Prospective Study of Serum Vitamin D and Cancer Mortality in the United States

D. Michal Freedman, Anne C. Looker, Shih-Chen Chang, Barry I. Graubard

Background Vitamin D has been hypothesized to reduce cancer mortality through its effects on incidence and/or survival. Epidemiologic studies of the association of 25-hydroxyvitamin D [25(OH)D] and the risk of cancer, however, have been largely limited to incident cancers at a few sites.

Methods A total of 16818 participants in the Third National Health and Nutrition Examination Survey who were 17 years or older at enrollment were followed from 1988–1994 through 2000. Levels of serum 25(OH)D were measured at baseline by radioimmunoassay. Cox proportional hazards regression models were used to examine the relationship between serum 25(OH)D levels and total cancer mortality (in the entire population or according to race/ethnicity, sex, age, and retinol status) and mortality from specific cancers. Because serum was collected in the south in cooler months and the north in warmer months, we examined associations by collection season. All statistical tests were two-sided.

Results We identified 536 cancer deaths in 146578 person-years. Total cancer mortality was unrelated to baseline vitamin D status in the entire population, men, women, non-Hispanic whites, non-Hispanic blacks, Mexican Americans, and in persons younger than 70 or 70 years or older. We found no interaction between vitamin D and season or vitamin D and serum retinol. Colorectal cancer mortality was inversely related to serum 25(OH)D level, with levels 80 nmol/L or higher associated with a 72% risk reduction (95% confidence interval = 32% to 89%) compared with lower than 50 nmol/L, P_{trend} = .02.

Conclusions Our results do not support an association between 25(OH)D and total cancer mortality, although there was an inverse relationship between 25(OH)D levels and colorectal cancer mortality.

J Natl Cancer Inst 2007;99:1594–602

Vitamin D has been hypothesized to reduce cancer mortality through its effects on incidence and/or survival (1,2). Support for these hypotheses comes from a diversity of scientific approaches: Animal and in vitro studies have shown that vitamin D plays a variety of biologic roles that potentially reduce cancer incidence and promote survival, including suppressing tumor progression by reducing cell proliferation and stimulating apoptosis and cell differentiation (3,4). Exposure to sunlight is one of the major sources of vitamin D (5), and ecologic studies have demonstrated an inverse association between indicators of residential ultraviolet radiation and cancer mortality at various sites, including the colon, ovary, prostate, and breast (6–9). Subsequent ecologic and observational studies found an inverse gradient for solar radiation surrogates (e.g., residence, outdoor work) for mortality due to these and other cancer sites (10–12). Recent analytic epidemiologic studies found improved survival for several cancers if diagnosis or treatment occurred during nonwinter seasons, compared with winter, leading to the speculation that therapy may positively interact with vitamin D (2,13–16). The observation that some individuals who are obese or dark-skinned tend to have lower vitamin D levels and higher cancer mortality has led some investigators to suggest that these associations may be mediated by vitamin D levels (1,17). Finally, a recent Health Professionals Follow-Up Study (HPFS) that was based on predicted 25-hydroxyvitamin D [25(OH)D] values (the major circulating form of vitamin D) estimated that a 25 nmol/L difference in predicted 25(OH)D was associated with a 29% reduction in total cancer mortality risk in men (18).

After its photochemical synthesis in the skin, vitamin D_3 is hydroxylated in the liver to the major circulating form, 25(OH)D, and further hydroxylated in the kidneys, to the active or hormonal form of vitamin D [1,25 dihydroxyvitamin D_3 or $1,25(OH)_2D$]. In recent years, it has been established that hormonal vitamin D is also synthesized from 25(OH)D in several extrarenal tissues (19). Because circulating 25(OH)D levels are related to dietary intake

Affiliations of authors: Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, MD (DMF, SCC, BIG); National Center for Health Statistics, Centers for Disease Control and Prevention, Hyattsville, MD (ACL).

Correspondence to: D. Michal Freedman, PhD, MPH, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Executive Plaza South Rm 7036, 6120 Executive Blvd, Bethesda, MD 20892-7238 (e-mail: mf101e@nih.gov).

See "Funding" and "Notes" following "References."

DOI: 10.1093/jnci/djm204

Published by Oxford University Press 2007.

and sun exposure [and serve as a substrate for $1,25(OH)_2D$ in other organs], whereas circulating $1,25(OH)_2D$ is homeostatically controlled, circulating 25(OH)D is considered to be reflective of an individual's vitamin D status.

Despite the potential benefits of vitamin D, to our knowledge, the relationship between measured vitamin D levels and total cancer mortality has not been examined prospectively. In this study, we examined the relationship between serum 25(OH)D and cancer mortality in persons aged 17 years and older from the Third National Health and Nutrition Examination Survey (NHANES III), a nationally representative sample of the noninstitutionalized US population.

CONTEXT AND CAVEATS

Prior knowledge

Based on epidemiologic studies, vitamin D has been hypothesized to reduce cancer mortality.

Study design

Cox proportional hazard regression models were used to examine the relationship between serum vitamin D level measured in participants in a nationwide survey of health and nutrition and cancer mortality.

Contribution

This study found that total cancer mortality was not related to vitamin D status but that higher levels of vitamin D may be associated with a reduced risk of colorectal cancer mortality.

Implications

Inverse associations between a surrogate for vitamin D status and cancer mortality reported previously are not supported by the results of this study.

Limitations

This study lacked power to clarify associations between vitamin D status and particular cancers for which there were insufficient deaths, and it relied on a single measurement to reflect serum vitamin D status.

Methods

Study Population

The NHANES III survey, which was conducted between 1988 and 1994 by the National Center for Health Statistics of the Centers for Disease Control and Prevention, was designed to examine the health and nutritional status of the noninstitutionalized US population (20). In addition to providing representative estimates of health and nutrition for the total noninstitutionalized population, the survey was designed to provide separate estimates for three major racial/ethnic groups: non-Hispanic whites, non-Hispanic blacks, and Mexican Americans. It was conducted with oversampling from the latter two populations. Data were collected by interview and physical examinations (conducted in Mobile Examinations Centers) that included blood sampling for vitamin D and other serum constituents. All procedures were approved by the National Center for Health Statistics Institutional Review Board, and all subjects provided written informed consent.

We restricted eligibility to those who were aged 17 years and older at baseline and who had completed the physical examination in a Mobile Examination Center (n = 17 705). Those who had no 25(OH)D measurement (n = 875), were of unknown mortality status (n = 11), or were missing birthdate (n = 1) were excluded, resulting in a cohort of 16 818 subjects.

Vitamin D Measurement

Detailed information on the assay used to measure 25(OH)D has been provided elsewhere (21). Briefly, levels of serum 25(OH)D were assayed with a radioimmunoassay kit (DiaSorin, Stillwater, MN). The coefficient of variation 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). The highest coefficient of variation, 25%, corresponds to a mean 25(OH)D value close to the assay detection limit, which reflects the fact that small variability in the lower levels of measured 25(OH)D values can result in higher coefficients of variation.

Categorical 25(OH)D cut points were set at less than 50, 62.5, 80, 100, and 120 nmol/L. The three lowest categories (<50, 50–<62.5, and 62.5–<80 nmol/L) were chosen because they reflect alternative cut points for 25(OH)D insufficiency (in the absence of agreement on optimal levels) (21,22). Even lower values, e.g., 37.5 nmol/L, have been used as cut points, but small case numbers prevented us from using lower threshold values in our analyses. The highest cut points were selected to reflect the full distribution of 25(OH)D values, with 20%, 10%, and 7% of the cohort respectively, corresponding to 25(OH)D levels of 80 to less than 100, 100 to less than 120, or 120 nmol/L or higher. Subgroup analyses included fewer cut points, with the number of intervals varying according to the case number size in each subgroup.

Sample Collection

Between 1988 and 1994, blood samples were collected in different seasons, with collection in southern latitudes undertaken in the cooler months of November through March and collection in northern latitudes in warmer months (April–October) because the mobile vans used for data collection were sensitive to weather (21). Thus, season and latitude, both of which are related to 25(OH)D levels, are linked in the dataset, which limits the opportunity to assess each variable independent of the other. We refer to the two groups as "winter/lower latitude" and "summer/higher latitude."

Endpoint Ascertainment

Follow-up of the cohort continued from data collection until December 31, 2000, and was based on the NHANES III Linked Mortality file (International Statistical Classification of Diseases, 10th Revision) (with 113 underlying causes of death), which had been derived through probabilistic linkage with the National Death Index. Cancer mortality was based on International Statistical Classification of Diseases, Tenth Revision, codes (Table 2). Survival follow-up continued until the event of interest, an underlying cause of death due to cancer. Follow-up was censored at the date of death for persons who died of other diseases and at December 31, 2000, for persons who were not known to have died.

Confounding Variables

We considered race/ethnicity, latitude, body mass index (BMI), total body fat, education level, smoking patterns, levels of physical activity, disease history, calcium intake, and serum retinol levels as potential confounders. Race/ethnicity was based on self-report, and latitude, on the location of the physical examination. BMI was calculated as body weight (kilograms) divided by height (meters) squared, using height and weight measured during the physical exam (20). Total body fat (kilograms) was derived using bioelectrical impedance analysis data as previously described (23).

Education was based on self-reported number of years of schooling completed. Smoking status was derived from responses to questionnaire items that asked whether the respondents had ever smoked at least 100 cigarettes, and if so, whether they currently smoked. Pack-years were calculated from multiplying the reported number of cigarettes smoked per day by the years smoked. There were two physical activity variables, one based on the self-reported frequency of various activities and another derived from a self-rating of personal activity level compared with others of the same age and sex.

Dietary calcium and vitamin D intake from food and alcohol were based on a single 24-hour dietary recall. The University of Minnesota Nutrition Coordinating Center food composition database was used for the vitamin D composition data (24,25), while the U.S. Department of Agriculture food composition database provided the calcium and alcohol values (26). Serum retinol was measured by high-performance liquid chromatography at the National Center for Environmental Health, Centers for Disease Control and Prevention (27).

Statistical Analysis

Chi-square tests and multiple linear regression were used to assess associations between baseline demographic and health-related characteristics and serum 25(OH)D levels. Cox proportional hazards regression analysis was used to compute relative risks (RR) with 95% confidence intervals (CIs) for total and site-specific cancer mortality by categorical level of 25(OH)D, with age (beginning at baseline) as the time line (28). The proportional hazards assumption was tested by examining the interaction between the age attained during the study with the measured continuous 25(OH)D serum value and was found to be satisfied. We examined total cancer mortality in each of the two season/latitude groups, as well as for each sex, in non-Hispanic whites, non-Hispanic blacks, and Mexican Americans, and in age strata (<70 and ≥70 years [attained age], with these strata selected based on case numbers). To further assess whether 25(OH)D levels are associated with difference in cancer mortality by race, we examined mortality risk with and without adjusting for 25(OH)D measurements, after controlling for a number of potentially confounding factors generally comparable to those in the HPFS that sought to explore the role of vitamin D in explaining racial differences in cancer incidence and mortality (29). These factors were sex, smoking status, BMI, latitude, total calories, alcohol, dietary calcium intake, serum retinol and physical activity.

We also assessed site-specific cancer mortality, including mortality due to cancer of the lung, colon or rectum, other digestive organs, breast, and prostate, as well as mortality due to non-Hodgkin lymphoma or leukemia. Site-specific cancer mortality outcomes were selected based in part on suggested protective associations with dietary, circulating vitamin D, and other indicators of vitamin D exposure reported previously (e.g., colorectal, breast, prostate) (1,12,17,30). We combined deaths due to non-Hodgkin lymphoma and leukemia because of small numbers and because experimental and epidemiologic studies suggest a possible association between vitamin D or solar radiation and these hematopoetic cancers (11,31–35).

Potential confounders were assessed by examining Pearson and Spearman correlation coefficients with serum 25(OH)D for the unweighted sample. No correlation coefficient was greater than .24. We also assessed the potential confounders listed above to identify the ones that substantively affected the main effects. The final models were adjusted for sex, race/ethnicity, and smoking (never, former, current <20 pack-years, ≥20 pack-years, unknown pack-years). We also analyzed the relationships between 25(OH)D and cancer mortality after excluding the first year of follow-up because individuals who died within the first year may have had disease at baseline. However, exclusion of the first year of follow-up had little effect on risks, and results are not reported.

Trend tests were based on modeling the measured values of 25(OH)D both as a continuous variable and by assigning a value for the median of each category of measured values and modeling the resulting variable as continuous. Results were similar using both methods (when there were more than two categories), so only those based on the former are reported. All statistical tests were two-sided, and *P* values less than .05 were considered to be statistically significant.

To explore effect modification, we examined stratified analyses and multiplicative interaction by creating product terms. We assessed seasonal/latitude group for interaction with 25(OH)D for total cancer mortality. We also examined interaction of 25(OH)D level with serum retinol levels for total cancer and colorectal cancer mortality to explore a published hypothesis that retinol intake may counteract the cancer prevention effects of vitamin D (1).

All statistical tests using Cox proportional hazards analysis were based on the Wald-F test (36). The data were analyzed using the SUDAAN software program, a family of statistical procedures for analysis of data from complex sample surveys (release 9.0.1, Research Triangle Institute, Research Triangle Park, NC). Sample weights were used to conduct weighted analyses to account for the unequal probability of selecting individuals and for survey nonresponse (36).

Results

We identified 536 deaths due to cancer during 146 578 person-years of follow-up in the NHANES III study, of which 206 were in the winter/lower latitude group and 330 in the summer/higher latitude group. The distribution of 25(OH)D measurements varied to a statistically significant extent according to the factors presented in Table 1 ($P \leq .001$, except for education [$P = .01$] and alcohol [$P = .004$]). Men, whites, and more highly educated participants were more likely to have higher 25(OH)D values than women, both African Americans and Mexican Americans, and less educated participants, respectively (Table 1). Higher BMI was associated with

Table 1. Baseline demographic and health-related characteristics of study participants by serum 25-hydroxyvitamin D [25(OH)D] level, Third National Health and Nutrition Examination Survey (NHANES III), 1988–1994*

Characteristic	Serum 25(OH)D level (nmol/L)			
	<50	50 to <62.5	62.5 to <80	≥80
Sample size	5744	3143	3713	4218
Serum 25(OH)D levels†‡ (nmol/L)	38.3	56.6	71.3	104.4
Age†‡ (y)	45.2	45.9	44.1	40.8
Sex† (%)				
Men	34.7	46.5	49.7	55.3
Women	65.3	53.5	50.3	44.7
Race† (%)				
Non-Hispanic white	51.6	69.7	81.2	90.4
Non-Hispanic black	29.0	12.4	5.9	2.4
Mexican American	7.2	7.4	5.4	2.9
Other	12.2	10.5	7.6	4.3
Education, postsecondary† (%)	35.4	35.9	42.3	42.4
Latitude†‡	37.3	38.1	38.8	39.3
Smoking status† (%)				
Never	47.6	49.8	48.8	44.5
Former	22.0	23.6	25.4	26.9
Current	30.4	26.6	25.9	28.5
Alcohol†‡ (g/day)	9.2	8.3	9.2	12.8
BMI†‡ (kg/m^2)	27.8	27.3	26.3	25.2
Total body fat†‡ (kg)	26.0	24.1	22.5	20.1
Level of physical activity (same or more active than others of same age/sex)† (%)	71.1	75.6	76.8	80.4
Ever walk a mile, jog, swim, or bike in last month† (%)	52.3	59.3	66.0	71.9
Vitamin D from food†‡ (mcg/day)	3.7	4.5	5.2	5.9
Calcium from food†‡ (mg/day)	665.6	802.2	871.5	965.5
Serum vitamin A†‡ (μmol/L)	1.9	2.0	2.1	2.1

* All *P* values for differences by serum 25(OH)D level were significant at $P \leq .001$, except for education (*P* value = .01) and alcohol (*P* = .004), using chi-square test for categorical variables and linear regression analysis for continuous variables. BMI = body mass index.

† Weighted estimate.

‡ Mean value.

lower 25(OH)D serum measurement, with a mean BMI of 27.8 kg/m^2 in subjects with 25(OH)D lower than 50 nmol/L and a mean BMI of 25.2 kg/m^2 in those with 25(OH)D 80 nmol/L or higher. In contrast, reported levels of physical activity were higher in those with higher 25(OH)D, with 71.1% of those with circulating 25(OH)D lower than 50 nmol/L reporting a level of physical activity the same or greater than others of the same age/sex and 80.4% reporting such physical activity in those with circulating 25(OH)D 80 nmol/L or higher. Dietary vitamin D and calcium, as well as serum retinol, were higher in those with higher 25(OH)D levels (Table 1).

In the combined group of both season/latitude subpopulations, total cancer mortality was unrelated to 25(OH)D level (P_{trend} = .65) (Table 2). There was also no relationship to cancer mortality in either the winter/lower latitude or summer/higher latitude groups, and the test for interaction with season/latitude groups was not statistically significant (*P* = .36).

When we confined the analysis to men, there was similarly no statistically significant association between 25(OH)D and cancer mortality risk in the combined season/latitude subpopulations, although there was a suggestion of elevated risk in the two highest categories of 25(OH)D [80 to <100 nmol/L: RR = 1.21, 95% CI = 0.83 to 1.78; ≥100 nmol/L: RR = 1.35; 95% CI = 0.78 to 2.31] (Table 2). In women, there was also no association in the combined season/latitude subpopulations (Table 2). Because the test for interaction with season/latitudes groups was not statistically significant for men (*P* = .70) or for women (*P* = .26), we have not presented associations by sex and season/latitude groups. In addition, no association was seen in each age stratum (<70 or ≥70 years at attained age; data not shown).

When we considered racial/ethnic groups separately, there was also no association between 25(OH)D and total cancer mortality in non-Hispanic whites (P_{trend} = .80), non-Hispanic blacks (P_{trend} = .14), or Mexican Americans (P_{trend} = .37) (Table 3). As with sex, the test for interaction with season/latitude groups was not statistically significant for non-Hispanic whites, non-Hispanic blacks, or Mexican Americans, and we have therefore not presented associations by season/latitude group. Moreover, we found a statistically significantly elevated risk in non-Hispanic blacks compared with non-Hispanic whites that was not reduced by adjusting for serum 25(OH)D (data not shown).

We analyzed the relationship between serum 25(OH)D level and site-specific cancer mortality (Table 4). One hundred fifty-three subjects died of lung cancer; 66, of colorectal cancer; 72, of other digestive cancers; 28, of breast cancer; 47, of prostate cancer; 40, of non-Hodgkin lymphoma or leukemia; and 130, of other cancers. We found no relationship between 25(OH)D level and lung cancer mortality. There was a statistically significant inverse relationship between colorectal cancer mortality and 25(OH)D levels (P_{trend} = .02). Serum levels at or above 80 nmol/L

Table 2. Relative risks (RRs) and 95% confidence intervals (CIs) for cancer mortality according to baseline serum 25-hydroxyvitamin D [25(OH)D] level (nmol/L) by seasonal subpopulation and sex in the NHANES III Study, 1988–2000*

	Serum 25(OH)D (nmol/L)						
Cancer mortality†	**<50**	**50 to <62.5**	**62.5 to <80**	**80 to <100**	**100 to <120**	**≥120**	**P_{trend}**
Total cancer mortality, season/latitude combined							
No. of deaths	175	103	117	80	41	20	
RR	1.0	1.22	1.02	1.00	0.92	1.49	
95% CI		0.91 to 1.64	0.69 to 1.50	0.71 to 1.40	0.58 to 1.46	0.85 to 2.64	.65
	<50	**50 to <62.5**	**62.5 to <80**	**80 to <100**	**≥100**		
Winter/lower latitude							
No. of deaths	74	37	42	30	23		
RR	1.0	1.02	1.16	0.95	1.08		
95% CI		0.59 to 1.76	0.52 to 2.56	0.51 to 1.79	0.54 to 2.16		.44
Summer/higher latitude							
No. of deaths	101	66	75	50	38		
RR	1.0	1.29	0.96	1.00	1.09		
95% CI		0.90 to 1.85	0.65 to 1.41	0.65 to 1.54	0.66 to 1.81		.95
Males, season/latitude combined							
No. of deaths	88	57	71	58	44		
RR	1.0	1.03	0.99	1.21	1.35		
95% CI		0.73 to 1.44	0.57 to 1.74	0.83 to 1.78	0.78 to 2.31		.08
Females, season/latitude combined							
No. deaths	87	46	46	22	17		
RR	1.0	1.40	1.02	0.72	0.78		
95% CI		0.94 to 2.08	0.62 to 1.67	0.40 to 1.26	0.40 to 1.53		.12

* RRs were adjusted for age, race/ethnicity, sex, and smoking history (pack-years) using Cox proportional hazard regression. P_{trend} was based on 25(OH)D as a continuous variable. Categories were selected to reflect alternative cut points of 25(OH)D insufficiency and the full distribution of 25(OH)D values (21,22).

† Cancer mortality was based on International Statistical Classification of Diseases, Tenth Revision, codes for cancer sites as follows: C33–C34 (lung, trachea, and bronchus); C18–C21 (colon, rectum, and anus); C15, C16, C22, C25 (other digestive system, i.e., esophagus, stomach, liver, pancreatic cancer); C50 (breast); C61 (prostate); C82–C85, C91–C95 (non-Hodgkin lymphoma/leukemia); and C00–C14, C17, C23–C24, C26–C32, C37–C41, C43–C49, C51–C52, C54–C60, C62–C65, C67–C80, C88, C90, C97 (other cancers, including buccal, larynx, melanoma, gynecological sites, kidney, bladder, brain, multiple myeloma).

were associated with lower risk (RR = 0.28, 95% CI = 0.11 to 0.68) compared with levels lower than 50 nmol/L. Mortality for cancers at the other digestive cancer sites combined (including esophagus, stomach, liver, and pancreatic cancer) showed no association with 25(OH)D levels (P = .18).

Levels of 25(OH)D levels were stratified in two categories (<62.5 and ≥62.5 nmol/L) to assess breast cancer mortality, with the cut point reflecting one alternative level of 25(OH)D insufficiency (21). The higher category (with small case numbers, n = 8) was related to a statistically significantly lower risk of mortality due to breast cancer (RR = 0.28; 95% CI = 0.08 to 0.93), but the linear trend [modeling 25(OH)D level as continuous values] was not statistically significant (P_{trend} = .76). There was also no association between prostate cancer mortality and 25(OH)D levels (stratified as <62.5 and ≥62.5 nmol/L). Neither mortality due to non-Hodgkin lymphoma and leukemia combined nor mortality due to all cancers not in the major groupings demonstrated a relationship to 25(OH)D.

We examined the association between total cancer mortality and serum retinol (umol/L) in quartiles to address the hypothesis that retinol might counteract cancer prevention by vitamin D. There was a suggestion of declining risk (RR with increasing retinol = 0.94 [95% CI = 0.67 to 1.32]; 0.91 [95% CI = 0.61 to 1.35]; 0.73 [95% CI = 0.49 to 1.09]), but the linear trend was not statistically significant (P_{trend} = .17). The pattern was similar in both season/latitude groups (data not shown). The relationship between 25(OH)D (whether treated as a continuous or categorical variable) and total cancer mortality was not modified by serum retinol (in two categories, split at the median) (test of interaction, P = .13 and P = .41, respectively). There was also no interaction between 25(OH)D and serum retinol for colorectal cancer mortality (P = .76, P = .91).

Discussion

We found no association between 25(OH)D and total cancer mortality in the total NHANES III study population or in either season/latitude subpopulation. We did, however, find an inverse association with colorectal cancer mortality. Although there was also a statistically significant inverse association with female breast cancer mortality when subjects were divided into those with high and low levels of 25(OH)D, the linear trend was not statistically significant and the number of deaths due to breast cancer was small. For all other cancer sites and groups of sites that we examined, there was no association between mortality and 25(OH)D levels. To our knowledge, this study is the first to examine the relationship between measured serum vitamin D levels and cancer mortality for selected sites and for all sites combined.

The study of the relationship between vitamin D levels and cancer mortality has been based largely on latitude or other surrogates for exposure to solar radiation, not vitamin D itself (6,7,10).

Table 3. Relative risks (RR) and 95% confidence intervals (CIs) for total cancer mortality according to baseline serum 25-hydroxyvitamin D [25(OH)D] level (nmol/L) by racial/ethnic group in the Third National Health and Nutrition Examination Survey (NHANES III Study), 1988–2000*

	Serum 25(OH)D (nmol/L)				
Racial/ethnic group	**<50**	**50 to <80**	**80 to <100**	**≥100**	P_{trend}
Non-Hispanic white					
No. of deaths	61	135	57	45	
RR	1.0	1.12	1.02	1.10	
95% CI		0.78 to 1.60	0.66 to 1.56	0.70 to 1.74	.80
	<50	**50 to <80**	**≥80**		
Non-Hispanic black					
No. of deaths	80	42	22		
RR	1.0	0.84	1.41		
95% CI		0.53 to 1.33	0.80 to 2.48		.14
Mexican American					
No. of deaths	33	39	15		
RR	1.0	0.89	0.66		
95% CI		0.42 to 1.87	0.28 to 1.55		.37

* RRs were adjusted for age, sex, and smoking history (pack-years) using Cox proportional hazard regression. P_{trend} was based on 25(OH)D as a continuous variable. Categories were selected to reflect alternative cut points of 25(OH)D insufficiency and the full distribution of 25(OH)D values (21,22).

There has, however, been one randomized controlled 5-year trial of triannual high oral doses of vitamin D that evaluated cancer mortality. It found a somewhat lower cancer mortality risk (RR = 0.86, 95% CI = 0.61 to 1.20) in subjects who were given vitamin D, although the case numbers were small (n = 135 cancer deaths in both arms combined) and the difference in risk was not statistically significant (37). In addition, as noted above, one study estimated that there was a 29% reduction in total cancer mortality risk associated with an increment of 25 nmol/L in 25(OH)D based on a predictive model for vitamin D level (18).

Several studies have, however, addressed the association between serum vitamin D level and site-specific cancer incidence. Our finding that colorectal cancer mortality is inversely associated with 25(OH)D is consistent with a large number of observational epidemiologic studies (1,17,38). Several prospective studies of the relationship between serum/plasma 25(OH)D and colorectal cancer incidence have found a reduced risk associated with increased vitamin D. One of the largest of these was a nested case–control study with 193 cases of colorectal cancer within the Nurses' Health Study. It found that the odds ratio of colorectal cancer for women in the highest relative to the lowest quintile of 25(OH)D was 0.53 (95% CI = 0.27 to 1.04) and that there was a statistically significant linear inverse trend (P = .02) (39). Three smaller studies (n = 34, 57, and 146 cases) also found lower risks of colorectal cancer associated with higher 25(OH)D (40–42). In addition, circulating 25(OH)D was associated with reduced risk of colorectal adenomas in women, although not in men (43). However, the Women's Health Initiative randomized controlled trial of calcium (1000 mg) and vitamin D (400 IU) supplements over an average of 7 years of follow-up found no association between the intervention supplements and colorectal cancer incidence (44). Nonetheless, the case–control study of baseline serum 25(OH)D nested in the trial found a statistically significant inverse association of colorectal cancer risk with baseline level of serum 25(OH)D, suggesting that the null result of the trial may have been due to low trial doses of vitamin D, insufficient trial duration (45), or reduced risk being limited to those with low baseline levels.

Only one study has prospectively examined the relationship between circulating levels of 25(OH)D and the risk of breast cancer (46). In that study, which included 701 breast cancer patients, women in the third, fourth, or fifth quintiles of 25(OH)D had a somewhat lower risk of incident breast cancer than women in the two lowest quintiles, suggesting a possible threshold effect. Although we observed a substantially reduced risk of breast cancer mortality for those with 25(OH)D measurements at or above 62.5 nmol/L, the linear trend was not statistically significant and the number of breast cancer deaths was small, so the result must be interpreted with caution. In fact, the estimated relative risk of breast cancer mortality associated with a 50-nmol change in 25(OH)D (RR = 0.83) was bounded by confidence intervals (95% CI = 0.24 to 2.85) that are, too wide to rule out either the possible protective effect observed in the study by Bertone-Johnson et al. (46) in which the relative risk for the fifth quintile compared with the first quintile [a difference in 25(OH)D of approximately 50 nmol] was 0.73, or an increased risk with rising 25(OH)D.

The absence of other associations, including the lack of any relationship between 25(OH)D level and total cancer mortality, does not support the current interest in 25(OH)D as a prohormone that reduces the risk of and mortality due to a wide range of cancers. It is also inconsistent with the inverse association with cancer mortality risk observed in the HPFS, which was based on predicted 25(OH)D values rather than on actual measured values (18).

There was no association between total cancer mortality and 25(OH)D in non-Hispanic whites, non-Hispanic blacks, and Mexican Americans; thus, our results do not support the hypothesis that low vitamin D levels in blacks contribute to racial disparities in cancer mortality (29). The fact that the cancer mortality risk in non-Hispanic blacks relative to non-Hispanic whites did not fall after adjusting for serum 25(OH)D is also inconsistent with vitamin D being a major factor in cancer mortality differences.

Table 4. Relative risks (RRs) and 95% confidence intervals (CIs) for site-specific cancer mortality according to baseline serum 25-hydroxyvitamin D [25(OH)D] levels (nmol/L) in the Third National Health and Nutrition Examination Survey (NHANES III Study), 1988–2000*

	25(OH)D (nmol/L)				
Cancer site	**<50**	**50 to <80**	**80 to <100**	**≥100**	P_{trend}
Lung cancer					
No. of deaths	57	51	23	22	
RR	1.0	0.78	0.65	1.14	
95% CI		0.50 to 1.22	0.36 to 1.18	0.60 to 2.18	.41
	<62.5	**≥62.5**			
Digestive cancers other than colorectal cancer†					
No. of deaths	34	37			
RR	1.0	1.42			
95% CI		0.73 to 1.76			.18
Breast cancer					
No. of deaths	20	8			
RR	1.0	0.28			
95% CI		0.08 to 0.93			.76
Prostate cancer					
No. of deaths	22	25			
RR	1.00	0.91			
95% CI		0.39 to 2.14			.95
Non-Hodgkin lymphoma/leukemia					
No. of deaths	16	24			
RR	1.0	1.34			
95% CI		0.62 to 2.91			.96
	<50	**50 to <80**	**≥80**		
Colorectal cancer‡					
No. of deaths	28	24	14		
RR	1.0	0.44	0.28		
95% CI		0.20 to 0.95	0.11 to 0.68		.02
Other cancer sites§					
No. of deaths	38	60	32		
RR	1.0	1.83	1.86		
95% CI		0.93 to 3.61	0.75 to 4.63		.84

* RRs were adjusted for age, sex, race/ethnicity, and smoking history (pack-years) using Cox proportional hazard regression. P_{trend} was based on 25(OH)D as a continuous variable. Categories were selected to reflect alternative cut points of 25(OH)D insufficiency and the full distribution of 25(OH)D values (21,22).

† Includes esophagus, stomach, liver, and pancreatic cancer.

‡ Includes anal cancer mortality.

§ Includes buccal, larynx, melanoma, gynecological sites, kidney, bladder, brain, multiple myeloma, and others.

Retinol has been hypothesized to modify adversely the proposed protective association between vitamin D and cancer (1). The theoretical basis for this proposal stems from an interaction between the hormonal vitamin D/vitamin D receptor complex and the retinoid X receptor to form a heterodimer complex that interacts with DNA sequences. It has been proposed that if retinol levels are high, this molecule may compete with hormonal vitamin D for the retinoid X receptor and thus reduce the activity of vitamin D (1). Animal and human evidence cited in support of the hypothesis relates to effects of vitamin D on serum calcium and bone health (1). Although one nested case–control study (46) of breast cancer and 25(OH)D examined the effects of retinol levels and did not find that retinol modified the actions of 25(OH)D, our study is the first, to our knowledge, to examine whether serum retinol modifies the association between 25(OH)D and total cancer mortality. We found no interaction between serum 25(OH)D and retinol in relationship to overall cancer mortality (where there was also no main effect of 25(OH)D) or in relationship to mortality from colorectal cancer, which was inversely associated with 25(OH)D. Thus, our results do not support a role for retinol levels in modifying an association between 25(OH)D levels and cancer.

Our study had several strengths. Serum 25(OH)D measurements were obtained from an entire study population that was derived from a representative sample of the civilian, noninstitutionalized US population. We studied a wide range of covariates and assessed an endpoint, cancer mortality, with a substantial follow-up period (median 8.9 years) so that our results would be sensitive to potential associations of vitamin D with survival.

Our study, however, recorded small numbers of cancer deaths for certain cancer sites and racial and season/latitude subgroups, and this limited our ability to detect associations. Other limitations included the imprecision in measurements of 25(OH)D, our limited ability to assess risk at particularly low values of 25(OH)D because of the absence of winter values for 25(OH)D in northern

latitudes, and a measurement of 25(OH)D based on a blood sample that was collected at a single point in time, an imperfect surrogate for long-term indicators of 25(OH)D. A recent cohort study, however, measured 25(OH)D concentrations at two times that were 3 years apart and found a Pearson correlation coefficient of .70 between samples ($P<.001$) (47).

In summary, we did not find a relationship between prospective serum 25(OH)D and total cancer mortality, although we observed a negative dose–response relationship between 25(OH)D and colorectal cancer mortality. Serum retinol did not modify the relationship between 25(OH)D and total cancer or colorectal mortality. Additional studies with large numbers of samples of measured 25(OH)D serum levels, preferably at multiple time points, are needed to confirm the total cancer mortality findings of this paper and to obtain more accurate risk estimates for mortality from specific cancers.

References

(1) Giovannucci E. The epidemiology of vitamin D and cancer incidence and mortality: a review (United States). Cancer Causes Control 2005; 16:83–95.

(2) Robsahm TE, Tretli S, Dahlback A, Moan J. Vitamin D3 from sunlight may improve the prognosis of breast-, colon- and prostate cancer (Norway). Cancer Causes Control 2004;15:149–58.

(3) Trump DL, Hershberger PA, Bernardi RJ, Ahmed S, Muindi J, Fakih M, et al. Anti-tumor activity of calcitriol: pre-clinical and clinical studies. J Steroid Biochem Mol Biol 2004;89–90:519–26.

(4) Ordonez-Moran P, Larriba MJ, Pendas-Franco N, Aguilera O, Gonzalez-Sancho JM, Munoz A. Vitamin D and cancer: an update of in vitro and in vivo data. Front Biosci 2005;10:2723–49.

(5) Holick MF. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease 2. Am J Clin Nutr 2004;80 Suppl:1678S–88S.

(6) Garland CF, Garland FC. Do sunlight and vitamin D reduce the likelihood of colon cancer? Int J Epidemiol 1980;9:227–31.

(7) Lefkowitz ES, Garland CF. Sunlight, vitamin D, and ovarian cancer mortality rates in US women. Int J Epidemiol 1994;23:1133–6.

(8) Hanchette CL, Schwartz GG. Geographic patterns of prostate cancer mortality. Evidence for a protective effect of ultraviolet radiation. Cancer 1992;70:2861–9.

(9) Garland FC, Garland CF, Gorham ED, Young JF. Geographic variation in breast cancer mortality in the United States: a hypothesis involving exposure to solar radiation. Prev Med 1990;19:614–22.

(10) Grant WB. Ecologic studies of solar UV-B radiation and cancer mortality rates. Recent Results Cancer Res 2003;164:371–7.

(11) Freedman DM, Zahm SH, Dosemeci M. Residential and occupational exposure to sunlight and mortality from non-Hodgkin's lymphoma: composite (threefold) case-control study. BMJ 1997;314:1451–5.

(12) Freedman DM, Dosemeci M, McGlynn K. Sunlight and mortality from breast, ovarian, colon, prostate and non-melanoma skin cancer: a composite death certificate based case-control study. Occup Environ Med 2002; 59:257–62.

(13) Porojnicu AC, Robsahm TE, Ree AH, Moan J. Season of diagnosis is a prognostic factor in Hodgkin's lymphoma: a possible role of sun-induced vitamin D. Br J Cancer 2005;93:571–4.

(14) Zhou W, Suk R, Liu G, Park S, Neuberg DS, Wain JC, et al. Vitamin D is associated with improved survival in early-stage non-small cell lung cancer patients. Cancer Epidemiol Biomarkers Prev 2005;14:2303–9.

(15) Moan J, Porojnicu AC, Robsahm TE, Dahlback A, Juzeniene A, Tretli S, et al. Solar radiation, vitamin D and survival rate of colon cancer in Norway. J Photochem Photobiol B 2005;78:189–93.

(16) Lim HS, Roychoudhuri R, Peto J, Schwartz G, Baade P, Moller H. Cancer survival is dependent on season of diagnosis and sunlight exposure. Int J Cancer 2006;119:1530–6.

(17) Garland CF, Garland FC, Gorham ED, Lipkin M, Newmark H, Mohr SB, et al. The role of vitamin D in cancer prevention. Am J Public Health 2006;96:252–61.

(18) Giovannucci E, Liu Y, Rimm EB, Hollis BW, Fuchs CS, Stampfer MJ, et al. Prospective study of predictors of vitamin D status and cancer incidence and mortality in men. J Natl Cancer Inst 2006;98:451–9.

(19) Zehnder D, Bland R, Williams MC, McNinch RW, Howie AJ, Stewart PM, et al. Extrarenal expression of 25-hydroxyvitamin d(3)-1 alpha-hydroxylase. J Clin Endocrinol Metab 2001;86:888–94.

(20) National Center for Health Statistics. Plan and operation of the Third National Health and Nutrition Examination Survey, 1988–1994. Vital Health Statistics 1(32). Hyattsville, MD: National Center for Health Statistics; 1994. Report No.: DHHS Publication No. (PHS) 94–1308.

(21) Looker AC, Dawson-Hughes B, Calvo MS, Gunter EW, Sahyoun NR. Serum 25-hydroxyvitamin D status of adolescents and adults in two seasonal subpopulations from NHANES III. Bone 2002;30:771–7.

(22) Heaney RP. The vitamin D requirement in health and disease. J Steroid Biochem Mol Biol 2005;97:13–9.

(23) Looker AC. Body fat and vitamin D status in black versus white women 1. J Clin Endocrinol Metab 2005;90:635–40.

(24) Buzzard IM, Feskanich D. Maintaining a food composition database for multiple research studies: the NCC food table. In: Rand WM, Windham CT, Wyse BW, Young VR, editors. Food composition data: a user's perspective. Tokyo, Japan: The United Nations University; 1987. p. 115–22.

(25) University of Minnesota, Nutrient Coordinating Center. Nutrient database versions 15–27. Minneapolis, MN; 1996.

(26) US Department of Agriculture, Agricultural Research Service. Survey nutrient databases for NHANES III, phase 1 (1993) and phase 2 (1995). Riverdale, MD: US Department of Agriculture, Agricultural Research Service; 1993.

(27) Gunter EW, Lewis BL, Koncikowski SM. Laboratory methods used for the Third National Health and Nutrition Examination Survey (NHANES III), 1988–1994 [CD-ROM]. Hyattsville, MD: Center for Disease Control and Prevention; 1996.

(28) Korn EL, Graubard BI, Midthune D. Time-to-event analysis of longitudinal follow-up of a survey: choice of the time-scale. Am J Epidemiol 1997;145:72–80.

(29) Giovannucci E, Liu Y, Willett WC. Cancer incidence and mortality and vitamin D in black and white male health professionals. Cancer Epidemiol Biomarkers Prev 2006;15:2467–72.

(30) Grant WB, Garland CF. Evidence supporting the role of vitamin D in reducing the risk of cancer. J Intern Med 2002;252:178–9.

(31) Hartge P, Lim U, Freedman DM, Colt JS, Cerhan JR, Cozen W, et al. Ultraviolet radiation, dietary vitamin D, and risk of non-Hodgkin lymphoma (United States). Cancer Causes Control 2006;17:1045–52.

(32) Smedby KE, Hjalgrim H, Melbye M, Torrang A, Rostgaard K, Munksgaard L, et al. Ultraviolet radiation exposure and risk of malignant lymphomas. J Natl Cancer Inst 2005;97:199–209.

(33) Hu S, Ma F, Collado-Mesa F, Kirsner RS. Ultraviolet radiation and incidence of non-Hodgkin's lymphoma among Hispanics in the United States. Cancer Epidemiol Biomarkers Prev 2004;13:59–64.

(34) Hughes AM, Armstrong BK, Vajdic CM, Turner J, Grulich AE, Fritschi L, et al. Sun exposure may protect against non-Hodgkin lymphoma: a case-control study. Int J Cancer 2004;112:865–71.

(35) Luong QT, Koeffler HP. Vitamin D compounds in leukemia. J Steroid Biochem Mol Biol 2005;97:195–202.

(36) Korn EL, Graubard BI. Analysis of health surveys. New York: Wiley; 1999. p. 160–5.

(37) Trivedi DP, Doll R, Khaw KT. Effect of four monthly oral vitamin D3 (cholecalciferol) supplementation on fractures and mortality in men and women living in the community: randomised double blind controlled trial. BMJ 2003;326:469.

(38) Giovannucci E. The epidemiology of vitamin D and colorectal cancer: recent findings. Curr Opin Gastroenterol 2006;22:24–9.

(39) Feskanich D, Ma J, Fuchs CS, Kirkner GJ, Hankinson SE, Hollis BW, et al. Plasma vitamin D metabolites and risk of colorectal cancer in women. Cancer Epidemiol Biomarkers Prev 2004;13:1502–8.

(40) Garland CF, Comstock GW, Garland FC, Helsing KJ, Shaw EK, Gorham ED. Serum 25-hydroxyvitamin D and colon cancer: eight-year prospective study. Lancet 1989;2:1176–8.
(41) Tangrea J, Helzlsouer K, Pietinen P, Taylor P, Hollis B, Virtamo J, et al. Serum levels of vitamin D metabolites and the subsequent risk of colon and rectal cancer in Finnish men. Cancer Causes Control 1997;8: 615–25.
(42) Braun MM, Helzlsouer KJ, Hollis BW, Comstock GW. Colon cancer and serum vitamin D metabolite levels 10-17 years prior to diagnosis. Am J Epidemiol 1995;142:608–11.
(43) Peters U, Hayes RB, Chatterjee N, Shao W, Schoen RE, Pinsky P, et al. Circulating vitamin D metabolites, polymorphism in vitamin D receptor, and colorectal adenoma risk. Cancer Epidemiol Biomarkers Prev 2004;13: 546–52.
(44) Wactawski-Wende J, Kotchen JM, Anderson GL, Assaf AR, Brunner RL, O'Sullivan MJ, et al. Calcium plus vitamin D supplementation and the risk of colorectal cancer. N Engl J Med 2006;354:684–96.
(45) Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. Am J Clin Nutr 2006;84:18–28.
(46) Bertone-Johnson ER, Chen WY, Holick MF, Hollis BW, Colditz GA, Willett WC, et al. Plasma 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D and risk of breast cancer. Cancer Epidemiol Biomarkers Prev 2005;14:1991–7.
(47) 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;15:255–65.

Funding

Intramural Research Program of the National Institutes of Health, National Cancer Institute; US Public Health Service of the Department of Health and Human Services.

Notes

We thank Donna J. LaVoie (MT)ASCP and Della B.Twite (MT)ASCP for performing the NHANES III 25(OH)D assays, Lisa Kahle of Information Management Services, Inc, for biomedical computer assistance, and Drs Martha Linet and Elaine Ron for insightful comments. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention. The National Center for Health Statistics of the Centers for Disease Control and Prevention was responsible for the data collection and design of the NHANES III.

Manuscript received March 7, 2007; revised July 20, 2007; accepted September 26, 2007.