter the quintile risk estimates. The odds ratio for a 1000 nmol/L increment in DBP was 0.99 (95% CI 0.94–1.04, p=0.65). The molar ratio of 25(OH)D:DBP was positively associated with prostate cancer risk, although the odds ratio for the highest quintile was not statistically significant (although those for the third and fourth quintiles were) (Table 2). Using estimated “free 25(OH)D”, with or without albumin, yielded odds ratios similar to that for the molar ratio, although the highest quintile was

statistically significant when a constant for albumin concentration was included (OR= 1.43, 95% CI 1.02–2.01).

Serum DBP modified the association between 25(OH)D and prostate cancer, with higher risk for elevated 25(OH)D observed primarily among men having DBP concentrations above the median (OR=1.81, 95% CI 1.18–2.79 for highest vs. lowest quintile, p-trend = 0.001; p-interaction = 0.04). This was also true when 25(OH)D was modeled using pre-defined cutpoints or the season-standardized values (data not shown). When further stratified by aggressive vs. non-aggressive disease, the associations mirrored the overall pattern for 25(OH)D where the elevated risk was noted only when DBP was above the median: OR=1.94, 95% CI 0.89–4.24 for highest vs. lowest quintile, p-trend= 0.05, for non-aggressive disease, and OR=1.91, 95% CI 1.00–3.66, p-trend =0.02 for aggressive disease. The pattern of interaction for the 25(OH)D:DBP molar ratio reflected that of 25(OH)D and showed elevated risks when DBP was above the median (OR=1.71, 95% CI 1.00–2.92 for the highest vs. lowest quintile, p-trend 0.01), although the interaction was not statistically significant.

Although serum DBP was not associated with prostate cancer overall, an effect modification was noted where risk decreased significantly with higher serum DBP among men whose 25(OH)D concentrations were below the median (OR=0.59, 95% CI 0.38–0.90 for highest vs. lowest quintile, p-trend = 0.003, Table 3). By contrast, prostate cancer risk increased with circulating DBP when 25(OH)D concentrations were above the median (p-interaction = 0.02), although 95% CIs for higher DBP levels did not exclude one and the trend test was not significant. This increased risk was more apparent when limited to aggressive cancers (OR=1.92, 95% CI 0.97–3.79, for highest vs. lowest quintile), compared with non-aggressive cancers (OR=1.21, 95% CI 0.65–2.25). None of the other factors tested modified the association between circulating DBP and risk of prostate cancer (data not shown).

Discussion

As we previously reported, 25(OH)D is positively associated with prostate cancer risk,[7] but the present analysis shows this association to be substantially stronger and statistically significant only when concentrations of the vitamin D transport molecule in circulation, DBP, are also higher. Serum DBP concentrations were not associated with risk of prostate cancer overall. The relationship differed qualitatively based on vitamin D status, however, such that higher DBP was related to decreased risk among men with lower 25(OH)D concentrations and possibly elevated risk in men with higher vitamin D levels. Although these DBP-25(OH)D interactions differ from our findings for pancreatic cancer,[21] they may indicate organ site-specific differences in vitamin D uptake or signaling and may have biologically relevant implications for the vitamin D-prostate cancer relation.

Several studies examined circulating 25(OH)D prospectively, yet meta-analyses have not concluded that higher vitamin D status is related to decreased prostate cancer risk,[3–5] with summary relative risk estimates of 0.99 (95% CI 0.95–1.03),[5] 1.03 (0.96–1.11),[3] and 1.04 (0.99–1.10)[4] for 25 nmol/L increments in 25(OH)D. In fact, more than half of the individual studies in the latest analysis observed elevated risk for the highest vitamin D category,[4] and recent findings from three large studies not included in the prior meta-analyses indicate no association,[22] a non-linear positive association,[23] and a 50– 60% increased prostate cancer risk for men with the highest vitamin D concentrations (OR 1.56, 95% CI 1.15–2.72).[7] A review of evidence regarding vitamin D intake revealed an inverse association with prostate cancer risk in only one of nine studies, and this was for supplemental vitamin D intake >600 IU/day;[6] the authors suggested that the level of intake may not have been sufficiently high in most other studies. As compared with measuring circulating 25(OH)D concentrations,

however, issues inherent to imprecision in the estimation of vitamin D intake include few foods containing vitamin D with widely varying vitamin D content, the major food sources (e.g., fatty fish) are not typically eaten frequently, and some food composition databases contain inaccurate data for vitamin D.[2] Greater exposure to sunlight or UVB has been more consistently associated with lower risk of prostate cancer,[6] but most of these studies are ecological in nature, using aggregate and not individual level data and therefore susceptible to confounding and exposure misclassification (e.g., due to sunscreen or other sun protection behaviors).[2] In contrast, there are only two investigations of DBP and prostate cancer risk with both a nested case-control study of DBP,[24] and another that examined the 25(OH)D:DBP molar ratio (or "free 25-D index") but not DBP itself,[25] showing no association.

DBP, also known as the group-specific component of serum, or Gc-globulin, is a 58 kDa glycoprotein that is synthesized and secreted primarily by the liver[9,10] and transports vitamin D and metabolites in circulation. DBP concentrations appear to be stable throughout the lifespan and do not vary by season,[9,20] but do show a diurnal pattern.[9] Only 0.4% of $1,25(OH)_2D$ and 0.04% of 25(OH)D circulate freely (i.e., not bound to DBP), due to the high affinity of DBP for $1,25(OH)_2D$ and 25(OH)D (higher for the latter).[10] DBP circulates at much higher concentrations (μM) compared with that of vitamin D (nM), resulting in less than 5% of DBP carrying vitamin D or its metabolites.[10] As a result, DBP serves as a reservoir that binds and delivers additional 25(OH)D to target tissues, thereby protecting against short-term vitamin D deficiency by increasing its half-life.[9] Other DBP functions include its scavenging role in binding actin and serving as a cochemotactic factor,[9,10,26] as well as its deglycosylation and conversion to a tumor-inhibiting macrophage activating factor.[27–29] The latter has been shown to prevent tumor growth in cell culture, animal, and human studies for many organ sites (including prostate, breast, pancreas, and colorectum) by inhibiting angiogenesis and cellular proliferation and migration, and via activation of tumoricidal macrophages.[27–29] Whether any of these anti-cancer properties of DBP are relevant to the present findings remains to be determined.

Over 120 rare variants for the gene encoding DBP, *GC*, have been identified.[9] The three most common variants are *Gc1F, Gc1S*, and *Gc2*, which correspond to haplotypes based on alleles in two SNPs - rs7041 and rs4588.[26] These and other variants (*e.g.*, rs2282679) appear functionally related to the affinity of DBP for vitamin D, and have been associated with circulating 25(OH)D, $1,25(OH)_2D$, and DBP concentrations in genome-wide association and candidate gene studies.[9,26,30–35] Further examination of genetic patterns, in conjunction with circulating DBP, may reveal additional associations of importance.

Our finding of elevated prostate cancer risk for higher 25(OH)D status primarily among men with DBP concentrations above the median is intriguing. Although speculative, having more DBP may facilitate vitamin D uptake by prostatic epithelium; for example, through increased endocytosis of DBP-bound 25(OH)D mediated by the megalin-cubilin cell membrane complex similar to that occurring in the renal proximal tubules.[36,37] Male reproductive tract and prostate tissue have been shown to express megalin,[38–40] which, as in the kidney and other organs, has a multi-ligand function for absorption of other molecules including proteins, lipids, vitamins, and steroid hormones relevant to organ-specific epithelium (e.g., SHBG-bound testosterone).[41,42] This raises the possibility that higher extracellular concentrations of DBP plus 25(OH)D result in upregulation of megalin-mediated internalization of not only the DBP-25(OH)D complex but of SHBG-bound testosterone, thereby providing a direct androgenic stimulus for prostate tumor progression and growth. Because circulating levels of 25(OH)D and testosterone are correlated,[43] it is also possible that intracellular concentrations are similarly associated. Additional research into these potential biological mechanisms will be useful, including examination of

functional variants in the gene encoding megalin, low density lipoprotein receptor-related protein 2 (*LRP2*), which have been associated with prostate cancer progression and mortality.[44]

The prospective design, inclusion of a large number of prostate cancer cases, and up to 20 years of follow-up are strengths of this study. Measurement of 25(OH)D and DBP in pre-diagnostic serum samples reduced the likelihood of reverse causality and enabled us to examine the influence of 25(OH)D and DBP simultaneously. In addition, blood samples were collected in the morning after an overnight fast. The ATBC Study is a cohort of male smokers, potentially limiting the generalizability of our findings to nonsmokers; however, DBP was not correlated with smoking dose or duration, and these factors did not confound any of the associations, including in the stratified models. A prostate-specific antigen (PSA) detection bias is unlikely given that PSA screening was uncommon in Finland during the majority of the observation period as compared with the United States, and very few prostate cancer cases were PSA-screen detected.[45] We tested and controlled for a number of potential confounding factors, based on data collected prior to cancer diagnosis; therefore, residual confounding by smoking or other unmeasured factors in our data in also unlikely, although still possible. DBP and 25(OH)D were measured in single blood samples drawn at baseline, which may not be representative of long-term status; however, DBP concentrations fluctuate little over time,[46,47] and several studies have reported correlations for 25(OH)D measured 3 years (r=0.70),[48] 5 years (r=0.53),[49] and 14 years (r=0.52)[50] apart. Circulating 25(OH)D values in the ATBC Study are lower than in many other populations[16], due to the low prevalence of vitamin D supplement use, the high geographic latitude, and few study clinic blood collections in the peak summer months. By contrast, DBP concentrations are in line with other populations.[9,10]

In summary, while serum DBP concentrations were not directly associated with prostate cancer risk overall in this prospective study, we observed an interaction between circulating 25(OH)D and DBP wherein the elevated risk for higher circulating 25(OH)D shown here and previously reported[7] was restricted to men with higher DBP concentrations. In addition, high DBP concentrations were associated with significantly reduced prostate cancer risk when 25(OH)D concentrations were lower, and with nearly significant elevations in prostate cancer risk when 25(OH)D was higher. Our findings indicate that the biological impact of vitamin D status on prostate cancer is modulated by its major blood transport protein and warrants examination in other populations that include nonsmokers and men with higher 25(OH)D concentrations.

Acknowledgments

This work was supported by the Intramural Research Program of the National Cancer Institute at the National Institutes of Health. Additionally, this research was supported by U.S. Public Health Service contracts (N01-CN-45165, N01-RC-45035, N01-RC-37004, HHSN261201000006C, and HHSN261200800001E) from the National Cancer Institute, National Institutes of Health. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

Abbreviations

25(OH)D	25-hydroxyvitamin D
(ATBC) Study	Alpha-Tocopherol, Beta-Carotene Cancer Prevention
BMI	body mass index
CI	confidence intervals

OR	odds ratio
PSA	prostate-specific antigen
DBP	vitamin D binding protein

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Novelty and impact statement

Although vitamin D has been hypothesized to reduce cancer risk, the epidemiologic evidence for prostate cancer is inconsistent. We previously observed increased prostate cancer risk with higher vitamin D status in a prospective cohort study; here we find that this association is substantially stronger when vitamin D binding protein (DBP) concentrations are also higher. Our data suggest that DBP, the primary vitamin D transporter, modulates the impact of vitamin D status on prostate cancer.

Table 1

Selected baseline characteristics across vitamin D binding protein quintiles in controls, ATBC Study, 1985–2005[a]

	Quintile of serum vitamin D binding protein[b]					
Characteristic	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	**p-value**
N	193	193	193	193	192	
Age, years	58	57	58	57	57	0.82
Height, cm	174	173	173	174	173	0.69
Weight, kg	77.3	80	77.3	76.9	76.0	0.37
Body mass index, kg/m^2	26.0	26.2	25.6	25.4	25.8	0.10
Education, % > elementary	23.3	21.2	23.8	17.1	22.9	0.49
Cigarettes/day	20	20	20	20	20	0.91
Years of smoking	38	35	38	37	37	0.23
History of diabetes, %	4.2	2.6	4.7	6.2	1.6	0.14
History of BPH, %	5.7	4.2	2.6	3.6	4.2	0.64
Family history of prostate cancer, %	2.6	2.6	4.7	3.6	1.6	0.45
Leisure activity, moderate and heavy, %	58.6	55.4	65.8	59.1	56.3	0.26
Vitamin D supplement use, %	9.8	6.2	5.2	5.7	5.2	0.14
Calcium supplement use, %	12.4	9.8	9.3	9.3	12.0	0.77
Energy intake, kcal/day	2629	2709	2641	2576	2518	0.69
Dietary vitamin D intake, ug/day	4.7	5.1	4.6	4.7	4.6	0.37
Total vitamin D intake (diet and supplements, ug/day)	5.1	5.3	4.8	4.9	4.9	0.03
Calcium intake, mg/day	1313	1373	1304	1324	1343	0.61
Total calcium intake (diet and supplements, mg/day)	1313	1380	1304	1325	1343	0.64
Fat intake, g/day	120	124	119	115	116	0.21
Ethanol intake, g/day	8.3	8.9	9.3	10.7	9.0	0.93
Season of blood draw, % May-October	44.6	34.2	42.5	37.8	41.1	0.25
Serum biomarkers						
DBP, nmol/L	3414	4622	5555	6588	8422	<0.0001
25(OH)D, nmol/L	34.6	29.8	35.5	32.2	33.7	0.09
25(OH)D:DBP molar ratio (×10^3)[c]	10.4	6.53	6.52	4.84	3.98	<0.0001
α-tocopherol, mg/L	11.7	11.3	11.9	11.4	11.6	0.85

Characteristic	Quintile of serum vitamin D binding protein[b] Q1	Q2	Q3	Q4	Q5	p-value
β-carotene, ug/L	201	178	201	163	186	0.19
Total cholesterol, mmol/L	6.10	6.22	6.41	6.29	6.33	0.42
HDL cholesterol, mmol/L	1.13	1.12	1.11	1.12	1.13	0.71
Retinol, ug/L	551	558	574	592	582	0.02

[a] values are medians or proportions

[b] Quintile cuts for DBP were: Q1: ≤ 4077, Q2: >4077 and ≤ 5113, Q3: > 5113 and ≤ 6058, Q4: > 6058 and ≤ 7320 Q5: >7320 nmol/L

[c] a proxy for free 25(OH)D

Table 2

Odds ratios and 95% confidence intervals for the association between serum 25(OH)D, DBP, and the 25(OH)D:DBP molar ratio, and risk of prostate cancer, ATBC Study, 1985–2005

	Quintile 1	**Quintile 2**	**Quintile 3**	**Quintile 4**	**Quintile 5**	**p-trend**
	OR (95% CI)	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	
25(OH)D						
Range [a]						
# cases/# controls	162/190	192/192	197/189	188/185	209/192	
Model 1[b]	1.00 (reference)	1.27 (0.93–1.72)	1.31 (0.97–1.78)	1.28 (0.93–1.76)	1.45 (1.06–1.99)	0.04
Model 2[c]	1.00 (reference)	1.27 (0.93–1.73)	1.32 (0.97–1.79)	1.29 (0.94–1.78)	1.47 (1.07–2.02)	0.03
DBP						
Range, nmol/L	≤ 4077	> 4077 & ≤ 5113	> 5113 & ≤ 6058	> 6058 & ≤ 7320	> 7720	
# cases/# controls	183/189	229/190	162/189	193/191	181/189	
Model 1[b]	1.00 (reference)	1.26 (0.93–1.70)	0.88 (0.65–1.19)	1.01 (0.74–1.39)	0.96 (0.70–1.33)	0.40
Model 2[c]	1.00 (reference)	1.28 (0.94–1.73)	0.86 (0.64–1.17)	0.99 (0.72–1.36)	0.94 (0.68–1.29)	0.29
25(OH)D:DBP molar ratio ($\times 10^3$) [d]						
Range	≤ 3.24	> 3.24 & ≤ 4.94	> 4.94 & ≤ 7.24	> 7.24 & ≤ 10.52	> 10.52	
# cases/# controls	145/189	185/191	249/190	190/190	179/188	
Model 1[b]	1.00 (reference)	1.32 (0.96–1.80)	1.80 (1.33–2.44)	1.45 (1.04–2.01)	1.36 (0.97–1.91)	0.04

[a] Season-specific quintile cutpoints for 25(OH)D were Q1: ≤16.3, Q2: >16.3 and ≤23.8, Q3: >23.8 and ≤33.3, Q4: >33.3 and ≤45.6, Q5: >45.6 nmol/L for the less sunny months (November-April); Q1: ≤25.9, Q2: >25.9 and ≤35.7, Q3: >35.7 and ≤48.3, Q4: >48.3 and ≤59.9, Q5: >59.9 nmol/L for sunnier months (May-October).

[b] Model 1 is conditioned on the matching factors and adjusted for age (continuous), family history of prostate cancer (yes, no, missing), and serum α-tocopherol (continuous).

[c] Model 2 is the same as Model 1, with additional adjustment for either 25(OH)D or DBP, respectively (continuous).

[d] a proxy for free 25(OH)D

Table 3

Odds ratios and 95% confidence intervals for the association between serum 25(OH)D, DBP, and the 25(OH)D:DBP molar ratio and risk of prostate cancer – stratified models, ATBC Study, 1985–2005

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	p-trend	p-interaction
	OR(95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)		
25(OH)D, nmol/L							
Range[a]							
DBP below median							
# cases/# controls	100/105	114/92	99/99	81/96	94/90		
Model 1[b]	1.00 (reference)	1.43 (0.96–2.13)	1.15 (0.77–1.73)	0.97 (0.64–1.46)	1.22 (0.81–1.84)	0.97	0.04
Model 2[c]	1.00 (reference)	1.43 (0.96–2.13)	1.15 (0.77–1.73)	0.97 (0.64–1.47)	1.22 (0.81–1.84)	0.97	
DBP above median							
# cases/# controls	62/89	78/103	98/92	107/94	117/104		
Model 1[b]	1.00 (reference)	1.16 (0.74–1.82)	1.68 (1.08–2.61)	1.75 (1.13–2.72)	1.81 (1.18–2.79)	0.001	
Model 2[c]	1.00 (reference)	1.16 (0.74–1.82)	1.67 (1.07–2.61)	1.75 (1.13–2.71)	1.81 (1.17–2.78)	0.001	
DBP							
Range, nmol/L	≤ 4077	> 4077 & ≤ 5113	> 5113 & ≤ 6058	> 6058 & ≤ 7320	> 7320		
25(OH)D below median							
# cases/# controls	99/97	132/106	75/91	81/93	64/99		
Model 1[b]	1.00 (reference)	1.20 (0.82–1.77)	0.79 (0.52–1.21)	0.82 (0.54–1.25)	0.59 (0.38–0.90)	0.003	0.02
Model 2[c]	1.00 (reference)	1.22 (0.83–1.80)	0.80 (0.52–1.22)	0.82 (0.54–1.25)	0.58 (0.38–0.90)	0.002	
25(OH)D above median							
# cases/# controls	85/96	97/87	87/102	113/100	117/93		
Model 1[b]	1.00 (reference)	1.28 (0.85–1.95)	0.99 (0.65–1.50)	1.25 (0.83–1.87)	1.47 (0.98v2.20)	0.10	
Model 2[c]	1.00 (reference)	1.29 (0.85–1.95)	0.99 (0.66–1.51)	1.25 (0.84–1.87)	1.47 (0.98–2.21)	0.10	
25(OH)D:DBP molar ratio ($\times 10^3$)[d]							
Range	≤ 3.24	> 3.24 & ≤ 4.94	> 4.94 & ≤ 7.24	> 7.24 & ≤ 10.52	> 10.52		
DBP below median							
# cases/# controls	51/57	69/68	114/84	112/118	142/155		

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	p-trend	p-interaction
	OR(95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)		
Model 1[b]	1.00 (reference)	1.23 (0.73–2.07)	1.60 (0.98–2.59)	1.18 (0.74–1.90)	1.14 (0.72–1.79)	0.91	0.71
DBP above median							
# cases/# controls	94/136	116/125	135/109	79/75	38/37		
Model 1[b]	1.00 (reference)	1.45 (0.99–2.10)	1.87 (1.29–2.73)	1.62 (1.06–2.48)	1.71 (1.00–2.92)	0.01	

[a] Season-specific quintile cutpoints for 25(OH)D were Q1: ≤16.3, Q2: >16.3 and ≤23.8, Q3: >23.8 and ≤33.3, Q4: >33.3 and ≤45.6, Q5: >45.6 nmol/L for the less sunny months (November-April); Q1: ≤25.9, Q2: >25.9 and ≤35.7, Q3: >35.7 and ≤48.3, Q4: >48.3 and ≤59.9, Q5: >59.9 nmol/L for sunnier months (May-October).

[b] Model 1 is calculated using unconditional regression, adjusted for age (continuous), family history of prostate cancer (yes, no, missing), serum α-tocopherol (continuous), and date of blood collection (continuous).

[c] Model 2 is the same as Model 1, with additional adjustment for either 25(OH)D or DBP, respectively (continuous).

[d] a proxy for free 25(OH)D