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Original Contribution

Circulating 25-Hydroxyvitamin D and Risk of Epithelial Ovarian Cancer

Cohort Consortium Vitamin D Pooling Project of Rarer Cancers

Wei Zheng*, Kim N. Danforth, Shelley S. Tworoger, Marc T. Goodman, Alan A. Arslan, Alpa V. Patel, Marjorie L. McCullough, Stephanie J. Weinstein, Laurence N. Kolonel, Mark P. Purdue, Xiao-Ou Shu, Kirk Snyder, Emily Steplowski, Kala Visvanathan, Kai Yu, Anne Zeleniuch-Jacquotte, Yu-Tang Gao, Susan E. Hankinson, Chinonye Harvey, Richard B. Hayes, Brian E. Henderson, Ronald L. Horst, and Kathy J. Helzlsouer

* Correspondence to Dr. Wei Zheng, Vanderbilt Epidemiology Center, Vanderbilt University Medical Center, 2525 West End Avenue, 8th Floor, Nashville, TN 37203 (e-mail: wei.zheng@vanderbilt.edu).

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A role for vitamin D in ovarian cancer etiology is supported by ecologic studies of sunlight exposure, experimental mechanism studies, and some studies of dietary vitamin D intake and genetic polymorphisms in the vitamin D receptor. However, few studies have examined the association of circulating 25-hydroxyvitamin D (25(OH)D), an integrated measure of vitamin D status, with ovarian cancer risk. A nested case-control study was conducted among 7 prospective studies to evaluate the circulating 25(OH)D concentration in relation to epithelial ovarian cancer risk. Logistic regression models were used to estimate odds ratios and 95% confidence intervals among 516 cases and 770 matched controls. Compared with 25(OH)D concentrations of 50–<75 nmol/L, no statistically significant associations were observed for <37.5 (odds ratio (OR) = 1.21, 95% confidence interval (CI): 0.87, 1.70), 37.5–<50 (OR = 1.03, 95% CI: 0.75, 1.41), or ≥75 (OR = 1.11, 95% CI: 0.79, 1.55) nmol/L. Analyses stratified by tumor subtype, age, body mass index, and other variables were generally null but suggested an inverse association between 25(OH)D and ovarian cancer risk among women with a body mass index of ≥25 kg/m^2 ($P_{interaction}$ < 0.01). In conclusion, this large pooled analysis did not support an overall association between circulating 25(OH)D and ovarian cancer risk, except possibly among overweight women.

case-control studies; cohort studies; ovarian neoplasms; prospective studies; vitamin D

Abbreviations: CI, confidence interval; ICD-O, *International Classification of Diseases for Oncology*; 25(OH)D, 25-hydroxyvitamin D; 1,25(OH)$_2$D, 1,25-dihydroxyvitamin D; OR, odds ratio; VDPP, Cohort Consortium Vitamin D Pooling Project of Rarer Cancers.

The pathogenesis of ovarian cancer, one of the most common gynecologic malignancies, is poorly understood, although hormonal factors, inflammation, and wound healing are thought to play an important role in its etiology (1). Tubal ligation, parity, and oral contraceptive use are associated with a reduced risk of ovarian cancer, while postmenopausal hormone use and a family history of breast and/or ovarian cancer are associated with an increased risk (2). Experimental studies suggest that vitamin D may influence ovarian carcinogenesis (3–9) through growth inhibition or induction of apoptosis (3, 7, 10–18). Evidence from ecologic studies of ultraviolet B, which initiates vitamin D production in the skin, and ovarian cancer mortality also supports a role of vitamin D in ovarian carcinogenesis (19–23). Results from studies of dietary vitamin D intake and ovarian cancer risk have been inconsistent but provide some support for an association (24–28). These studies, however, were limited as sunlight exposure, the principal source of vitamin D in humans, often was not considered. Because blood vitamin D concentrations are influenced by both dietary and nondietary factors, they provide an integrated measure of internal vitamin D exposure.

One prospective pooled study combining 3 cohorts (224 cases and 603 controls) examined plasma concentrations of 25-hydroxyvitamin D (25(OH)D) (29), a measure of overall vitamin D status, and 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), the most biologically active form of vitamin D, in relation to ovarian cancer risk. Overall, no statistically significant associations were observed across fourths of 25(OH)D or $1,25(OH)_2D$ and ovarian cancer risk. However, an inverse association of 25(OH)D with risk was noted among overweight women ($P_{trend} = 0.04$). Further, having higher ($\geq$32 ng/mL or approximately $\geq$80 nmol/L) versus lower ($<$32 ng/mL) 25(OH)D levels had a borderline inverse association with risk of serous tumors (relative risk = 0.64, 95% confidence interval (CI): 0.39, 1.05).

Overall, null associations between serum or plasma 25(OH)D and ovarian cancer risk were also observed in 2 other nested case-control studies, which included 201 (30) and 170 (31) cases of ovarian cancer. However, additional evidence that vitamin D may play a role in ovarian carcinogenesis comes from genetic association studies, which have observed associations between polymorphisms in the vitamin D receptor (*VDR*) and ovarian cancer risk (32–34).

Because ovarian cancer is relatively rare, it is necessary to pool cases and controls from multiple prospective studies to obtain a large enough sample size to carefully assess the association between circulating vitamin D concentrations and risk of ovarian cancer. The association between circulating vitamin D and ovarian cancer was examined in a nested case-control study combining cases from 7 cohort studies, with over 500 cases from diverse geographic locations.

MATERIALS AND METHODS

Study design and population

A detailed description of the study design and cohorts included in the Cohort Consortium Vitamin D Pooling Project of Rarer Cancers (VDPP) is provided elsewhere in this issue (35). Seven cohorts were included in the VDPP ovarian cancer study: the CLUE Study (CLUE), the Cancer Prevention Study II Nutrition Cohort (CPS-II), the Multiethnic Cohort Study (MEC), the Nurses' Health Study, the New York University Women's Health Study (NYU-WHS), the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO), and the Shanghai Women's Health Study (SWHS). Cases were female cohort members diagnosed with primary ovarian cancer (*International Classification of Diseases for Oncology* (ICD-O) code 56.9) after blood collection. Analyses were restricted to epithelial ovarian cases; 14 cases with missing histology were included because approximately 90% of malignant ovarian tumors are surface epithelial-stromal tumors (36). With the exception of the Nurses' Health Study, histologic subtypes were classified as follows: serous (ICD-O codes 8441, 8442, 8460, 8461, 8462); endometrioid (ICD-O codes 8380, 8381, 8560, 8570); mucinous (ICD-O codes 8470, 8471, 8472, 8473, 8480, 8481, 8490); clear cell (ICD-O codes 8310 and 8313); and other epithelial (ICD-O codes 8010, 8020, 8050, 8060, 8140, 8260, 8323, 8440, 8450, 9000; missing histology codes). Histological subtype classification in the Nurses' Health Study was conducted by a gynecologic pathologist on the basis of the review of surgical and pathology reports.

Within each cohort, controls were matched to cases by using the incidence-density method. Controls were selected from women with at least 1 intact ovary and no history of cancer (except for nonmelanoma skin cancer or in situ cervical cancer) at the time of case diagnosis and matched individually to cases at a 1:1 ratio on age at blood collection ($\pm$1 year), race/ethnicity (white/black/Asian/other), and date of blood draw ($\pm$30 days), with the exception of Nurses' Health Study participants who were selected prior to commencement of the VDPP. In the Nurses' Health Study, 3 controls were matched to each case on age ($\pm$1 year), month of blood collection ($\pm$1 month), time of day of blood draw ($\pm$2 hours), fasting status, menopausal status, and postmenopausal hormone use at blood draw; although race/ethnicity was not a matching factor, over 99% of Nurses' Health Study participants were white.

Of the initially identified 546 cases and 808 controls, 30 cases and 38 matched controls were excluded because the cases were later found to have nonepithelial or mixed ovarian tumors. The final analysis included 516 cases and 770 controls, with the number of cases in individual cohorts ranging from 18 to 127 (Table 1).

Measurement of circulating 25(OH)D

A direct, competitive chemiluminescence immunoassay using the DiaSorin LIAISON 25 OH Vitamin D TOTAL Assay (37) was used to measure 25(OH)D in 125 μL of serum or plasma. Samples were assayed at Heartland Assays, Inc., except for Nurses' Health Study samples, which had been assayed previously in the laboratory of Dr. Bruce Hollis using the same method as above (29). Quality control samples, which comprised 5% of the total sample number within each cohort set (10% for the Nurses' Health Study), came from 2 sources. First, each cohort provided masked quality control samples for the batch(es) containing its participants (more details are provided elsewhere (29, 35)). Additionally, 2 samples of ''level 1'' (~60 nmol/L) or ''level 2'' (~35 nmol/L) vitamin D standard, obtained from the National Institute of Standards and Technology (NIST), were included in each batch of 100 samples, except the Nurses' Health Study batches. As described by Gallicchio et al. (35), the intrabatch and interbatch coefficients of variation were 9.3% and 12.7%, respectively, for NIST level 1 samples and 11.0% and 13.6%, respectively, for NIST level 2 samples. For all cohorts except the Nurses' Health Study, the median intrabatch coefficient of variation was 9.9% (range: 3.8%–16.4%), and the median interbatch coefficient of variation was 13.2% (range: 4.8%–17.0%). For the Nurses' Health Study, the intra- and interbatch coefficients of variation were 6.9% and 9.3%, respectively.

Statistical analyses

Demographic characteristics and major risk factors were compared between cases and controls using the Wald test, obtained from conditional logistic regression models that excluded women with missing data on the characteristic being compared. Associations between 25(OH)D and

Table 1. Characteristics of Participants, by Cohort, in the Investigation of Ovarian Cancer Within the Cohort Consortium Vitamin D Pooling Project of Rarer Cancers

Cohort	No. of Cases	No. of Controls	Time From Blood Collection to Cancer Diagnosis, median years (interquartile range)	Circulating 25(OH)D, median nmol/L (interquartile range)	
				Cases	Controls
CLUE	102	102	9.0 (4.9–13.4)	58.9 (42.7–69.7)	58.4 (45.9–70.4)
CPS-II[a]	27	27	2.2 (0.7–3.5)	55.7 (42.9–78.9)	53.9 (42.1–65.3)
MEC	18	18	2.2 (1.3–3.2)	47.6 (31.5–59.0)	48.3 (29.7–60.5)
NHS	127	381	7.3 (3.3–10.6)	65.5 (49.0–79.8)	65.8 (51.3–80.8)
NYU-WHS	94	94	10.4 (5.7–14.9)	48.0 (34.5–62.1)	47.0 (32.8–66.4)
PLCO	74	74	2.6 (1.0–5.1)	53.6 (40.0–73.3)	50.9 (40.9–60.7)
SWHS	74	74	4.3 (2.5–6.3)	36.8 (25.6–48.5)	38.5 (29.5–53.1)
Total	516	770	5.9 (2.7–10.2)	53.2 (38.9–68.7)	57.0 (43.3–72.5)

Abbreviations: CPS-II, Cancer Prevention Study II Nutrition Cohort; MEC, Multiethnic Cohort Study; NHS, Nurses' Health Study; NYU-WHS, New York University Women's Health Study; 25(OH)D, 25-hydroxyvitamin D; PLCO, Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial; SWHS, Shanghai Women's Health Study.

[a] Three CPS-II participants were missing time from blood draw to diagnosis.

ovarian cancer were evaluated by estimating odds ratios and 95% confidence intervals from conditional logistic regression models (SAS, versions 9.1.3 and 9.2; SAS Institute, Inc., Cary, North Carolina); all reported *P* values were 2 sided. Models were adjusted for duration of oral contraceptive use and number of pregnancies. Additional adjustment for other major ovarian cancer risk factors, including family history of ovarian cancer, postmenopausal hormone use, age at menarche, age at menopause, body mass index, history of diabetes, cigarette smoking, alcohol consumption, physical activity, and education, did not appreciably change the results. Tubal ligation was not adjusted for in the models because information for this variable was available only from a few cohorts. However, tubal ligation is unlikely to be correlated with circulating 25(OH)D levels and therefore is unlikely to be a confounder in our study.

Circulating 25(OH)D was classified into 4 a priori categories based on clinically relevant cutpoints for the main analyses: <37.5, 37.5–<50, 50–<75, and ≥75 nmol/L. The referent group was 50–<75 nmol/L, because this group includes the mean level of the US population (62.91±0.81 nmol/L for men and 61.54±0.85 nmol/L for women), based on 2000–2004 National Health and Nutrition Examination Survey data (38). Trend tests were run by using an ordinal variable assigning the four 25(OH)D groups to values of 1–4, respectively. Additionally, the final models for all cases and serous tumors were analyzed by using finer vitamin D categories (<25, 25–<37.5, 37.5–<50, 50–<75, 75–<100, and ≥100 nmol/L) for consistency with the VDPP analyses for other cancer sites.

Analyses also were performed by using 25(OH)D categories constructed according to cohort- and season-specific quartiles among controls. In addition, as described in Gallicchio et al. (35), the residual method was used to carefully adjust for season, and season-adjusted residual data were then cut into cohort-specific quartiles. Results from these analyses were similar to those using the clinically defined categories and, therefore, are not presented. To assess the potential influence of preclinical disease on the observed association, we performed analyses that excluded the cases that occurred during either the first 2 or 5 years of follow-up.

Stratified analyses were performed by tumor subtype, age at blood draw, season of blood draw, race/ethnicity, body mass index, and oral contraceptive use to evaluate potential interactions. Odds ratios and 95% confidence intervals were estimated by unconditional logistic regression models, adjusting for the 4 common matching factors (age, race/ethnicity, date of blood draw, study cohort), duration of oral contraceptive use, and number of pregnancies. The stratification variable was included as a covariate in these models, except that the duration of oral contraceptive use was included as a covariate in analyses of ever oral contraceptive users. Interaction tests were conducted by including interaction terms of the 25(OH)D variable (created using the median value among controls for each category) with stratification level indicator variables (one for each level) in the multiple regression model (35). The log-likelihood test was used to compare models with and without the interaction terms.

A meta-analysis approach was used to combine data across cohorts (39). Data from the Women's Health Study (WHS), which had been included in a prior study with the Nurses' Health Study (29), were included in the meta-analyses. For each cohort, the odds ratios and 95% confidence intervals were estimated for the bottom (<37.5 nmol/L) and top (≥75 nmol/L) 25(OH)D categories compared with the reference (50–<75 nmol/L) category. Pooled odds ratios and 95% confidence intervals were obtained using inverse-variance weighted random-effects models. Heterogeneity across studies was evaluated. Sensitivity analyses were conducted by excluding one cohort at a time to evaluate the impact of each study on the overall results.

RESULTS

The median interval between blood draw and cancer diagnosis was 5.9 years, ranging from 2.2 to 10.4 years across

Table 2. Selected Characteristics of Case and Control Subjects in the Investigation of Ovarian Cancer Within the Cohort Consortium Vitamin D Pooling Project of Rarer Cancers

Characteristics	Cases (N = 516)			Controls (N = 770)			P Value[a]
	No.	%	Median (Interquartile Range)	No.	%	Median (Interquartile Range)	
Age at blood draw, years[b]			58.0 (50.5–65.0)			57.0 (51.0–64.0)	Matched
Season of blood draw							Matched
Winter (December–May)	184	35.7		300	39.0		
Summer (June–November)	332	64.3		470	61.0		
Race							Matched[c]
White	399	77.3		644	83.6		
Black	11	2.1		13	1.7		
Asian	82	15.9		83	10.8		
Other	12	2.3		11	1.4		
Missing	12	2.3		19	2.5		
Body mass index, kg/m^2							0.54
<25	248	48.1		400	52.0		
25–<30	141	27.3		222	28.8		
≥30	76	14.7		101	13.1		
Missing	51	9.9		47	6.1		
Age at menarche, years[b]			13 (12–14)			13 (12–14)	0.19
Age at menopause, years[b]			49 (44–52)			50 (45–52)	0.01
Ever had full-term pregnancy							<0.01
No	62	12.0		51	6.6		
Yes	365	70.7		632	82.1		
Missing	89	17.2		87	11.3		
No. of full-term pregnancies among parous women[b]			2 (2–3)			3 (2–4)	<0.001
Ever used oral contraceptive							0.82
No	303	58.7		424	55.1		
Yes	157	30.4		275	35.7		
Missing	56	10.9		17	9.2		
Duration of oral contraceptive use among oral contraceptive users, years[b]			2.8 (0.5–7.0)			3.0 (1.0–7.5)	<0.01
Ever used hormone therapy							0.14
No	133	25.8		148	19.2		
Yes	151	29.3		234	30.4		
Missing	232	45.0		388	50.4		
Smoking status							0.99
Never	296	57.4		408	53.0		
Former	147	28.4		248	32.2		
Current	65	12.6		92	11.9		
Missing	8	1.6		22	2.9		
Family history of ovarian cancer							<0.001
No	351	68.0		573	74.4		
Yes	20	3.9		7	0.9		
Missing	145	28.1		190	24.7		
Total vitamin D intake, IU/day[b]			310.7 (154.2–700.2)			320.2 (171.7–640.3)	0.48
Total calcium intake, mg/day[b]			1,004.5 (617.0–1,678.0)			1,074.0 (668.6–1,649.0)	0.91
Total intake of dairy products, g/day[b]			183.8 (66.8–301.4)			219.8 (83.5–369.8)	0.52

[a] P values were derived from the Wald statistic, generated by using conditional logistic regression models among women with no missing data on the characteristic being compared. Cases and controls were matched on age (±1 year), race/ethnicity (white, black, Asian, other), date of blood draw (±30 days), and study cohort, except for the Nurses' Health Study, which was matched on age (±1 year), month of blood collection (±1 month), time of day of blood draw (±2 hours), fasting status, menopausal status, and postmenopausal hormone use at blood draw.

[b] Median (25th–75th percentiles).

[c] Nurses' Health Study participants were not matched on race, although over 99% of included participants were white.

participating cohorts (Table 1). The median concentrations of circulating 25(OH)D differed considerably across study cohorts, ranging from 38.5 to 65.8 nmol/L among controls, with the lowest concentration observed in the Shanghai Women's Health Study, the only cohort that included exclusively Asian women. Other cohorts, including primarily European Americans, generally had a median concentration of 25(OH)D of approximately 50 nmol/L or higher.

Cases and controls were similar in age, race, and season of blood draw because of the matched study design (Table 2). The median age at blood draw in the study population was 58 years for cases and 57 for controls. The majority of study participants were white, and in 64% of cases and 61% of controls blood samples were obtained during the summer months. Other characteristics, including education, body mass index, postmenopausal hormone use, and smoking, generally were similar between cases and controls. However, cases were younger at menopause and had fewer full-term pregnancies compared with controls. Cases also were slightly less likely to use oral contraceptives and, among users, had used oral contraceptives for shorter durations. Additionally, more cases than controls had a family history of ovarian cancer among first-degree relatives.

Overall, there was no statistically significant association between circulating 25(OH)D and ovarian cancer risk. Compared with women with 25(OH)D concentrations of 50–<75 nmol/L, the odds ratios were 1.21 (95% CI: 0.87, 1.70) among women with <37.5 nmol/L, 1.03 (95% CI: 0.75, 1.41) for women with 37.5–<50 nmol/L, and 1.11 (95% CI: 0.79, 1.55) for women with ≥75 nmol/L (P_{trend} = 0.56). Results with expanded cutpoints are shown in Table 3. Results remained similar when each cohort was dropped in turn from the analysis in sensitivity analyses (data not shown) and in analyses excluding the first 2 or 5 years of follow-up. Results were unchanged when only serous tumors were included (P_{trend} = 0.54). The sample sizes for endometrioid (n = 50 cases) and mucinous (n = 47 cases) tumors were too small for reliable analyses.

To evaluate potential heterogeneity in associations, analyses were stratified by a number of factors, including age at blood draw, season of blood draw, race/ethnicity, body mass index, and oral contraceptive use (Table 4). In some instances, there were too few women in a stratum to reliably evaluate associations (e.g., winter blood draw and nonwhites), and therefore results from those strata have not been presented. Overall, findings were consistent with the main analyses and did not support a relation between circulating vitamin D concentrations and ovarian cancer risk. However, analyses among overweight women suggested an inverse association between circulating 25(OH)D and ovarian cancer risk (P_{trend} = 0.01). Among women with a body mass index ≥25 kg/m^2, ovarian cancer risk was nonsignificantly increased among women with <37.5 nmol/L (odds ratio (OR) = 1.53, 95% CI: 0.92, 2.55) and nonsignificantly decreased among women with ≥75 nmol/L (OR = 0.70, 95% CI: 0.36, 1.35), compared with women whose 25(OH)D concentrations were between 50 and <75 nmol/L. No statistically significant association, however, was observed among women with a body mass index below 25. The interaction test for body mass index and 25(OH)D was

Table 3. Odds Ratios and 95% Confidence Intervals for the Association Between Circulating 25(OH)D and Risk of Ovarian Cancer Within the Cohort Consortium Vitamin D Pooling Project of Rare Cancers, Overall and Serous Tumors

	Circulating 25(OH)D, nmol/L																				P_{trend}
	<25			25–<37.5			37.5–<50			50–<75			75–<100			≥100				
	No. of Cases	No. of Controls	OR	95% CI	No. of Cases	No. of Controls	OR	95% CI	No. of Cases	No. of Controls	OR	95% CI	No. of Cases	No. of Controls	OR	95% CI	No. of Cases	No. of Controls	OR	95% CI	
All tumors	38	43			80	83			113	154			190	320			74	134			21	36			
Crude[a]			1.12	0.67, 1.86			1.27	0.88, 1.83			1.03	0.76, 1.41			1.00	Referent			1.10	0.77, 1.57			1.18	0.66, 2.13	0.66
Multivariate adjusted[b]			1.08	0.64, 1.81			1.27	0.88, 1.85			1.03	0.75, 1.40			1.00	Referent			1.10	0.77, 1.59			1.11	0.61, 2.05	0.65
Serous subtype	18	24			43	47			56	77			95	174			41	75			9	23			
Crude[a]			0.93	0.46, 1.89			1.30	0.78, 2.14			1.15	0.75, 1.78			1.00	Referent			1.17	0.72, 1.89			0.86	0.37, 1.99	0.66
Multivariate adjusted[b]			0.96	0.46, 1.98			1.44	0.86, 2.41			1.15	0.74, 1.79			1.00	Referent			1.23	0.75, 2.01			0.97	0.41, 2.28	0.64

Abbreviations: CI, confidence interval; 25(OH)D, 25-hydroxyvitamin D; OR, odds ratio.

[a] Derived from conditional logistic regression models. Cases and controls were matched on age (±1 year), race/ethnicity (white, black, Asian, other), date of blood draw (±30 days), and study cohort, except for the Nurses' Health Study, which was matched on age (±1 year), month of blood collection (±1 month), time of day of blood draw (±2 hours), fasting status, menopausal status, and postmenopausal hormone use at blood draw.

[b] Derived from conditional logistic regression models adjusted for duration of oral contraceptive use and number of pregnancies.

Table 4. Odds Ratios and 95% Confidence Intervals for the Association Between Circulating 25(OH)D and Ovarian Cancer Risk From Stratified Analyses in the Cohort Consortium Vitamin D Pooling Project of Rarer Cancers

Stratification Factor	Circulating 25(OH)D, nmol/L																P_{trend}
	<37.5				37.5–<50				50–<75				≥75				
	No. of Cases	No. of Controls	OR	95% CI	No. of Cases	No. of Controls	OR	95% CI	No. of Cases	No. of Controls	OR	95% CI	No. of Cases	No. of Controls	OR	95% CI	
Age at blood draw																	
<50 years	30	29			18	31			48	62			25	35			
Multivariate adjusted[a]			0.85	0.40, 1.84			0.52	0.24, 1.09			1.00	Referent			0.91	0.46, 1.80	0.55
≥50 years	88	97			95	123			142	258			70	135			
Multivariate adjusted[a]			1.34	0.91, 1.98			1.20	0.84, 1.71			1.00	Referent			1.15	0.79, 1.68	0.31
Blood draw in summer	60	60			70	83			133	212			69	115			
Multivariate adjusted[a]			1.25	0.80, 1.97			1.10	0.73, 1.65			1.00	Referent			1.13	0.76, 1.68	0.63
Race/ethnicity, white	62	72			85	124			161	284			91	164			
Multivariate adjusted[a]			1.18	0.78, 1.79			0.99	0.70, 1.42			1.00	Referent			1.09	0.77, 1.52	0.83
Body mass index																	
<25 kg/m^2	44	53			42	77			94	162			68	108			
Multivariate adjusted[a]			1.09	0.63, 1.88			0.72	0.44, 1.18			1.00	Referent			1.21	0.79, 1.86	0.37
≥25 kg/m^2	67	64			61	69			72	136			17	54			
Multivariate adjusted[a]			1.53	0.92, 2.55			1.45	0.90, 2.35			1.00	Referent			0.70	0.36, 1.35	0.01
Oral contraceptive use																	
Never	81	76			72	88			100	167			50	93			
Multivariate adjusted[a]			1.40	0.89, 2.20			1.18	0.77, 1.80			1.00	Referent			1.08	0.69, 1.70	0.24
Ever	26	33			28	53			67	121			36	68			
Multivariate adjusted[a]			1.15	0.59, 2.26			0.71	0.39, 1.28			1.00	Referent			1.01	0.58, 1.75	0.87

Abbreviations: CI, confidence interval; 25(OH)D, 25-hydroxyvitamin D; OR, odds ratio.

[a] Derived from unconditional logistic regression models adjusting for matching variables (age, race/ethnicity, date of blood draw, and study cohort), duration of oral contraceptive use, and number of pregnancies. Age at blood draw and season of blood draw were not adjusted for in models stratified on these variables.

statistically significant under the multiplicative model ($P_{interaction} < 0.01$).

In meta-analyses that included data from the Women's Health Study and the 7 cohort studies in the VDPP project, results remained null for overall risk (Figure 1). There was no evidence of heterogeneity across studies for either the <37.5 vs. 50–<75 nmol/L or ≥75 vs. 50–<75 nmol/L analyses ($P_{heterogeneity} = 0.83$ and 0.71, respectively), although individual point estimates did vary, likely in part because of small sample sizes among some cohorts. Results were similar to those from pooled analyses presented in Table 3, indicating no apparent association between circulating 25(OH)D concentration and ovarian cancer risk.

DISCUSSION

In this large, pooled analysis of 7 prospective cohort studies, circulating 25(OH)D concentrations were not associated with ovarian cancer risk overall or in analyses stratified by tumor subtype, age at blood draw, or oral contraceptive use. However, stratified analyses by body mass index suggested a possible inverse association between circulating vitamin D and ovarian cancer risk among overweight and obese women.

Despite the biologic plausibility, results from this pooled analysis did not support an association between circulating 25(OH)D and ovarian cancer risk overall. These results are consistent with those from other studies to date (29–31). In a pooled analysis of the Nurses' Health Study, the Nurses' Health Study II, and the Women's Health Study by Tworoger et al. (29), fourths of 25(OH)D or $1,25(OH)_2D$ were not significantly associated with ovarian cancer risk overall. Both our study and the study by Tworoger et al. (29) included the same Nurses' Health Study participants (about 25% of the cases in the current study). However, excluding Nurses' Health Study cases and controls from our study did not materially alter the results obtained from all studies combined. Similarly, overall null associations were observed between serum 25(OH)D and ovarian cancer risk in a nested case-control study in the Finnish Maternity Cohort (30). Serum or plasma 25(OH)D was not associated with ovarian cancer risk in a case-control study nested within the New York University Women's Health Study and the Northern Sweden Health and Disease Study, nor was there evidence of an interaction with vitamin D receptor polymorphisms or haplotypes (31). Excluding subjects (18% of cases in the current pooled analysis) did not alter the results reported in this paper.

Among women with a body mass index of ≥25 kg/m^2, Tworoger et al. (29) observed a significant inverse association between 25(OH)D quartiles and ovarian cancer risk ($P_{trend} = 0.04$). A similar inverse association was found in this study ($P = 0.01$). In a sensitivity analysis excluding the Nurses' Health Study participants, who were also included in the study by Tworoger et al. (29), our results became statistically nonsignificant ($P_{trend} = 0.16$) but the point estimates remained similar. Thus, data from these 2 studies suggest that vitamin D concentrations may be inversely associated with ovarian cancer risk among overweight and obese individuals.

Although body mass index is not a clear risk factor for ovarian cancer (40), some data suggest adiposity is inversely associated with circulating 25(OH)D concentrations, likely because vitamin D is fat soluble (41–44). In rats fed vitamin D supplements, adipose tissue concentrations of vitamin D increased significantly; interestingly, the study reported that vitamin D was released from adipose tissue, particularly during fasting. This suggests that adipose tissue may be an important factor in determining long-term vitamin D status (45). Thus, it is possible that women with a higher body mass index may have more bioavailable vitamin D at the tissue level or that circulating concentrations may better reflect long-term tissue exposure in this subpopulation. More research is needed to elucidate this potential relation.

The median level of 25(OH)D was the lowest among participants from the Shanghai Women's Health Study. Excluding women from this study, however, did not materially affect the study results. Results stratified by age at blood draw and oral contraceptive use suggested no statistically significant interaction of these factors on the association of circulating vitamin D with ovarian cancer risk. Further, no associations were observed for specific histologic subtypes, although power was limited for some subtype analyses.

Strengths of the current study included a large sample size, the use of prediagnostic blood samples, and a wide exposure range due to inclusion of studies in different geographic locations. Additionally, information was available on numerous potential demographic and lifestyle factors, allowing evaluation of potential confounding effects by established ovarian cancer risk factors as well as potential effect modification.

One limitation of a pooled analysis is that data were collected using different methods across studies. Because of the different study methods, harmonization of data was a challenge, and information on some potential confounders, such as tubal ligation, was not collected in all studies and therefore not included in analyses. However, information was available on most established risk factors for ovarian cancer, and it is unlikely that factors such as tubal ligation are correlated with circulating vitamin D levels.

Even with a relatively large number of cases, sample sizes for some subanalyses were small, such as those stratified by tumor subtype and other ovarian cancer risk factors.

Only one blood measurement per person was taken, and within-person variation in circulating 25(OH)D could have obscured a true association. However, the correlation over 3 years is around 0.70 for 25(OH)D (46), and unpublished results from 2 studies in our analysis (New York University Women's Health Study and Nurses' Health Study) showed high intraclass correlations (35), suggesting that concentrations are relatively stable over time. Additionally, seasonal variation was addressed in multiple ways, including matching cases and controls on season and constructing cutpoints based on season-specific quartiles among controls. Finally, as with any observational study, it is not possible to completely eliminate potential confounding effects, although results were similar in crude and fully adjusted models.

In conclusion, this pooled analysis did not find evidence of a strong overall association between circulating 25(OH)D

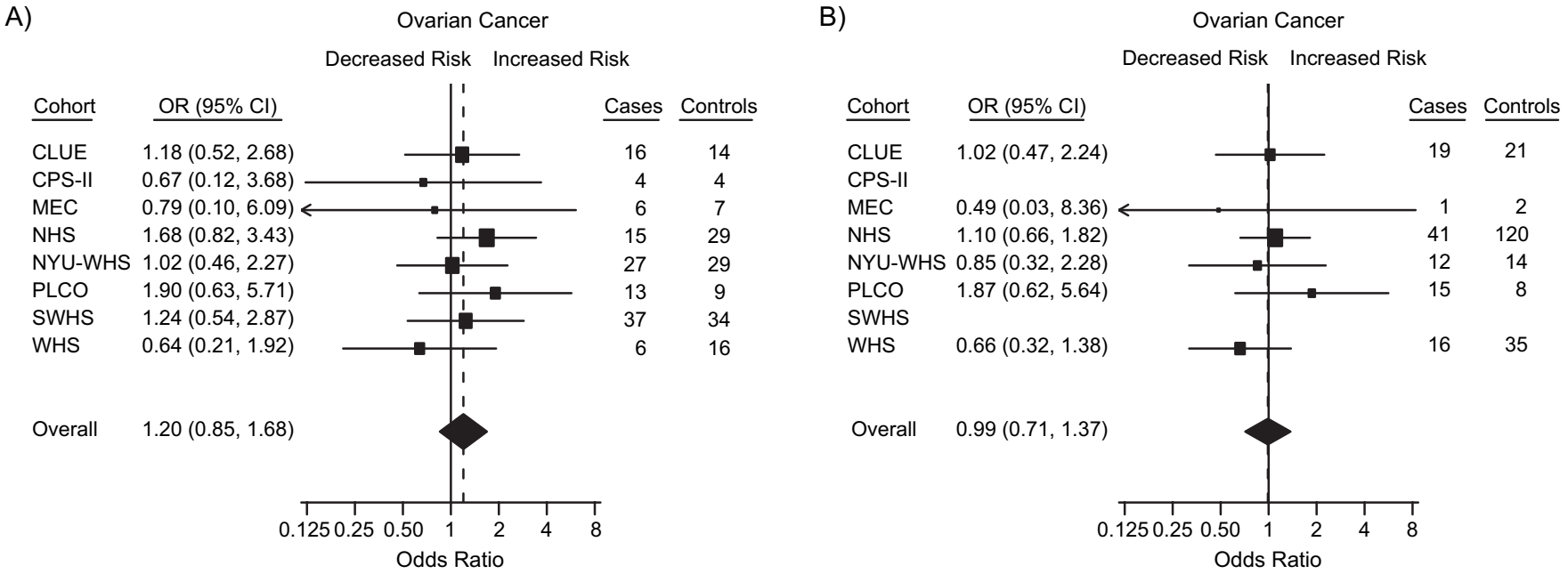

Figure 1. Forest plots for the meta-analysis of the association between circulating 25-hydroxyvitamin D (25(OH)D) and risk of ovarian cancer within the Cohort Consortium Vitamin D Pooling Project of Rarer Cancers. Risk estimates, by cohort, for subjects with circulating 25(OH)D concentrations <25 nmol/L (A) and ≥75 nmol/L (B) are compared with the referent group (50–<75 nmol/L). Odds ratios and 95% confidence intervals were derived from conditional logistic regression models adjusted for duration of oral contraceptive use and number of pregnancies. Cases and controls were matched on age (±1 year), race/ethnicity (white, black, Asian, other), date of blood draw (±30 days), and study cohort, except for the Nurses' Health Study, which was matched on age (±1 year), month of blood collection (±1 month), time of day of blood draw (±2 hours), fasting status, menopausal status, and postmenopausal hormone use at blood draw. The black squares show the odds ratios, the bars show the 95% confidence intervals, and the size of each square is inversely proportional to the variance of the log odds ratio estimate in each cohort. The overall estimates (diamonds) come from a meta-analysis using random-effects modeling. CPS-II and SWHS data are not included in the highest versus referent category forest plot (B) because of unstable risk estimates due to small numbers. CI, confidence interval; CPS-II, Cancer Prevention Study II Nutrition Cohort; MEC, Multiethnic Cohort Study; NHS, Nurses' Health Study; NYU-WHS, New York University Women's Health Study; OR, odds ratio; PLCO, Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial; SWHS, Shanghai Women's Health Study; WHS, Women's Health Study.

and ovarian cancer risk. However, there was some suggestion that low circulating vitamin D might be associated with an increased risk of ovarian cancer among overweight women.

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Author affiliations: Division of Epidemiology, Department of Medicine, Vanderbilt Epidemiology Center, Vanderbilt-Ingram Cancer Center, Vanderbilt University School of Medicine, Nashville, Tennessee (Wei Zheng, Xiao-Ou Shu); Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, Maryland (Kim N. Danforth, Mark P. Purdue, Stephanie J. Weinstein, Kai Yu); Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts (Shelley S. Tworoger, Susan E. Hankinson); Department of Epidemiology, Harvard School of Public Health, Boston, Massachusetts (Shelley S. Tworoger, Susan E. Hankinson); Cancer Research Center of Hawaii, University of Hawaii, Honolulu, Hawaii (Marc T. Goodman, Laurence N. Kolonel); Department of Obstetrics and Gynecology, New York University School of Medicine, New York, New York (Alan A. Arslan); Department of Environmental Medicine, New York University School of Medicine, New York, New York (Alan A. Arslan, Anne Zeleniuch-Jacquotte); New York University Cancer Institute, New York, New York (Alan A. Arslan, Anne Zeleniuch-Jacquotte); Epidemiology Research Program, American Cancer Society, Atlanta, Georgia (Alpa V. Patel, Marjorie L. McCullough); Information Management Services, Inc., Silver Spring, Maryland (Kirk Snyder, Emily Steplowski); Department of Epidemiology, Shanghai Cancer Institute, Shanghai, China (Yu-Tang Gao); Division of Cancer Control and Population Sciences, National Cancer Institute, Bethesda, Maryland (Chinonye Harvey); New York University Medical Center, New York, New York (Richard B. Hayes); Keck School of Medicine, University of Southern California, Los Angeles, California (Brian E. Henderson); Heartland Assays, Inc., Ames, Iowa (Ronald L. Horst); Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland (Kathy J. Helzlsouer, Kala Visvanathan); and the Prevention and Research Center, Weinberg Center for Women's Health and Medicine, Mercy Medical Center, Baltimore, Maryland (Kathy J. Helzlsouer).

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