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ORIGINAL PAPER

Serum 25-hydroxyvitamin D concentrations and lung cancer risk in never-smoking postmenopausal women

Ting-Yuan David Cheng[1] · Xiaoling Song[2] · Shirley A. A. Beresford[2] · Gloria Y. F. Ho[3,4] · Karen C. Johnson[5] · Mridul Datta[6] · Rowan T. Chlebowski[7] · Jean Wactawski-Wende[8] · Lihong Qi[9] · Marian L. Neuhouser[2]

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Abstract

Purpose Vitamin D has been implicated in lowering lung cancer risk, but serological data on the association among never-smoking women are limited. We report results examining the association of serum 25-hydroxyvitamin D [25(OH)D] concentrations with lung cancer risk among female never smokers. We also examined whether the association was modified by vitamin D supplementation and serum vitamin A concentrations.

Methods In the Women's Health Initiative, including the calcium/vitamin D (CaD) Trial, we selected 298 incident cases [191 non-small cell lung cancer (NSCLC) including 170 adenocarcinoma] and 298 matched controls of never smokers. Baseline serum 25(OH)D was assayed by a chemiluminescent method. Logistic regression was used to estimate odds ratios (ORs) for quartiles and predefined clinical cutoffs of serum 25(OH)D concentrations.

Results Comparing quartiles 4 versus 1 of serum 25(OH)D concentrations, ORs were 1.06 [95% confidence interval (CI) 0.61–1.84] for all lung cancer, 0.94 (95% CI 0.52–1.69) for NSCLC, and 0.91 (95% CI 0.49–1.68) for adenocarcinoma. Comparing serum 25(OH)D ≥ 75 (high) versus <30 nmol/L (deficient), ORs were 0.76 (95% CI 0.31–1.84) for all lung cancer, 0.71 (95% CI 0.27–1.86) for NSCLC, and 0.81 (95% CI 0.31–2.14) for adenocarcinoma. There is suggestive evidence that CaD supplementation (1 g calcium + 400 IU D_3/day) and a high level of circulating vitamin A may modify the associations of 25(OH)D with lung cancer overall and subtypes (p interaction <0.10).

Conclusions In this group of never-smoking postmenopausal women, the results did not support the hypothesis of an association between serum 25(OH)D and lung cancer risk.

Keywords 25-Hydroxyvitamin D · Lung cancer · Postmenopausal women · Never smokers · Histology

Introduction

Carcinogens from environmental exposures, including second-hand tobacco smoke, are closely tied to lung cancers

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✉ Ting-Yuan David Cheng
tingyuan.cheng@ufl.edu

[1] Department of Epidemiology, University of Florida, 2004 Mowry Road, 4th Floor, RM4213, P. O. Box 100231, Gainesville, FL 32610, USA

[2] Public Health Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA

[3] Feinstein Institute for Medical Research, Manhasset, NY, USA

[4] Hofstra North Shore-LIJ School of Medicine, Hempstead, NY, USA

[5] Department of Preventive Medicine, University of Tennessee Health Science Center, Memphis, TN, USA

[6] Department of Nutrition Science, Purdue University, West Lafayette, IN, USA

[7] City of Hope National Medical Center, Duarte, CA, USA

[8] Department of Epidemiology and Environment Health, University at Buffalo, The State University of New York, Buffalo, NY, USA

[9] Division of Biostatistics, Department of Public Health Sciences, University of California-Davis, Davis, CA, USA

occurring in never smokers, but a large fraction of these lung cancers may be attributable to other unknown risk factors [1]. Low vitamin D status has been hypothesized as a risk factor for lung cancer because there are abundant vitamin D metabolic enzymes as well as the vitamin D receptor (VDR) expressed in the lung [2, 3]. Humans obtain vitamin D from diet, and through cutaneous synthesis as ultraviolet B radiation exposure converts 7-dehydrocholesterol to vitamin D. Through enzymatic reactions in the liver and kidney, vitamin D is converted to 25-hydroxyvitamin D [25(OH)D], the standard biomarker for assessing vitamin D status, and further to 1,25-dihydroxyvitamin D [4]. 1,25-Dihydroxyvitamin D has antiproliferative properties [5]. 1,25-Dihydroxyvitamin D also binds to the VDR, pairing with retinoid X receptor (RXR) to regulate the transcription of target genes that control proliferation, apoptosis, and angiogenesis [6, 7].

Although epidemiological data are inconsistent on the association of 25(OH)D concentrations with lung cancer risk (Supplementary Table 1) [8–17], a meta-analysis of ten prospective studies showed an inverse, dose–response relationship (5% reduction in incidence or mortality for each 10 nmol/L increment) [18]. To date, two studies have reported findings for women [8, 9], of which only one study examined the association of 25(OH)D concentrations with lung cancer incidence [8]. Serum 25(OH)D concentrations ≥47 versus ≤30 nmol/L were associated with a lower lung cancer risk in a cohort study of Finnish women ($n = 3{,}730$) [relative risk = 0.16, 95% confidence interval (CI) 0.04–0.59] [8]; however, the number of lung cancer cases was very small ($n = 25$). Data from the Third National Health and Nutrition Examination Survey (NHANES III) showed no association of serum 25(OH)D with lung cancer mortality in women [hazard ratio (HR) 0.64, 95% CI 0.23–1.79, ≥80 vs. <50 nmol/L] [9]. However, in another analysis of the NHANES III data, a significant decrease in lung cancer mortality with higher serum 25(OH)D was observed in never smokers and former smokers who quit for 20 years or longer and had higher serum 25(OH)D levels (HR 0.31, 95% CI 0.13–0.76, >80 vs. <44 nmol/L) [10]. Lung cancer incidence was not assessed in the NHANES III and, in some participants, blood samples might have been obtained after the lung cancer diagnosis. Despite these limitations, the findings suggest that serum 25(OH)D may also play a role in lung cancer etiology in never smokers. Research is needed on the association of 25(OH)D concentrations with lung cancer risk among female never smokers for whom the risk factors remain elusive.

Here, we report results examining the association of serum 25(OH)D concentrations with lung cancer risk among never smokers in the Women's Health Initiative (WHI). WHI is a large study of 161,808 postmenopausal women in the U.S. and includes an Observational Study (OS) and three overlapping clinical trials (CTs) [19]. Among CTs, the calcium/vitamin D (CaD) Trial was a randomized, placebo-controlled trial testing supplementation with daily 1 g of calcium carbonate and 400 IU of vitamin D_3 for preventing fracture and colorectal cancer [20, 21]. Thus, we also examined whether the association of serum 25(OH)D concentrations with lung cancer risk differed by the CaD Trial intervention. In addition, we investigated whether serum vitamin A (retinol), which supplies ligands necessary for the VDR–RXR heterodimer, modified the relationship between serum 25(OH)D and lung cancer risk.

Methods

Study population

We conducted a case–control study nested in the WHI CTs and OS [19, 22]. The eligibility criteria for both the CTs and the OS included being postmenopausal, aged 50–70 years with a life expectancy of at least 3 years, no history of breast cancer, and no history of other cancers within the previous 10 years. At baseline (1993–1998), women were enrolled in the Hormonal Therapy Trials ($n = 27{,}347$) and Dietary Modification Trial ($n = 48{,}835$). After 1 year, CT participants were invited to join the CaD Trial ($n = 36{,}282$). For the CaD Trial, women who had a history of hypercalcemia, kidney stones, or corticosteroid or calcitriol use and women who chose to continue vitamin D supplementation over 600 IU/d were ineligible. Personal use of calcium and vitamin D supplements (initially up to 600 IU/d, and later, 1,000 IU/d) was permitted during the trial. Women either ineligible or unwilling to participate in the CTs were enrolled in the OS for prospective follow-up ($n = 93{,}676$). The protocol was approved by the institutional review boards of each clinical center and the WHI Clinical Coordinating Center. All women provided written informed consent. The current study was approved by the institutional review board of the Fred Hutchinson Cancer Research Center.

Outcome assessment and case and control selection

Participants reported all new cancer diagnoses, including lung cancer, semiannually in the CTs and annually in the OS until 2005 when the outcomes were thereafter reported annually. Trained study physicians, blinded to WHI study components, at local clinics confirmed and adjudicated cases by reviewing medical records [23]. Tumor histology and stage were coded using the International Classification of Disease for Oncology, second edition. For the current study, inclusion criteria were cases who were never smokers and had adequate serum samples at baseline. Smoking status was based on self-report assessed by a standardized questionnaire [24], and never smokers were defined as those who

had not smoked >100 cigarettes in their lifetime and did not smoke at the time of questionnaire completion [25]. A total of 369 incident lung cancer cases out of 2491 ascertained as of 30 September, 2012 were eligible; from these cases, 300 were randomly selected. Controls were women who never smoked, provided baseline serum samples, and did not have a diagnosis of cancer other than non-melanoma skin cancer up to the time of the case's diagnosis date. One control was selected for each case from the risk set at the time of the case's event, with additional matching on age (±1 year) and study (CT/OS). Two case–control pairs were excluded due to missing data on serum 25(OH)D concentrations and body mass index (BMI), leaving 298 case–control pairs for statistical analyses.

Blood sample processing and analysis

Whole blood was collected after an overnight, 12-h fast at baseline. The samples were processed to serum, aliquoted in dim/yellow light at the clinical centers, and stored at −70 °C within 2 h of collection and at −80 °C for long-term storage. Serum 25(OH)D was assayed in duplicate using LIAISON® 25 OH vitamin D TOTAL Assay (DiaSorin, Stillwater, MN), a chemiluminescent assay providing the combined concentrations of both $25(OH)D_2$ and $25(OH)D_3$. Serum retinol and retinyl palmitate, the main component of retinyl esters [26, 27], were measured by high-performance liquid chromatography as described previously [28]. We did not assay other retinyl esters because their serum concentrations were very low in WHI participants. All assays were performed at the Fred Hutchinson Cancer Research Center Biomarker Laboratory, which participates in the National Institute of Standards and Technology Micronutrients Measurement Quality Assurance Program for fat-soluble vitamins and carotenoids in human serum and the Vitamin D Metabolites Quality Assurance Program. Cases and matched controls were analyzed in the same batch; laboratory staff was blinded to the case/control status of samples. Average interassay coefficients of variation (CV) were 5.5% for 25(OH)D, 2.6% for retinol, and 14.6% for retinyl palmitate in 30 pairs of blinded duplicate samples.

Questionnaire and anthropometric data collection

Demographic characteristics, including age, race/ethnicity, education, history of chronic diseases, and health-related behaviors, including physical activity, smoking history, and use of postmenopausal hormones and other medications, were collected at baseline by standardized questionnaires [19]. OS participants additionally reported (1) whether they ever or currently lived and worked with a smoker after the age of 18 years, and (2) numbers of years living and working with a smoker, as an assessment of second-hand smoke exposure. Recreational physical activity was estimated as metabolic equivalent task (MET)-hours per week. Dietary intakes were assessed at baseline by a self-administered, 122-item food frequency questionnaire, validated by four 24-h dietary recalls and a 4-day food record [29]. Supplemental vitamin use was assessed by a simplified inventory method [30]. Weight and height were measured at baseline clinic visits by trained staff using a standardized protocol; BMI was computed as [weight (kg)/height^2 (m^2)].

Statistical analysis

Differences in baseline levels of exposure variables and potential confounders between cases and controls were examined with *t* test (continuous variables) and Chi-square test (categorical variables). Correlations were evaluated between serum concentrations of 25(OH)D, retinol, and retinyl palmitate and intake values of vitamin D and vitamin A from diet and supplements. We used conditional logistic regression models to estimate odds ratios (ORs) and 95% CIs for lung cancer risk. Cases and controls were grouped by quartiles of serum 25(OH)D concentrations; the quartile cutoffs were selected based on the distribution in control participants. In addition, we used season-specific quartiles to examine the serum 25(OH)D–lung cancer association as a secondary analysis [31]. We also tested the 25(OH)D–lung cancer association using a priori selected clinical cut points serum 25(OH)D concentrations defined as deficient (<30 nmol/L), inadequate (30 to <50 nmol/L), sufficient (50 to <75 nmol/L), and high (≥75 nmol/L). The cutoffs for deficient, inadequate, and sufficient statuses were based on the Institute of Medicine classification [32], and the high status has been suggested to be beneficial for various health outcomes including cancer [33]. Tests of linear trend across increasing categories of serum 25(OH)D concentrations were conducted using the Wald tests by modeling the median values of each category as a single continuous variable in the models. The selection of covariates was based on biomedical knowledge and their influence on risk estimates [1, 34]. Covariates in the final regression model included race/ethnicity (black/African American, Hispanic/Latino, non-Hispanic white, others), baseline body mass index (<25, 25 to <30, 30 to <35, ≥35 kg/m^2), the CaD Trial randomization assignment (yes, no), serum retinol concentrations (quartiles), and season of blood draw (summer, winter). Other potential risk factors for lung cancer including recreational physical activity, use of postmenopausal hormonal replacement therapy, Hormonal Therapy Trials randomization assignments, and history of lung or respiratory tract diseases were not included in the final model because they did not change risk estimates. Unconditional logistic regression was used in the analyses for histological subtypes, i.e., NSCLC and adenocarcinoma, to maximize the sample

size. In sensitivity analyses, we evaluated whether preclinical cancer status affects serum 25(OH)D concentrations by excluding lung cancer cases diagnosed within 2 years after the baseline blood draw and their matched controls. Because African American women have lower serum 25(OH)D concentrations than women of other races in general [35], we also examined the association of serum 25(OH)D with lung cancer risk after excluding African Americans. Additionally, we adjusted second-hand smoke exposure for OS participants in whom this information was collected.

To evaluate the hypothesized effect modification, we stratified the association of serum 25(OH)D concentrations by (a) the CaD Trial intervention and (b) circulating vitamin A, i.e., serum concentrations of retinol and retinyl palmitate and the ratio of retinyl palmitate to retinol, because the serum retinyl palmitate/retinol ratio can indicate excess circulating vitamin A [27]. For the analysis of the CaD Trial, the intervention group was women who were randomized to the intervention arm of the trial. The no intervention group included women who were randomized to the placebo arm of the trial and those who did not participate in the trial; the average intake values of vitamin D from supplements at baseline were similar between these two groups (186 and 174 IU/d, respectively). We grouped participants as below or above the sufficient level (50 nmol/L) of serum 25(OH)D, instead of using a finer cutoff, because of the relatively small number of lung cancer cases that occurred in the intervention arm ($n = 31$). For evaluating the effect modification of serum vitamin A, we grouped participants using the quartiles of serum 25(OH)D concentrations and stratified by the median values of serum concentrations of retinol and retinyl palmitate and the retinyl palmitate/retinol ratio to maintain an approximately even number of participates between groups. The interaction was assessed using Wald tests (1 df) of the cross-product term between serum 25(OH)D categories and CaD Trial intervention or above/below the median of serum retinol and retinyl palmitate concentrations and the retinyl palmitate/retinol ratio. Statistical analyses were conducted using SAS 9.4 (Cary, NC).

Results

Among cases, non-small cell lung cancer (NSCLC) was the major histological subtype ($n = 191$; 72% among cases with known histology); within NSCLC, adenocarcinoma was the most common subtype ($n = 170$; 89% of NSCLC) (Supplementary Table 2). Cases and controls did not differ with regard to demographic and lifestyle characteristics, history of lung diseases and hormone use, or serum biomarkers (Table 1). Serum 25(OH)D concentrations were higher among women who had the blood draw in summer than among those who had the blood draw in winter, but there was no difference between the cases and controls. Serum 25(OH)D concentrations had a modest correlation with total vitamin D intake from diet and supplements ($r = 0.34$; Supplementary Table 3).

The unadjusted model showed that there was no association between serum 25(OH)D in quartiles and lung cancer, NSCLC, and adenocarcinoma risk (Table 2). After adjusting for the covariates, there remained no association between serum 25(OH)D and any lung cancer. Women in quartile (Q) 4 had adjusted ORs of 1.06 (95% CI 0.61–1.84) for all lung cancer, 0.94 (95% CI 0.52–1.69) for NSCLC, and 0.91 (95% CI 0.49–1.68) for adenocarcinoma, compared with those in Q1. In the secondary analysis, similar risk estimates were derived from season-specific quartiles: adjusted OR of Q4 versus Q1 = 1.06 (95% CI 0.60–1.85) for all lung cancer, 1.02 (95% CI 0.56–1.84) for NSCLC, and 1.00 (95% CI 0.54–1.85) for adenocarcinoma (data not shown). Also, there was no association after we excluded cases within 2 years after blood draw and their matched controls (adjusted OR 0.97, 95% CI 0.51–1.84, Q4 vs. Q1) or African Americans (adjusted OR 1.15, 95% CI 0.68–1.94, Q4 vs. Q1) (all lung cancer; data not shown). In the OS, additional adjustment of ever living and working with a smoker in adulthood did not change the risk estimates for lung cancer (without adjustment: OR 1.11, 95% CI 0.54–2.29; with adjustment: 1.16, 95% CI 0.55–2.46; both Q4 vs. Q1; data not shown). For the models with clinical cutoffs (Table 3), no association with lung cancer was observed for women with ≥75 nmol/L compared with those with <30 nmol/L of serum 25(OH)D concentrations (adjusted OR 0.76, 95% CI 0.31–1.84). Similar ORs were also observed for NSCLC (adjusted OR 0.71, 95% CI 0.27–1.86) and adenocarcinoma (adjusted OR 0.81, 95% CI 0.31–2.14) among women with high versus deficient levels of serum 25(OH)D.

Among women with serum 25(OH)D concentrations <50 nmol/L, the CaD Trial intervention versus no intervention was not associated with lung cancer risk (OR 0.99, 95% CI 0.52–1.90, Table 4). In women with serum 25(OH)D ≥ 50 nmol/L at baseline and receiving the CaD intervention, there was a trend for lower lung cancer risk (OR 0.42, 95% CI 0.16–1.14), compared with those with serum 25(OH)D < 50 nmol/L at baseline and no exposure to the intervention supplements (p interaction = 0.07). The ORs were 0.39 (95% CI 0.12–1.29; p interaction = 0.09) for NSCLC and 0.35 (95% CI 0.09–1.30; p interaction = 0.06) for adenocarcinoma.

There was no clear pattern of effect modification of serum retinol, retinyl palmitate, and the retinyl palmitate/retinol ratio on the association of serum 25(OH)D with the overall risk of lung cancer (Table 5). Among women with lower serum retinol palmitate/retinol ratio (below median), those with higher versus lower serum 25(OH)D tended to have a lower risk of NSCLC (OR 0.62, 95% CI 0.28–1.39 in Q4

Table 1 Baseline characteristics of lung cancer cases and controls in the WHI CTs and OS

Characteristic	Cases ($n=298$)	Controls ($n=298$)	p value[a]
Age, years (mean ± SD)	65.5 ± 7.1	65.6 ± 7.1	1.00
Region (%)			
Northeast	72 (24.2)	67 (22.5)	0.70
South	72 (24.2)	77 (25.8)	
Midwest	62 (20.8)	71 (23.8)	
West	92 (30.9)	83 (27.9)	
Race/ethnicity (%)			0.63
Black/African American	18 (6.0)	17 (5.7)	
Hispanic/Latino	7 (2.3)	10 (3.3)	
Non-Hispanic white	254 (85.3)	258 (86.6)	
Others	19 (6.4)	13 (4.4)	
Education (%)			0.63
High school or less	12 (4.0)	17 (5.7)	
School after high school	153 (51.3)	159 (53.4)	
College degree or higher	131 (44.0)	119 (39.9)	
Unknown	2 (0.7)	3 (1.0)	
Body mass index, kg/m^2 (mean ± SD)	27.1 ± 5.4	27.4 ± 5.4	0.61
Recreational physical activity[b], MET-h/week (mean ± SD)	13.4 ± 14.4	13.0 ± 12.4	0.74
Ever living with a smoker in adulthood[c] (%)	103 (58.5)	109 (61.9)	0.51
Ever working with a smoker in adulthood[c] (%)	123 (69.9)	113 (64.2)	0.26
Alcohol use (%)			0.67
Non-drinkers	50 (16.8)	56 (18.8)	
Past drinkers	44 (14.8)	51 (17.1)	
Current drinkers	202 (67.8)	190 (63.8)	
Oral contraceptive use (ever, %)	103 (34.6)	100 (33.6)	0.80
Use of hormone therapy (%)			0.44
Never	125 (41.9)	139 (46.6)	
Past	55 (18.5)	43 (14.4)	
Current, estrogen alone	71 (23.8)	75 (25.2)	
Current estrogen + progesterone	47 (15.8)	41 (13.8)	
Participated in Hormone Therapy Trial (%)	55 (18.5)	51 (17.1)	0.67
Participated in calcium/vitamin D Trial (%)	58 (19.5)	69 (23.2)	0.27
History of lung disease[d] (ever, %)	34 (11.4)	24 (8.1)	0.17
Serum 25(OH)D, nmol/L (mean ± SD)	47.6 ± 16.2	47.1 ± 17.8	0.68
[Median (IQR)]	47.4 (35.5–59.3)	45.3 (33.9–58.9)	
Blood draw in summer[e] (mean ± SD)	50.4 ± 16.3	48.0 ± 17.8	0.22
[Median (IQR)]	50.8 (37.9–63.3)	46.6 (34.6–59.6)	
Blood draw in winter[e] (mean ± SD)	44.8 ± 15.8	45.9 ± 17.8	0.57
[Median (IQR)]	43.1 (33.4–56.5)	43.9 (32.6–58.3)	
Serum retinol, μg/dL (mean ± SD)	67.6 ± 14.2	66.4 ± 15.4	0.37
[Median (IQR)]	65.8 (58.1–76.2)	64.4 (55.8–75.7)	
Serum retinyl palmitate, μg/dL (mean ± SD)	4.08 ± 3.05	3.92 ± 3.74	0.54
[Median (IQR)]	3.47 (2.48–4.65)	2.99 (2.29–4.30)	
Serum retinyl palmitate/retinol ratio, % (mean ± SD)	6.1 ± 4.6	5.9 ± 5.6	0.67
[Median (IQR)]	5.1 (2.8–7.2)	4.7 (3.7–6.2)	

IQR interquartile range, *SD* standard deviation

[a] *t* tests for continuous variables and Chi-square tests for categorical variables

[b] 9 cases and 11 controls had missing values

[c] Assessed only among women in the OS (176 case–control pairs)

[d] Ever diagnosed for asthma and/or emphysema

[e] Summer months were May, June, July, August, September, and October; winter months were November, December, January, February, March, and April

Table 2 Odds ratios for lung cancer risk according to the quartiles of serum 25(OH)D concentrations

Serum 25(OH)D	*n* (cases)	*n* (controls)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	*p* trend
All lung cancer[a]					
Quartile 1[d] (ref.)	64	73	1.00	1.00	0.85
Quartile 2	74	75	1.13 (0.40–1.81)	1.11 (0.65–1.88)	
Quartile 3	84	75	1.27 (0.81–2.00)	1.15 (0.69–1.90)	
Quartile 4	76	75	1.16 (0.72–1.87)	1.06 (0.61–1.84)	
Non-small cell lung cancer[b,c]					
Quartile 1 (ref.)	43	73	1.00	1.00	0.78
Quartile 2	49	75	1.11 (0.66–1.87)	1.07 (0.61–1.88)	
Quartile 3	54	75	1.22 (0.73–2.04)	1.05 (0.60–1.85)	
Quartile 4	45	75	1.02 (0.60–1.73)	0.94 (0.52–1.69)	
Adenocarcinoma[b]					
Quartile 1 (ref.)	39	73	1.00	1.00	0.81
Quartile 2	42	75	1.05 (0.61–1.80)	0.98 (0.55–1.76)	
Quartile 3	50	75	1.25 (0.74–2.12)	1.05 (0.58–1.88)	
Quartile 4	39	75	0.97 (0.56–1.68)	0.91 (0.49–1.68)	

N number of participants, *OR* odds ratio, *CI* confidence interval

[a]Odds ratios were estimated by conditional logistic regression models, adjusted for race/ethnicity, BMI, CaD Trial allocations, serum retinol concentrations, and season of blood draw

[b]Odds ratios were estimated by unconditional logistic regression, adjusted for the covariates and matching factors (age and CT/OS status)

[c]Non-small cell lung cancer included adenocarcinoma, squamous cell carcinoma, and large cell carcinoma

[d]Serum 25(OH)D concentration cutoffs were Q1: <33.9, Q2: 33.9 to <45.3, Q3: 45.3 to <58.9, Q4: ≥58.9 nmol/L

Table 3 Associations of serum 25(OH)D concentrations, categorized as predefined clinical cutoffs, with lung cancer risk

Serum 25(OH)D, nmol/L	*N* (cases)	*N* (controls)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	*p* trend
All lung cancer[a]					
<30 (ref.)	43	55	1.00	1.00	0.91
30 to <50	120	121	1.31 (0.82–2.09)	1.22 (0.72–2.05)	
50 to <75	121	102	1.57 (0.96–2.57)	1.34 (0.77–2.35)	
≥75	14	20	0.86 (0.38–1.90)	0.76 (0.31–1.84)	
Non-small cell lung cancer[b,c]					
<30 (ref.)	31	55	1.00	1.00	0.69
30 to <50	79	121	1.16 (0.69–1.96)	0.99 (0.56–1.76)	
50 to <75	72	102	1.25 (0.73–2.14)	1.01 (0.55–1.87)	
≥75	9	20	0.80 (0.32–1.97)	0.71 (0.27–1.86)	
Adenocarcinoma[b]					
<30 (ref.)	28	55	1.00	1.00	0.88
30 to <50	69	121	1.12 (0.65–1.93)	0.92 (0.51–1.67)	
50 to <75	64	102	1.23 (0.71–2.14)	0.99 (0.53–1.86)	
≥75	9	20	0.88 (0.36–2.19)	0.81 (0.31–2.14)	

N number of participants, *OR* odds ratio, *CI* confidence interval

[a]Odds ratios were estimated by conditional logistic regression models, adjusted for race/ethnicity, BMI, CaD Trial allocations, and serum retinol concentrations, and season of blood draw

[b]Odds ratios were estimated by unconditional logistic regression, adjusted for the covariates and matching factors (age and CT/OS status)

[c]Non-small cell lung cancer included adenocarcinoma, squamous cell carcinoma, and large cell carcinoma

Table 4 Effect modification of the calcium/vitamin D (CaD) Trial intervention on the association of serum 25(OH)D concentrations with lung cancer risk

Serum 25(OH)D		Calcium/vitamin D Trial[c]		
		No intervention	Intervention	*p* interaction
All lung cancer[a]				
<50 nmol/L	*N* (cases/controls)	139/152	24/24	
	OR (95% CI)	1.00 (referent)	0.99 (0.52–1.90)	0.07
≥50 nmol/L	*N* (cases/controls)	128/107	7/15	
	OR (95% CI)	1.22 (0.84–1.77)	0.42 (0.16–1.14)	
Non-small cell lung cancer[b]				
<50 nmol/L	*N* (cases/controls)	93/152	17/24	
	OR (95% CI)	1.00 (referent)	1.14 (0.54–2.39)	0.09
≥50 nmol/L	*N* (cases/controls)	77/107	4/15	
	OR (95% CI)	1.13 (0.74–1.72)	0.39 (0.12–1.29)	
Adenocarcinoma[b]				
<50 nmol/L	*N* (cases/controls)	82/152	15/24	
	OR (95% CI)	1.00 (referent)	1.20 (0.55–2.60)	0.06
≥50 nmol/L	*N* (cases/controls)	70/107	3/15	
	OR (95% CI)	1.20 (0.77–1.86)	0.35 (0.09–1.30)	

[a]Odds ratios were estimated by conditional logistic regression models, adjusted for race/ethnicity, BMI, serum retinol concentrations, and season of blood draw

[b]Odds ratios were estimated by unconditional logistic regression, adjusted for the covariates and matching factors (age and CT/OS status)

[c]The no intervention group included women who were randomly assigned to the placebo arm in the CaD Trial and those who did not participate in the trial. The intervention group included women who were randomly assigned to the calcium and vitamin D arm in the CaD Trial

vs. Q1 of 25(OH)D). The pattern was similar for adenocarcinoma. However, women with both higher serum 25(OH)D and high retinyl palmitate/retinol ratio tended to have a higher risk of lung cancer, compared with women with both low serum 25(OH)D concentrations (quartile 1) and low retinyl palmitate/retinol ratio (p interaction = 0.06 for both NSCLC and adenocarcinoma).

Discussion

In this sample of never-smoking postmenopausal women, the data did not support the hypothesis that higher versus lower serum 25(OH)D concentrations were associated with a lower risk of lung cancer. Our effect modification analysis for the CaD Trial intervention showed that a trend towards a lower yet non-significant risk of lung cancer was observed for women with higher serum 25(OH)D concentrations and receiving vitamin D supplementation, compared with those with lower serum 25(OH)D concentrations and not receiving the trial supplement. It is important to note that there were very few cases and controls with higher serum 25(OH)D concentrations at baseline and in the intervention arm so the finding must be interpreted with caution. The CaD supplementation increased serum 25(OH)D concentration on average from 45.5 nmol/L in the placebo arm to 60.8 nmol/L in the active intervention arm at 2 years post randomization [36]. However, the vitamin D supplement dose used in the trial (400 IU/d) might not have been high enough to elevate serum 25(OH)D concentrations to a level that can decrease cancer risk [37]. In a post hoc analysis with 7 years of follow-up, the intervention versus placebo was not associated with lung cancer incidence (HR 0.86, 95% CI 0.67–1.12) [38]. The potentially suboptimal dose for lung cancer prevention can explain the null association between the trial supplement and lung cancer risk in women with lower baseline serum 25(OH)D concentrations. A large randomized, double-blind, placebo-controlled trial is currently ongoing with a higher dose of vitamin D supplementation (2,000 IU/d) in the primary prevention of cancer and cardiovascular disease [39], and it will need several years to know the effect on lung cancer risk.

Our findings on high versus deficient levels of serum 25(OH)D are in the same direction as a prospective cohort study in the WHI reporting an inverse association of estimated dietary vitamin D exposure with lung cancer risk in never smokers [40]. The majority of lung cancer cases in these two studies were the same group of women. The dietary vitamin D study showed that a higher total vitamin D intake (food + supplements) starting from 600 IU/day was significantly associated with a lower risk of lung cancer among never smokers [HR = 0.55 (95% CI 0.31–0.96) for 600 to <800 (41 cases) vs. <100 IU/day; HR 0.37 (95% CI = 0.18–0.77) for ≥800 (14 cases) vs. <100 IU/day] [40].

Table 5 Effect modification of serum retinol, retinyl palmitate, and the retinyl palmitate/retinol ratio on the association of serum 25(OH)D concentrations with lung cancer risk

		Serum 25(OH)D concentrations				*p* interaction
		Q1	Q2	Q3	Q4	
All lung cancer[a]						
Serum retinol						
Below median (<66 μg/dL)	*N*, case/control	39/44	30/37	34/27	27/34	0.80
	OR (95% CI)	1.00 (referent)	0.92 (0.48–1.78)	1.44 (0.72–2.86)	0.98 (0.47–2.04)	
Above median (≥66 μg/dL)	*N*, case/control	25/29	44/38	50/48	49/41	
	OR (95% CI)	0.97 (0.46–2.02)	1.41 (0.70–2.82)	1.19 (0.65–2.20)	1.41 (0.72–2.76)	
Serum retinyl palmitate						
Below median (<3.0 μg/dL)	*N*, case/control	37/51	33/40	26/28	23/32	0.80
	OR (95% CI)	1.00 (referent)	1.11 (0.57–2.16)	1.25 (0.62–2.54)	1.03 (0.51–2.06)	
Above median (≥3.0 μg/dL)	*N*, case/control	27/22	41/35	58/47	53/43	
	OR (95% CI)	1.77 (0.83–3.75)	1.74 (0.91–3.36)	1.74 (0.96–3.17)	1.75 (0.92–3.35)	
Serum retinyl palmitate/retinol ratio						
Below median (<0.05)	*N*, case/control	39/47	38/41	37/39	31/37	0.81
	OR (95% CI)	1.00 (referent)	1.18 (0.61–2.29)	1.17 (0.61–2.24)	1.14 (0.59–2.21)	
Above median (≥0.05)	*N*, case/control	25/26	36/34	47/36	45/38	
	OR (95% CI)	1.22 (0.61–2.46)	1.35 (0.70–2.60)	1.64 (0.88–3.08)	1.51 (0.76–3.00)	
Non-small cell lung cancer[b]						
Serum retinol						
Below median (<66 μg/dL)	*N*, case/control	26/44	18/37	21/27	13/34	0.43
	OR (95% CI)	1.00 (referent)	0.84 (0.39–1.83)	1.23 (0.56–2.68)	0.68 (0.29–1.58)	
Above median (≥66 μg/dL)	*N*, case/control	17/29	31/38	33/48	32/41	
	OR (95% CI)	0.95 (0.43–2.11)	1.37 (0.67–2.81)	1.16 (0.57–2.34)	1.37 (0.66–2.82)	
Serum retinyl palmitate						
Below median (<3.0 μg/dL)	*N*, case/control	28/51	21/40	15/28	11/32	0.30
	OR (95% CI)	1.00 (referent)	0.94 (0.45–1.98)	0.95 (0.42–2.15)	0.66 (0.28–1.58)	
Above median (≥3.0 μg/dL)	*N*, case/control	15/22	28/35	39/47	34/43	
	OR (95% CI)	1.16 (0.50–2.71)	1.49 (0.73–3.05)	1.47 (0.75–2.91)	1.52 (0.76–3.07)	
Serum retinyl palmitate/retinol ratio						
Below median (<0.05)	*N*, case/control	30/47	26/41	23/39	14/37	0.06
	OR (95% CI)	1.00 (referent)	0.94 (0.46–1.91)	0.86 (0.41–1.78)	0.62 (0.28–1.39)	
Above median (≥0.05)	*N*, case/control	13/26	23/34	31/36	31/38	
	OR (95% CI)	0.69 (0.30–1.59)	1.07 (0.51–2.22)	1.29 (0.64–2.63)	1.30 (0.64–2.65)	
Adenocarcinoma[b]						
Serum retinol						
Below median (<66 μg/dL)	*N*, case/control	23/44	16/37	19/27	11/34	
	OR (95% CI)	1.00 (referent)	0.84 (0.36–1.82)	1.26 (0.56–2.82)	0.66 (0.27–1.61)	0.44
Above median (≥66 μg/dL)	*N*, case/control	16/29	27/38	31/48	28/41	
	OR (95% CI)	1.03 (0.46–2.32)	1.35 (0.64–2.08)	1.25 (0.61–2.57)	1.42 (0.67–3.01)	
Serum retinyl palmitate						
Below median (<3.0 μg/dL)	*N*, case/control	25/51	16/40	12/28	11/32	0.40
	OR (95% CI)	1.00 (referent)	0.77 (0.35–1.70)	0.80 (0.33–1.91)	0.73 (0.30–1.77)	
Above median (≥3.0 μg/dL)	*N*, case/control	14/22	26/35	38/47	28/43	
	OR (95% CI)	1.13 (0.47–2.70)	1.53 (0.74–3.19)	1.61 (0.80–3.22)	1.44 (0.69–2.99)	
Serum retinyl palmitate/retinol ratio						
Below median (<0.05)	*N*, case/control	27/47	22/41	20/39	13/37	0.06

Table 5 (continued)

		Serum 25(OH)D concentrations				*p* interaction
		Q1	Q2	Q3	Q4	
	OR (95% CI)	1.00 (referent)	0.85 (0.41–1.77)	0.79 (0.37–1.69)	0.63 (0.28–1.46)	
Above median (≥0.05)	*N*, case/control	12/26	20/34	30/36	26/38	
	OR (95% CI)	0.67 (0.28–1.58)	1.03 (0.48–2.20)	1.41 (0.68–2.90)	1.25 (0.60–2.62)	

[a]Odds ratios were estimated by conditional logistic regression, adjusted for matching factors (age and study status), race/ethnicity, BMI, CaD Trial allocation, and season of blood draw

[b]Odds ratios were estimated by unconditional logistic regression, adjusted for the covariates and matching factors (age and CT/OS status)

Significant inverse associations were also observed for both NSCLC and adenocarcinoma. In the current study, high 25(OH)D concentrations (≥75 nmol/L) were not associated with lower lung cancer risk (OR 0.76, 95% CI 0.31–1.84, compared with <30 nmol/L). Serum 25(OH)D concentrations at 75 nmol/L or higher have been suggested as the level that may lower cancer risk in general [33]. Given the smaller observed effect size in the serological study than the dietary vitamin D study, we may need a study with a larger number of lung cancer cases to observe a significant association for serum 25(OH)D in relation to lung cancer risk. The biological reasons are unknown for the weaker association of serum 25(OH)D concentrations compared to vitamin D intake with lung cancer risk. In studies examining dietary exposure, it is possible that other dietary factors that correlated with vitamin D intake contributed the lower risk of lung cancer. Also, serum 25(OH)D represents vitamin D exposure from both diet and cutaneous photosynthesis. Thus, serological studies examining serum 25(OH)D are important to confirm the association. Studies considering other components of the vitamin D pathway, such as genetic variants of enzymes, vitamin D protein carrier, and VDR in lung tissue, are needed to elucidate the association.

Although the NSCLC subtype, mainly adenocarcinoma, examined in this study was not significantly associated with serum 25(OH)D concentrations, it is important to consider histological subtypes of lung cancer while studying the risk associated with vitamin D. Vitamin D inhibits lung cancer signaling pathways including epidermal growth factor receptor mutations [41, 42], which are more related to NSCLC than small cell lung cancer [43]. Also, the expression of VDR is stronger in NSCLC, particularly adenocarcinoma [2], than in small cell lung cancer [44]. Epidemiological studies suggest that vitamin D exposure is more strongly associated with NSCLC than small cell lung cancer in smokers [12, 45] and with adenocarcinoma than other subtypes in never smokers [40]. In the U.S. and other parts of the world, the incidence of adenocarcinoma among women is increasing [46], an observation that cannot be entirely attributable to smoking behavior [47]. Research is warranted on low vitamin D intake and serum 25(OH)D concentrations as risk factors for adenocarcinoma.

We observed a trend towards effect modification for serum retinol palmitate/retinol ratio on the association of serum 25(OH)D with NSCLC and adenocarcinoma in the study population. Retinol is crucial to the function of VDR–RXR heterodimer complex by supplying 9-*cis*-retinoic acid to RXR. However, excess retinol may interrupt the function of VDR–RXR heterodimer by forming RXR–RXR homodimer [6], although it is unclear what levels of vitamin A metabolites would promote the biological change. In the NHANES III, serum retinyl ester concentrations ≥7.0 μg/dL or the retinyl esters/retinol ratio ≥0.08, the level exceeding the capacity of liver storage and potentially causing toxicity [27], were shown to attenuate the inverse association of serum 25(OH)D with lung cancer mortality [10]. In the WHI, a similar pattern was also observed for a high level of dietary and supplemental vitamin A intake [40]. However, in part due to older age, fewer participants in WHI had an excess level of circulating vitamin A than in the NHANES III (15 vs. 43% with serum retinyl esters/retinol ratio ≥0.08). Thus, we were unable to use this more biologically meaningful cutoff than the median level to examine the effect modification. The level above which retinol and its metabolites can hinder the function of vitamin D and influence lung cancer risk remains inconclusive and may vary in different populations according to their vitamin A status and other characteristics such as aging.

This study has several strengths. The analysis was conducted in the largest sample, to date, of female never smokers with data on serum 25(OH)D. We used a prospective design so that the bias of reverse causality was unlikely. Study physicians blinded to study components adjudicated the incidence and histology of lung cancer. However, several limitations should be noted. First, we were unable to address potential effects of the long-term systematic or random variation of the biomarkers because they were measured at WHI baseline. In the WHI, serum samples collected 5 years apart exhibit a modest within-person correlation for 25(OH)D ($r = 0.61$) [48], suggesting that one-point blood measurements moderately represent

long-term serum 25(OH)D concentrations. Second, we were unable to adjust for second-hand smoke exposure in all participants because the data were only collected in the OS participants, although the adjustment was unlikely to materially change the study findings, as shown in the sensitivity analysis. Third, in the CaD Trial, the distinction between women assigned to intervention versus placebo may have been reduced, as the trial allowed participants to take personal vitamin D supplement up to 1,000 IU/d. Also, in the nonintervention group, some women were ineligible for the CaD Trial because they were taking higher doses, i.e., >600 IU/d, of vitamin D supplements. These potential issues of misclassification may have attenuated our results in the effect modification analysis of the CaD Trial. Lastly, our effect modification analyses should be interpreted as an exploratory work because of the reduced sample size after stratification.

In conclusion, there was no clear association between serum 25(OH)D concentrations and lung cancer risk in a sample of never-smoking postmenopausal women. Future studies should examine the effects of higher serum 25(OH)D concentrations (≥75 nmol/L) on adenocarcinoma in never-smoking women.

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Compliance with ethical standards

Conflict of interest There are no conflicts of interest to disclose.

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