

Serum Vitamin D Concentration and Prostate Cancer Risk: A Nested Case–Control Study

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For the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial Project Team

- Background** Epidemiological studies have yielded inconsistent associations between vitamin D status and prostate cancer risk, and few studies have evaluated whether the associations vary by disease aggressiveness. We investigated the association between vitamin D status, as determined by serum 25-hydroxyvitamin D [25(OH)D] level, and risk of prostate cancer in a case–control study nested within the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial.
- Methods** The study included 749 case patients with incident prostate cancer who were diagnosed 1–8 years after blood draw and 781 control subjects who were frequency matched by age at cohort entry, time since initial screening, and calendar year of cohort entry. All study participants were selected from the trial screening arm (which includes annual standardized prostate cancer screening). Conditional logistic regression was used to estimate adjusted odds ratios (ORs) with 95% confidence intervals (CIs) by quintile of season-standardized serum 25(OH)D concentration. Statistical tests were two-sided.
- Results** No statistically significant trend in overall prostate cancer risk was observed with increasing season-standardized serum 25(OH)D level. However, serum 25(OH)D concentrations greater than the lowest quintile (Q1) were associated with increased risk of aggressive (Gleason sum ≥ 7 or clinical stage III or IV) disease (in a model adjusting for matching factors, study center, and history of diabetes, ORs for Q2 vs Q1 = 1.20, 95% CI = 0.80 to 1.81, for Q3 vs Q1 = 1.96, 95% CI = 1.34 to 2.87, for Q4 vs Q1 = 1.61, 95% CI = 1.09 to 2.38, and for Q5 vs Q1 = 1.37, 95% CI = 0.92 to 2.05; $P_{\text{trend}} = .05$). The rates of aggressive prostate cancer for increasing quintiles of serum 25(OH)D were 406, 479, 780, 633, and 544 per 100,000 person-years. In exploratory analyses, these associations with aggressive disease were consistent across subgroups defined by age, family history of prostate cancer, diabetes, body mass index, vigorous physical activity, calcium intake, study center, season of blood collection, and assay batch.
- Conclusion** The findings of this large prospective study do not support the hypothesis that vitamin D is associated with decreased risk of prostate cancer; indeed, higher circulating 25(OH)D concentrations may be associated with increased risk of aggressive disease.

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Vitamin D is a prohormone that can be supplied from dietary sources and generated endogenously from sunlight exposure (1). The primary circulating form of vitamin D is 25-hydroxyvitamin D [25(OH)D]. Prostate and renal cells can convert 25(OH)D to 1,25-dihydroxyvitamin D [1,25(OH)₂D], which influences the expression of many proteins that are involved in cellular differentiation, proliferation, and apoptosis (2). Although 1,25(OH)₂D is the biologically active form of vitamin D, serum 25(OH)D is considered to be the better biomarker of vitamin D status because it reflects endogenous and exogenous vitamin D sources (1).

There is evidence from laboratory studies that high doses of 1,25(OH)₂D inhibit proliferation and differentiation in human prostate cancer cell lines (3), primary cultures of prostatic cells (4), and rodent models of prostate cancer (5). However, epidemiological studies investigating the association between vitamin D and prostate

cancer risk have been inconclusive. Indicators of high ambient UV exposure (a determinant of vitamin D status) have been associated with reduced risks of (6–8) and mortality from (9) prostate cancer.

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A prospective study from Scandinavian countries reported an inverse association (10) and a U-shaped association (11) of 25(OH)D with prostate cancer risk. A recent report from the Health Professionals Follow-up Study showed that men with deficiency levels of circulating 25(OH)D (defined as below 37.5 nmol/L) were at a lower risk for total and poorly differentiated prostate cancers than men with higher levels (12). Several other nested case-control studies of prostate cancer showed no evidence of an association with 25(OH)D status (13–18). However, most studies were based on small numbers of subjects, and little is known about the differential association of vitamin D with respect to prostate tumor characteristics, such as stage and histological grade.

We examined whether vitamin D status, as determined by serum 25(OH)D concentration, was associated with risk of prostate cancer in a nested case-control study within the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial, based on men who were screened for prostate cancer regularly following a standardized protocol. Given the large sample size, we examined whether the associations of 25(OH)D with prostate cancer risk differed according to tumor aggressiveness.

Subjects and Methods

Study Setting

The PLCO Cancer Screening Trial is a large randomized controlled multicenter trial in the United States of approximately 155 000 men and women at sites in Birmingham, AL; Denver, CO; Detroit, MI; Honolulu, HI; Marshfield, WI; Minneapolis, MN; Pittsburgh, PA; Salt Lake City, UT; St Louis, MO; and Washington, DC, that was designed to evaluate selected methods for the early detection of these four cancers (19,20). Enrollment began November 1, 1993, and ended June 30, 2001. Participants were randomly assigned to either the screening or control arm. The men in the screening arm were offered prostate cancer screening by serum prostate-specific antigen (PSA) at entry and annually for 5 years and digital rectal examination (DRE) at entry and annually for 3 years. Men with a positive screening result (PSA >4 ng/mL or DRE suspicious for prostate cancer) were referred to their medical care providers for prostate cancer diagnostic evaluation. Incident prostate cancer cases were ascertained from annually mailed questionnaires to participants. We acquired all medical and pathology records related to prostate cancer diagnosis for all men with suspected prostate cancer by screening examination or annual questionnaire. Data were abstracted by trained medical record specialists. Screening arm participants were asked to provide a blood sample at each screening visit. All participants were followed up to October 1, 2003. The institutional review boards of the US National Cancer Institute and the 10 study centers approved the trial, and all participants provided written informed consent.

Study Population

Details of the selection of case and control subjects have been described elsewhere (21). Briefly, of the 38 350 men assigned to the screening arm of the trial, case and control subjects were selected from men who were of non-Hispanic white race/ethnicity; who had no prior history of prostate of cancer before randomization; who

CONTEXT AND CAVEATS

Prior knowledge

Although data from laboratory studies have suggested that vitamin D inhibits prostate cell proliferation and differentiation, epidemiological studies have yielded mixed results on the association between vitamin D status and prostate cancer risk.

Study design

Nested case-control study in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. All of the men in this analysis were receiving regular prostate cancer screening.

Contributions

An increase in season-standardized serum 25-hydroxyvitamin D level was not associated with a decreased risk of prostate cancer. There was some evidence that men with vitamin D levels above the lowest quintile had an increased risk of prostate cancer with aggressive characteristics, but no clear monotonic trend was evident.

Implications

Higher levels of serum 25-hydroxyvitamin D may not reduce the risk of prostate cancer; indeed, it is possible that higher levels are associated with increased risk of aggressive disease.

Limitations

Only a single baseline vitamin D measurement was available. Whether vitamin D levels could affect prostate-specific antigen levels in some cancers, causing a diagnosis bias, is not known. As with all epidemiology studies, unmeasured confounders could account for the results.

had at least one (PLCO) prostate cancer screen (PSA testing) before October 1, 2003; who had completed a baseline questionnaire about risk factors for cancer; and who had provided a blood sample.

We selected 1200 prostate cancer patients for this study, including all eligible case patients with aggressive cancer [Gleason sum ≥ 7 or clinical stage III or IV (22)] and a randomly selected subset (70.4% of total available nonaggressive prostate cancers) of patients with nonaggressive disease (clinical stage I or II tumors with Gleason sum <7) because of our interest in the more clinically significant but less common aggressive forms of prostate cancer. We selected control subjects by incidence density sampling (23) with a case-control ratio of 1:1 frequency matched by age at cohort entry (5-year intervals), time since initial screening (1-year time window), and calendar year of cohort entry. For this serum-based study, we excluded men with prevalent prostate cancer (defined as disease diagnosed within the first year of follow-up after the initial screening) and their corresponding control subjects, which left 749 case patients and 781 control subjects.

Vitamin D Assay

Nonfasting baseline blood specimens collected at the clinical centers were processed and frozen within 2 hours of blood draw and stored at -70°C . Baseline serum 25(OH)D concentration was determined by radioimmunoassay (Heartland Assays, Ames, IA) (24). Case and control groups were assayed consecutively within batches. Laboratory personnel were blinded to case-control status. Multiple blinded quality-control samples from four different individuals were

included in all batches (total n = 80); the coefficients of variation for 25(OH)D samples were 5.9%.

Assessment of Questionnaire-Based Covariates

At enrollment, all participants were asked to complete a questionnaire that included questions about age, ethnicity, education, current and past smoking behavior, history of cancer and other diseases, use of selected drugs, recent history of screening examinations, and prostate-related health factors. Usual dietary intake during the 12 months before enrollment was assessed with a 137-item food-frequency questionnaire that included 14 additional questions about intake of vitamin and mineral supplements and 10 additional questions on meat cooking practices. Dietary nutrient intake was calculated by multiplying the daily frequency of each consumed food item by the nutrient value of the sex-specific portion size (25) using the nutrient database from the US Department of Agriculture (26).

Statistical Analysis

We compared the distribution of selected characteristics for case and control subjects using *t* tests for the continuous variables and χ^2

tests for categorical variables. Generalized linear models were used to determine whether the distribution of serum 25(OH)D level at baseline differed according to these selected characteristics to help identify potential confounders. Because 25(OH)D concentrations varied by season of blood collection, we used locally weighted polynomial regression models (Proc Loess, SAS Institute, version 9.1; Cary, NC) to describe the deviation of 25(OH)D from the predicted weekly average and calculated residuals of the regression (27). Using the residuals as the exposure variables of interest, we were able to use standardized cut points (ie, quintiles) for serum 25(OH)D irrespective of season of blood collection. Season-standardized 25(OH)D was calculated by adding the residuals to the overall population mean (58.32 nmol/L).

We used conditional logistic regression to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for prostate cancer according to quintile of season-standardized 25(OH)D based on the distribution among the control subjects. We also conducted subanalyses using season-specific quintile cutoffs of 25(OH)D. All analyses were conditioned on the matching factors (age at cohort entry, time since initial screening, and calendar year of cohort entry) and adjusted for study center and history of diabetes.

Table 1. Selected characteristics of prostate cancer case patients and control subjects in a case-control study nested within the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial*

Characteristic	Case patients (n = 749)	Control subjects (n = 781)	P†
Age at cohort entry, y, mean (SD)	67.8 (5.3)	67.6 (5.3)	.35 (matched)
PSA level at baseline screening, ng/mL, mean (SD)	4.0 (2.1)	1.7 (0.1)	<.001
Family history of prostate cancer, n (%)	93 (12.4)	41 (5.2)	<.001
History of diabetes, n (%)	48 (6.6)	75 (10.0)	.02
BMI, kg/m ² , mean (SD)	27.3 (3.6)	27.5 (3.9)	.19
Vigorous physical activity, h/wk, mean (SD)	2.5 (1.9)	2.4 (1.9)	.30
Daily aspirin and/or ibuprofen use, n (%)	380 (50.7)	410 (52.6)	.86
Smoking status, n (%)			
Never	256 (34.2)	229 (29.3)	.04
Current	48 (6.4)	74 (9.5)	
Former	372 (49.7)	410 (52.5)	
Cigar or pipe only	72 (9.6)	68 (8.7)	
Study center, n (%)			
Denver, CO	89 (11.9)	74 (9.5)	.09
Washington, DC	49 (6.5)	50 (6.4)	
Honolulu, HI and Birmingham, AL‡	13 (1.7)	9 (1.2)	
Detroit, MI	16 (9.0)	71 (9.1)	
Minneapolis, MN	154 (20.6)	198 (25.4)	
St Louis, MO	60 (8.0)	59 (7.6)	
Pittsburgh, PA	119 (15.9)	108 (13.8)	
Salt Lake City, UT	86 (11.5)	68 (8.7)	
Marshfield, WI	112 (15.0)	144 (18.4)	
Total energy intake, kcal/d, mean (SD)‡	2325 (765)	2328 (829)	.54
Vitamin D intake, IU/d, mean (SD)‡,§	416 (300)	418 (315)	.67
Season of blood collection, n (%)			
Winter	188 (25.1)	188 (24.1)	.22
Spring	201 (26.8)	179 (22.9)	
Summer	187 (25.0)	215 (27.5)	
Fall	173 (23.1)	199 (25.5)	
Serum 25-hydroxyvitamin D, nmol/L, mean (SD)	59.0 (19.1)	57.6 (18.9)	.18

* SD = standard deviation; PSA = prostate-specific antigen; BMI = body mass index.

† P value (two-sided) was based on *t* test or χ^2 test.

‡ Diet values were available for 704 case patients and 745 control subjects.

§ Adjusted for total energy intake; combined dietary and supplemental intakes.

|| The season categories were defined as winter: December, January, and February; spring: March, April, and May; summer: June, July, and August; and fall: September, October, and November.

Table 2. Median and interquartile range of serum 25-hydroxyvitamin D according to selected characteristics of control subjects in the Prostate, Lung, and Ovarian Cancer Screening Trial*

Characteristic	n	Median (IQR)	P†
Age at cohort entry, y			
55–59	126	56.5 (45.7–72.9)	.10
60–64	259	53.7 (43.4–67.1)	
65–69	253	57.4 (45.7–69.6)	
70–74	143	56.9 (44.7–70.9)	
No. of years since initial screening			
1–2	442	56.4 (44.9–69.4)	.94
3–4	186	54.2 (43.2–71.1)	
5–6	95	55.2 (44.7–67.9)	
7–9	29	57.9 (48.7–66.6)	
Calendar year of cohort entry			
1994–1995	317	54.2 (43.2–66.9)	.10
1996–1997	308	57.8 (47.5–71.0)	
1998–1999	127	56.2 (42.7–70.6)	
2000–2001	29	54.9 (45.9–64.6)	
Family history of prostate cancer			
No	740	56.2 (44.7–69.9)	.31
Yes	41	52.4 (42.2–63.4)	
History of diabetes			
No	679	55.9 (44.7–70.1)	.07
Yes	75	54.4 (43.7–62.2)	
BMI, kg/m²			
<25	208	60.8 (50.7–74.0)	<.001
25–29.9	394	55.9 (43.9–70.1)	
≥30	179	50.7 (41.4–62.2)	
Vigorous physical activity, h/wk			
<1	224	52.7 (39.4–65.5)	<.001
2–3	236	55.5 (44.7–68.4)	
≥4	321	59.2 (47.9–72.9)	
Daily aspirin or ibuprofen use			
No	370	56.4 (44.7–69.9)	.79
Yes	411	55.4 (44.7–69.1)	
Smoking status			
Never	229	55.4 (45.4–71.4)	.93
Current	74	54.8 (43.7–69.1)	
Former	410	56.3 (44.9–68.4)	
Cigar or pipe only	68	53.8 (43.1–67.1)	
Study center			
Denver, CO	74	58.9 (46.4–72.6)	.005
Washington, DC	50	55.4 (46.2–71.1)	
Honolulu, HI, and Birmingham, AL‡	9	86.6 (67.1–91.9)	
Detroit, MI	71	54.4 (40.7–62.9)	
Minneapolis, MN	198	57.0 (45.7–69.1)	
St Louis, MO	59	51.9 (43.4–61.2)	
Pittsburgh, PA	108	53.4 (41.2–67.8)	
Salt Lake City, UT	68	60.9 (46.8–70.0)	
Marshfield, WI	144	54.7 (45.7–73.4)	
Dietary vitamin D, IU/d			
<200	252	53.7 (41.1–66.5)	<.001
200–399	188	52.8 (43.4–65.0)	
400–599	167	58.2 (45.9–72.4)	
600–799	97	60.9 (49.7–76.6)	
800–999	28	66.5 (48.3–72.6)	
≥1000	49	59.2 (50.9–71.1)	

(Table continues)

Table 2 (continued).

Characteristic	n	Median (IQR)	P†
PSA level at baseline screening, ng/mL			
<2	576	55.4 (44.6–69.1)	.11
2–3.9	140	56.9 (45.2–67.9)	
≥4	65	56.9 (45.7–72.9)	
No. of screens per year§			
1	640	55.9 (44.6–69.8)	.34
<1	141	54.9 (44.9–68.1)	
25-hydroxyvitamin D assay batch			
1	78	56.2 (45.7–64.4)	.19
2	78	56.4 (45.9–74.1)	
3	83	54.2 (40.9–70.1)	
4	74	55.5 (44.4–72.4)	
5	85	52.7 (42.9–62.2)	
6	75	54.4 (43.7–66.9)	
7	84	59.9 (45.3–74.4)	
8	84	58.3 (47.8–68.9)	
9	81	57.2 (44.9–66.9)	
10	59	55.2 (43.2–78.1)	
Season of blood collection 			
Winter	188	49.9 (39.6–62.8)	<.001
Spring	179	52.4 (41.2–62.4)	
Summer	215	60.9 (51.7–74.9)	
Fall	199	60.4 (49.7–74.1)	

* IQR = interquartile range; BMI = body mass index; PSA = prostate-specific antigen.

† P values (two-sided) were based on generalized linear model.

‡ Hawaii and Alabama were combined due to small numbers.

§ Number of prostate cancer screening examinations (PSA test and/or digital rectal examination) up to diagnosis of prostate cancer (case patients) or selection as a control subject. Maximum period was limited to the period of active screening (years 0–5).

|| The season categories were defined as winter: December, January, and February; spring: March, April, and May; summer: June, July, and August; and fall: September, October, and November.

Because a small number of case patients were recruited from the Hawaii (n = 1) and Alabama (n = 12) study centers and serum 25(OH)D distributions for these centers were similar, we combined these two groups. The initial multivariable model (model 1) included study center and history of diabetes because both factors changed the estimated effect by 10% or more when added sequentially to the model. Factors that were found not to confound the associations of interest included the following: family history of prostate cancer (yes or no), body mass index (BMI; <25, 25–29.9, and ≥30 kg/m²), 25(OH)D assay batch (1–10), vigorous physical activity (0, 1, 2, 3, 4, and ≥5 h/wk), daily aspirin and/or ibuprofen use (none, aspirin only, ibuprofen only, and aspirin and ibuprofen both), smoking status (never, current, former, and cigar or pipe only), total energy (quintile, kcal/d), and dairy product (quintile, servings per day), vitamin D (<200, 200–399, 400–599, 600–799, 800–999, ≥1000 IU/d), and calcium (<750, 750–999, 1000–1499, 1500–1999, ≥2000 mg/d) intake. Nevertheless, we also developed a multivariable model in which we additionally adjusted for BMI, vigorous physical activity, and calcium intake (multivariable model 2). Tests for linear trend (1 df) were conducted by treating the median values of the exposure category as a continuous variable.

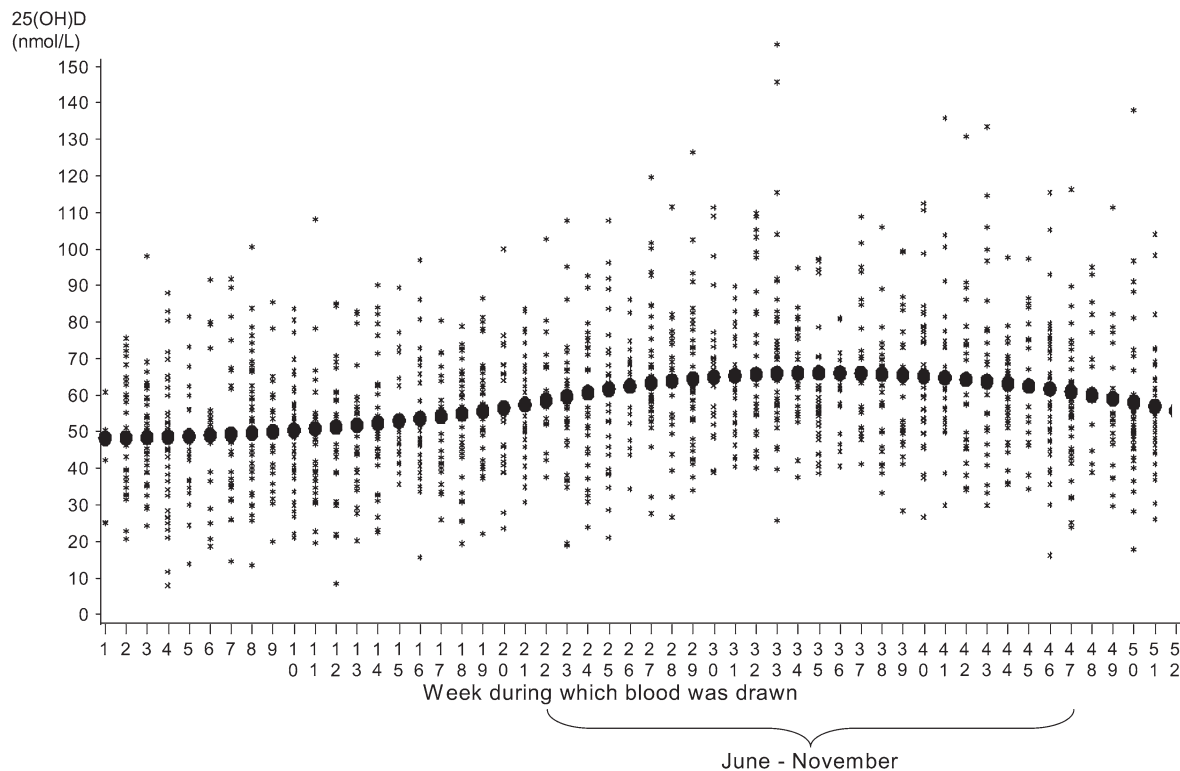


Figure 1. Association between 25-hydroxyvitamin D [25(OH)D] concentration and the week of the year of blood collection. Each **asterisk** represents an individual measurement of 25(OH)D concentration, with measurements plotted by the week of the year of blood collection. The **circles** represent the predicted mean serum 25(OH)D for each week of the year after smoothing using locally weighted polynomial regression.

To test for heterogeneity by disease aggressiveness, we used polytomous logistic regression with endpoints for nonaggressive and aggressive disease. In a sensitivity analysis, we used a more stringent definition of aggressive prostate cancer (Gleason sum >8 or clinical stage III or IV disease). In exploratory analyses, we also investigated associations separately by age, family history of prostate cancer, history of diabetes, BMI, vigorous physical activity, calcium intake, study center, season of blood collection, and assay batch. In these stratified analyses, we used unconditional logistic regression, adjusting for the matching variables and selected confounders. We formally tested for interactions using log-likelihood ratio tests. All statistical tests were two-sided, and *P* values less than .05 were considered to be statistically significant.

Results

Among the 749 men with incident prostate cancer included in this analysis, 434 were diagnosed during the second year of follow-up, 187 during the third and fourth years of follow-up, and 128 between the fifth and eighth years of follow-up (case patients who were diagnosed during the first year of follow-up were excluded from the study). A total of 466 men had aggressive disease (ie, Gleason sum ≥ 7 or stage III or IV), of whom 196 met the more stringent definition of aggressive disease (ie, Gleason sum ≥ 8 or stage III or IV). Compliance with the PLCO screening protocol was very high, with the average number of prostate cancer screens per year during the period of active screening being 0.97.

Case patients were more likely than control subjects to have a family history of prostate cancer and less likely to have a history of

diabetes; they also were less likely to smoke than the control subjects (Table 1). The mean serum concentration of 25(OH)D was slightly higher among case patients than control subjects, but the difference was not statistically significant. The distributions of the matching factors, that is, age at cohort entry, time since initial screening, and calendar year of cohort entry, did not differ between case patients and control subjects (data not shown).

Table 2 shows the median and interquartile range of 25(OH)D concentration according to selected baseline characteristics among the control subjects. The overall median serum 25(OH)D concentration was 55.9 nmol/L (interquartile range = 44.4–68.1 nmol/L). The distribution of 25(OH)D did not differ according to age at cohort entry, number of years since initial screening, or calendar year of cohort entry. Men who were diabetic, obese, or physically inactive had lower 25(OH)D concentrations than men who were nondiabetic, nonobese, and physically active, respectively. 25(OH)D concentration did not vary according to number of prostate cancer screens per year, PSA level, or 25(OH)D assay batch. 25(OH)D concentration was higher in samples collected during summer or fall than during winter or spring (*P* < .001). Loess regression models also revealed that serum concentrations of 25(OH)D varied during the time of the year of blood collection, with higher levels between June and November (about weeks 22–47, Figure 1).

In a minimally adjusted analysis, a weak positive trend (*P*_{trend} = .04) was noted between increasing quintile of season-standardized 25(OH)D and risk of prostate cancer (Table 3). In the multivariable analysis (multivariable model 1), the trends were similar but did not reach statistical significance. We conducted alternative

Table 3. ORs and 95% CIs for the association between serum 25-hydroxyvitamin D and prostate cancer, Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial*

Model	Quintile of serum 25(OH)D					<i>P</i> _{trend} [†]
	1	2	3	4	5	
Quintiles based on the season of blood collection—standardized values‡,§						
Range, nmol/L	12.8–42.5	42.5–51.3	51.4–60.5	60.6–71.7	71.8–129.5	
Case patients/control subjects	119/157	125/156	190/157	167/156	148/155	
Minimally adjusted model, OR (95% CI)	1.00 (referent)	1.12 (0.79 to 1.57)	1.61 (1.16 to 2.22)	1.42 (1.02 to 1.96)	1.32 (0.94 to 1.84)	.04
Multivariable model 1, OR (95% CI) ,¶	1.00 (referent)	1.12 (0.79 to 1.58)	1.57 (1.13 to 2.18)	1.38 (0.99 to 1.92)	1.25 (0.89 to 1.75)	.10
Multivariable model 2, OR (95% CI) ,¶,#	1.00 (referent)	1.10 (0.78 to 1.56)	1.53 (1.10 to 2.13)	1.33 (0.95 to 1.86)	1.18 (0.83 to 1.68)	.20
Quintiles based on the combined quintile seasonal cut points‡,**						
Range, nmol/L (winter and spring)	8.0–38.4	38.5–46.9	47.0–55.2	55.3–66.6	66.7–138.0	
Range, nmol/L (summer and fall)	16.2–48.7	48.8–56.4	56.5–65.6	65.7–77.9	78.0–156.0	
Case patients/control subjects	131/161	118/158	175/154	178/154	147/154	
Minimally adjusted model, OR (95% CI)	1.00 (referent)	0.93 (0.66 to 1.30)	1.43 (1.04 to 1.97)	1.43 (1.04 to 1.97)	1.22 (0.88 to 1.69)	.03
Multivariable model 1, OR (95% CI) ,¶	1.00 (referent)	0.92 (0.66 to 1.30)	1.39 (1.00 to 1.92)	1.41 (1.02 to 1.94)	1.14 (0.81 to 1.60)	.07
Multivariable model 2, OR (95% CI) ,¶,#	1.00 (referent)	0.90 (0.64 to 1.27)	1.36 (0.98 to 1.89)	1.34 (0.96 to 1.87)	1.08 (0.77 to 1.53)	.15

* OR = odds ratio; CI = confidence interval; 25(OH)D = 25-hydroxyvitamin D.

† Tests for linear trend (1 df) were conducted by treating the median values of the exposure category as a continuous variable.

‡ Quintiles based on distribution of control subjects.

§ Based on the residuals of the locally weighted polynomial regression models of the 25(OH)D concentrations by the week of the year of blood collection, the season-standardized 25(OH)D was calculated by adding the residuals to the overall population mean (58.32 nmol/L).

|| Odds ratios based on conditional logistic regression. Matching factors were age at cohort entry, time since initial screening, and calendar year of cohort entry.

¶ Odds ratios were additionally adjusted for study center and history of diabetes.

Odds ratios were additionally adjusted for body mass index, physical activity, and total calcium intake.

** Quintile based on merging participants within quintiles of each season stratum.

analyses using season-specific cut points, with similar results. Estimates remained unchanged when BMI, vigorous physical activity, and calcium intake were additionally adjusted for (multivariable model 2). Results were also unchanged when we restricted the analysis to case patients who were diagnosed at least 2 years after blood collection. The odds ratio for quintile 5 vs quintile 1 was 1.33 (95% CI = 0.86 to 2.08) for case patients who were diagnosed during the second year of follow-up and 1.29 (95% CI = 0.77 to 2.17) for case patients who were diagnosed after the second year of follow-up.

Serum 25(OH)D was not associated with risk for nonaggressive disease (Table 4); however, concentrations of 25(OH)D greater than the lowest quintile tended to be related to increased risk of aggressive (Gleason sum ≥ 7 or clinical stage III or IV) disease (ORs from multivariable model 1 for Q2 vs Q1 = 1.20, 95% CI = 0.80 to 1.81, for Q3 vs Q1 = 1.96, 95% CI = 1.34 to 2.87, for Q4 vs Q1 = 1.61, 95% CI = 1.09 to 2.38, and for Q5 vs Q1 = 1.37, 95% CI = 0.92 to 2.05; $P_{\text{trend}} = .05$). This association was also seen for both high-grade (Gleason score ≥ 7) and high-stage (stage III or IV) disease considered separately. Inclusion of a quadratic term for 25(OH)D did not improve model fit ($\chi^2 = 3.84$; $P = .26$). Results were similar when we used a more stringent definition of aggressive disease (Gleason sum ≥ 8 or stage III or IV). Results were also similar when we used season-specific cutoffs of 25(OH)D (data not shown). The rates of aggressive prostate cancer for

increasing quintiles of serum 25(OH)D were 406, 479, 780, 633, and 544 per 100 000 person-years.

In an exploratory analysis (data not shown), the positive association between serum 25(OH)D and aggressive prostate cancer was consistent across subgroups defined by age at study selection, family history of prostate cancer, diabetes, BMI, vigorous physical activity, calcium intake, study center, and season of blood collection (all $P_{\text{interaction}} > .10$) and age at diagnosis ($P_{\text{heterogeneity}} = .16$).

Discussion

The findings from this large prospective analysis do not support the hypothesis that higher levels of circulating 25(OH)D are associated with decreased risk of prostate cancer. Indeed, higher circulating 25(OH)D concentrations may be associated with increased risk of aggressive disease, although a clear monotonic dose–response relationship was lacking.

Interest in the relation of 25(OH)D to prostate cancer risk was raised by observations by Ahonen et al. (10), whose study in Finland showed that men with greater concentrations of this pro-hormone were at reduced risk of prostate cancer, consistent with a large body of experimental evidence pointing to a potential protective role for vitamin D in carcinogenesis. A subsequent larger study by this group (11) carried out in Finland, Sweden, and Norway also showed increased risks for men at the lowest concentrations but also

Table 4. ORs and 95% CIs for the association between serum 25-hydroxyvitamin D and prostate cancer stratified by selected tumor characteristics, the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial*

Model	Quintile of serum 25-hydroxyvitamin D†					P _{trend} ‡
	1	2	3	4	5	
Nonaggressive disease (Gleason sum <7 and stage <III)¶						
Case patients/control subjects	51/157	52/156	60/157	62/156	58/155	
Multivariable model 1, OR (95% CI)§	1.00 (referent)	0.95 (0.59 to 1.52)	1.16 (0.73 to 1.83)	1.23 (0.78 to 1.94)	1.11 (0.69 to 1.76)	.43
Multivariable model 2, OR (95% CI)§,	1.00 (referent)	0.92 (0.57 to 1.48)	1.13 (0.71 to 1.79)	1.17 (0.74 to 1.87)	1.05 (0.65 to 1.69)	.59
Aggressive disease with lenient definition (Gleason sum ≥7 or stage III or IV)¶						
Case patients/control subjects	68/157	73/156	130/157	105/156	90/155	
Multivariable model 1, OR (95% CI)§	1.00 (referent)	1.20 (0.80 to 1.81)	1.96 (1.34 to 2.87)	1.61 (1.09 to 2.38)	1.37 (0.92 to 2.05)	.05
Multivariable model 2, OR (95% CI)§,	1.00 (referent)	1.18 (0.78 to 1.79)	1.92 (1.31 to 2.82)	1.56 (1.05 to 2.31)	1.34 (0.87 to 1.98)	.09
High-stage aggressive disease (stage III or IV, any Gleason sum)						
Case patients/control subjects	17/157	18/156	37/157	34/156	31/155	
Multivariable model 2, OR (95% CI)§,	1.00 (referent)	1.16 (0.57 to 2.35)	2.09 (1.11 to 3.93)	1.98 (1.05 to 3.74)	1.83 (0.95 to 3.50)	.02
High-grade aggressive disease (Gleason sum ≥7, any stage)						
Case patients/control subjects	63/157	67/156	117/157	91/156	81/155	
Multivariable model 2, OR (95% CI)§,	1.00 (referent)	1.22 (0.79 to 1.86)	1.92 (1.30 to 2.85)	1.51 (1.00 to 2.26)	1.33 (0.88 to 2.01)	.10
Aggressive disease with stringent definition (Gleason sum ≥8 or stage III or IV)						
Case patients/control subjects	24/157	30/156	54/157	46/156	42/155	
Multivariable model 1, OR (95% CI)§	1.00 (referent)	1.37 (0.76 to 2.48)	2.17 (1.25 to 3.74)	1.88 (1.08 to 3.28)	1.78 (1.01 to 3.14)	.03
Multivariable model 2, OR (95% CI)§,	1.00 (referent)	1.31 (0.72 to 2.39)	2.10 (1.21 to 3.63)	1.79 (1.02 to 3.14)	1.66 (0.93 to 2.97)	.06

* OR = odds ratio; CI = confidence interval.

† Quintiles based on distribution of the season of the blood collection—standardized values among control subjects.

‡ Tests for trend (1 *df*) were conducted by treating the median values of the exposure category as a continuous variable.

§ Odds ratios were based on unconditional logistic regression, adjusted for the matching factors (age at cohort entry, time since initial screening, and calendar year of cohort entry) and study center and history of diabetes.

|| Odds ratios were additionally adjusted for body mass index, physical activity, and total calcium intake.

¶ We tested for heterogeneity using polytomous logistic regression with endpoints for nonaggressive and aggressive disease. The *P* value for the test of heterogeneity according to tumor aggressiveness was .05.

at the highest concentrations of serum 25(OH)D (Figure 2). Six other studies, conducted in the United States, showed no association between 25(OH)D and prostate cancer risk (13–18) (Figure 2). As recently summarized by Li et al. (16), the Nordic study populations (10,11) were distinguished by the large proportion of men deficient for serum vitamin D (ie, with serum levels <50 nmol/L—approximately 50% of the men were deficient, compared with only 20% for the US study populations). The range of 25(OH)D levels of the men in our study was similar to those of the other US investigations. Taken together, therefore, it appears that studies based on populations with generally adequate vitamin D status do not support evidence of an association between 25(OH)D and prostate cancer risk; however, an excess risk for prostate cancer at very low 25(OH)D, as suggested by the Nordic studies (10,11) remains noteworthy.

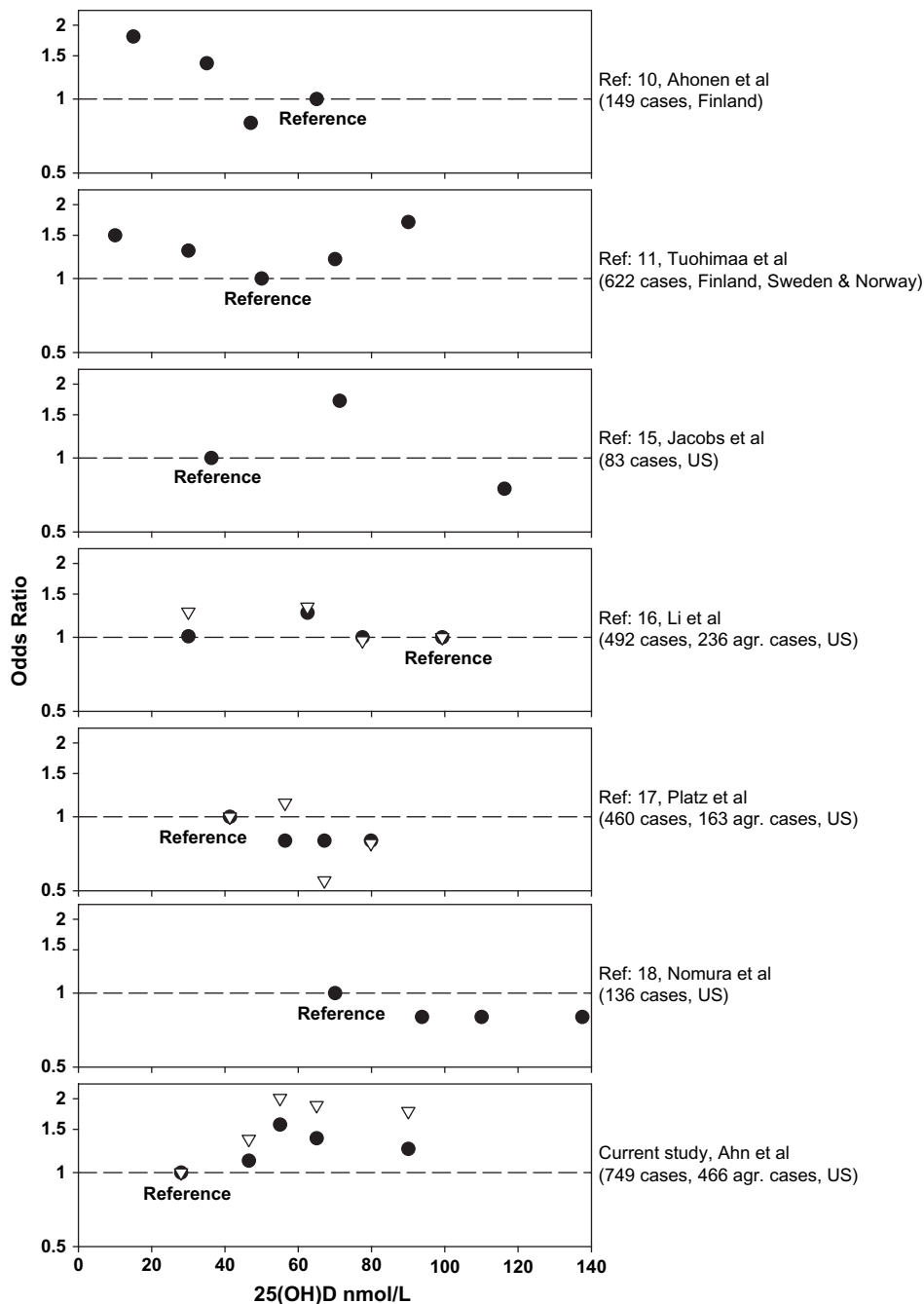
When we examined risks according to disease aggressiveness, we found that higher concentrations of 25(OH)D were associated with increased risk for aggressive disease. Previous studies did not stratify according to disease aggressiveness (10,11,15,18) or had a limited number of patients with aggressive disease (13,14,16,17). The Nordic study (11) showed a pattern of increased risk at the highest concentrations of 25(OH)D; given that PSA screening was not widespread in Northern Europe in the 1980s and early 1990s it is possible that a larger proportion of cancers in the Nordic study were aggressive. Recent findings from the Health Professionals Follow-up Study (12) showed that men with a deficiency in circulating 25(OH)D (ie, with levels <37.5 nmol/L) had a statistically significantly lower risk of poorly differentiated prostate cancers

than men with higher levels (OR = 0.42, 95% CI = 0.23 to 0.73). Therefore, circulating levels of 25(OH)D greater than 37.5 nmol/L were associated with increased risk of aggressive prostate cancer, consistent with our results.

Most attention has been given to potential reduced risks associated with higher 25(OH)D; however, the vitamin D signaling pathway interacts in a complex fashion with other signaling pathways, and their downstream effect on cellular differentiation, proliferation, and apoptosis are not entirely understood (28). Further studies on evaluating underlying mechanisms between vitamin D and aggressive prostate cancer are warranted.

This study, because it was conducted within a cancer screening trial, has several strengths. Unlike participants in previous investigations of the association of vitamin D with risk of prostate cancer, participants in this study had the same protocol for prostate cancer detection irrespective of lifestyle factors, substantially reducing the likelihood of screening-related detection bias. Also, because information on tumor grade and stage was available for all patients, misclassification of disease was unlikely. Other strengths include the use of prediagnostic serum samples, large sample size, and detailed information on demographic, dietary, and lifestyle factors. In addition to these strengths, risks observed in our study were relatively consistent with respect to time period of follow-up. Moreover, the distribution of 25(OH)D levels was similar to that seen other US studies (13–17), and the 25(OH)D concentration in our study varied as expected by other known factors, such as vitamin D intake, as well as by study center and level of vigorous

Figure 2. Odds ratios of prostate cancer according to serum 25-hydroxyvitamin D concentration from prospective studies. The **solid circles** represent odds ratios of total cancer, and the **triangles** represent odds ratios of aggressive cancer. Agr = aggressive disease.



physical activity, as surrogates of sunlight exposure (29). 25(OH)D is relatively stable during storage (30), and 25(OH)D concentration did not vary according to number of years since initial screening and calendar year of cohort entry after taking seasonality into account. Laboratory reproducibility was excellent, based on blinded quality control samples.

A limitation of our study is measurement of only a single serum sample; 25(OH)D measures at multiple time points would have resulted in more precise estimates of exposure. Because most cancers were diagnosed by PSA screening, we cannot completely rule out screening-related detection bias. For example, a positive PSA test may be less likely to yield a diagnosis of prostate cancer in men with low vitamin D because this group may be enriched

with obese or diabetic men, who tend to have lower PSA concentrations than nonobese or nondiabetic men (31). However, adjustment for BMI and physical activity did not change any of the risk estimates, and the association of 25(OH)D with prostate cancer risk was not modified by these factors. Thus, such bias is likely to be minimal.

In summary, results from this large prospective study of men who underwent standardized prostate cancer screening in the context of a screening trial do not support the hypothesis that higher serum vitamin D status is associated with decreased risk of prostate cancer. The study showed no association of vitamin D level with nonaggressive disease; however, it raises the possibility that higher vitamin D level may be associated with increased risks

for aggressive disease, although a clear monotonic dose–response relationship was lacking. Along with recent reports of adverse associations for higher vitamin D status and risk of pancreatic (32) and esophageal (33,34) cancer, caution should be taken in recommending high doses of vitamin D or sunlight exposure to the general public for prostate cancer prevention. Future analyses are warranted to confirm these results and to further clarify the effects of vitamin D on aggressive prostate cancer.

References

- DeLuca HF, Zierold C. Mechanisms and functions of vitamin D. *Nutr Rev.* 1998;56(2 pt 2):S4–S10.
- Lin R, White JH. The pleiotropic actions of vitamin D. *Bioessays.* 2004;26(1):21–28.
- Skowronski RJ, Peehl DM, Feldman D. Vitamin D and prostate cancer: 1,25 dihydroxyvitamin D3 receptors and actions in human prostate cancer cell lines. *Endocrinology.* 1993;132(5):1952–1960.
- Peehl DM, Skowronski RJ, Leung GK, Wong ST, Stamey TA, Feldman D. Antiproliferative effects of 1,25-dihydroxyvitamin D3 on primary cultures of human prostatic cells. *Cancer Res.* 1994;54(3):805–810.
- Oades GM, Dredge K, Kirby RS, Colston KW. Vitamin D receptor-dependent antitumour effects of 1,25-dihydroxyvitamin D3 and two synthetic analogues in three in vivo models of prostate cancer. *BJU Int.* 2002;90(6):607–616.
- John EM, Dreon DM, Koo J, Schwartz GG. Residential sunlight exposure is associated with a decreased risk of prostate cancer. *J Steroid Biochem Mol Biol.* 2004;89–90(1–5):549–552.
- John EM, Schwartz GG, Koo J, Van Den BD, Ingles SA. Sun exposure, vitamin D receptor gene polymorphisms, and risk of advanced prostate cancer. *Cancer Res.* 2005;65(12):5470–5479.
- Luscombe CJ, Fryer AA, French ME, et al. Exposure to ultraviolet radiation: association with susceptibility and age at presentation with prostate cancer. *Lancet.* 2001;358(9282):641–642.
- Hanchette CL, Schwartz GG. Geographic patterns of prostate cancer mortality. Evidence for a protective effect of ultraviolet radiation. *Cancer.* 1992;70(12):2861–2869.
- Ahonen MH, Tenkanen L, Teppo L, Hakama M, Tuohimaa P. Prostate cancer risk and prediagnostic serum 25-hydroxyvitamin D levels (Finland). *Cancer Causes Control.* 2000;11(9):847–852.
- Tuohimaa P, Tenkanen L, Ahonen M, et al. Both high and low levels of blood vitamin D are associated with a higher prostate cancer risk: a longitudinal, nested case-control study in the Nordic countries. *Int J Cancer.* 2004;108(1):104–108.
- Mikhak B, Hunter DJ, Spiegelman D, Platz EA, Hollis BW, Giovannucci E. Vitamin D receptor (VDR) gene polymorphisms and haplotypes, interactions with plasma 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D, and prostate cancer risk. *Prostate.* 2007;67(9):911–923.
- Corder EH, Guess HA, Hulka BS, et al. Vitamin D and prostate cancer: a prediagnostic study with stored sera. *Cancer Epidemiol Biomarkers Prev.* 1993;2(5):467–472.
- Gann PH, Ma J, Hennekens CH, Hollis BW, Haddad JG, Stampfer MJ. Circulating vitamin D metabolites in relation to subsequent development of prostate cancer. *Cancer Epidemiol Biomarkers Prev.* 1996;5(2):121–126.
- Jacobs ET, Giuliano AR, Martinez ME, Hollis BW, Reid ME, Marshall JR. Plasma levels of 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D and the risk of prostate cancer. *J Steroid Biochem Mol Biol.* 2004;89–90(1–5):533–537.
- Li H, Stampfer MJ, Hollis JB, et al. A prospective study of plasma vitamin D metabolites, vitamin D receptor polymorphisms, and prostate cancer. *PLoS Med.* 2007;4(3):e103.
- Platz EA, Leitzmann MF, Hollis BW, Willett WC, Giovannucci E. Plasma 1,25-dihydroxy- and 25-hydroxyvitamin D and subsequent risk of prostate cancer. *Cancer Causes Control.* 2004;15(3):255–265.
- Nomura AM, Stemmermann GN, Lee J, et al. Serum vitamin D metabolite levels and the subsequent development of prostate cancer (Hawaii, United States). *Cancer Causes Control.* 1998;9(4):425–432.
- Prorok PC, Andriole GL, Bresalier RS, et al. Design of the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial. *Control Clin Trials.* 2000;21(6)(suppl):273S–309S.
- Hayes RB, Sigurdson A, Moore L, et al. Methods for etiologic and early marker investigations in the PLCO trial. *Mutat Res.* 2005;592(1–2):147–154.
- Yeager M, Orr N, Hayes RB, et al. Genome-wide association study of prostate cancer identifies a second risk locus at 8q24. *Nat Genet.* 2007;39(5):645–649.
- Greene FI, Page DL, Fleming ID, Fritz A. *AJCC Cancer Staging Manual.* 5th ed. Philadelphia (PA): Lippincott-Raven; 1997.
- Rothman KJ, Greenland P. *Modern Epidemiology.* 2nd ed. Philadelphia (PA): Lippincott Williams & Wilkins; 1998.
- Hollis BW. Quantitation of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D by radioimmunoassay using radioiodinated tracers. *Methods Enzymol.* 1997;282:174–186.
- Subar AF, Midthune D, Kullendorff M, et al. Evaluation of alternative approaches to assign nutrient values to food groups in food frequency questionnaires. *Am J Epidemiol.* 2000;152(3):279–286.
- Design and operation: the continuing survey of food intakes by individuals and the diet and health knowledge survey, 1994–96. Nationwide Food Surveys Report 96–1. Beltsville, MD: Agriculture Research Service, US Department of Agriculture; 1998.
- Borkowf CB, Albert PS, Abnet CC. Using lowess to remove systematic trends over time in predictor variables prior to logistic regression with quantile categories. *Stat Med.* 2003;22(9):1477–1493.
- Deeb KK, Trump DL, Johnson CS. Vitamin D signalling pathways in cancer: potential for anticancer therapeutics. *Nat Rev Cancer.* 2007;7(9):684–700.
- Giovannucci E, Liu Y, Rimm EB, et al. Prospective study of predictors of vitamin D status and cancer incidence and mortality in men. *J Natl Cancer Inst.* 2006;98(7):451–459.
- Lissner D, Mason RS, Posen S. Stability of vitamin D metabolites in human blood serum and plasma. *Clin Chem.* 1981;27(5):773–774.
- Kristal AR, Chi C, Tangen CM, Goodman PJ, Etzioni R, Thompson IM. Associations of demographic and lifestyle characteristics with prostate-specific antigen (PSA) concentration and rate of PSA increase. *Cancer.* 2006;106(2):320–328.
- Stolzenberg-Solomon RZ, Vieth R, Azad A, et al. A prospective nested case-control study of vitamin D status and pancreatic cancer risk in male smokers. *Cancer Res.* 2006;66(20):10213–10219.
- Chen W, Dawsey SM, Qiao YL, et al. Prospective study of serum 25(OH)-vitamin D concentration and risk of oesophageal and gastric cancers. *Br J Cancer.* 2007;97(1):123–128.
- Abnet CC, Chen W, Dawsey SM, et al. Serum 25(OH)-vitamin D concentration and risk of esophageal squamous dysplasia. *Cancer Epidemiol Biomarkers Prev.* 2007;16(9):1889–1893.

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