American Journal of Epidemiology

Vol. 172, No. 1
DOI: 10.1093/aje/kwq114
Advance Access publication:
June 18, 2010

Original Contribution

Circulating 25-Hydroxyvitamin D and Risk of Endometrial Cancer

Cohort Consortium Vitamin D Pooling Project of Rarer Cancers

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Initially submitted October 23, 2009; accepted for publication April 12, 2010.

A nested case-control study, including 830 cases and 992 controls from 7 cohorts, was conducted to evaluate the association of circulating 25-hydroxyvitamin D (25(OH)D), the best indicator of vitamin D status, with risk of endometrial cancer. Matching factors included age at blood donation, date of blood donation, and race. Conditional logistic regression was used in the main analysis. The median concentration of 25(OH)D was slightly lower in cases (49.4 nmol/L) than in controls (50.8 nmol/L) ($P = 0.08$). However, there was no association between 25(OH)D concentration and disease risk, after adjustment for body mass index. Compared with the 50–<75 nmol/L 25(OH)D category, the body mass index-adjusted odds ratios and 95% confidence intervals were 1.08 (95% confidence interval: 0.73, 1.57) for the <25 nmol/L category and 0.90 (95% confidence interval: 0.51, 1.58) for the ≥100 nmol/L category ($P_{trend} = 0.99$). Similarly null results were observed after further adjustment for other known risk factors and in stratified analyses. Although an effect of circulating 25(OH)D at high concentrations cannot be ruled out (the highest category of 25(OH)D was ≥100 nmol/L, and for stratified analyses, ≥75 nmol/L), these results do not support a protective role of vitamin D against endometrial cancer.

case-control studies; endometrial neoplasms; prospective studies; vitamin D

Abbreviations: CI, confidence interval; 25(OH)D, 25-hydroxyvitamin D; $1,25(OH)_2D$, 1,25-dihydroxyvitamin D; VDPP, Cohort Consortium Vitamin D Pooling Project of Rarer Cancers.

Endometrial cancer is the most common gynecologic cancer in the United States, ranking fourth among all cancers in women in age-adjusted incidence (1). The large international variation in incidence rates (2) suggests that much of the risk may be modifiable. Factors associated with high estrogen and low progesterone levels, such as estrogen-only hormone replacement therapy and obesity, have been shown to increase the risk of endometrial cancer (3). However, the role of other modifiable factors, such as diet and environmental exposures, has not been fully investigated.

Limited data are available regarding the association of vitamin D with endometrial cancer risk. Exposure to ultraviolet B irradiation leads to induction of vitamin D precursor synthesis in the skin and is the main source of vitamin D in humans (4). Ecologic studies have described an inverse association between ultraviolet B irradiation and endometrial cancer incidence rates, suggesting a protective role of vitamin D against endometrial cancer (5, 6). Diet (mostly through fortification) and supplements are also sources of vitamin D (4). A recent review of the only 3 case-control studies that have examined the association between dietary intake of vitamin D and risk of endometrial cancer (7–9) concluded that the evidence available did not support an association but that it was too limited to draw firm conclusions (10).

Table 1. Characteristics of Participants, by Cohort, in the Investigation of Endometrial 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	192	192	10.0 (4.8–14.2)	51.9 (39.4–68.4)	56.8 (44.0–71.1)
CPS-II	51	51	2.2 (1.2–3.2)	60.8 (46.8–77.8)	63.5 (46.0–78.9)
MEC	39	39	1.4 (0.6–2.4)	58.0 (41.5–72.5)	61.3 (30.3–77.8)
NHS[a]	163	325	7.2 (4.2–10.6)	56.3 (37.2–68.5)	52.8 (39.7–69.0)
NYU-WHS	139	139	10.7 (5.9–13.1)	41.9 (28.8–60.0)	46.7 (31.1–63.0)
PLCO	147	147	2.6 (0.6–4.7)	51.3 (39.7–65.2)	52.1 (37.1–64.8)
SWHS	99	99	4.7 (2.1–6.6)	29.9 (22.7–41.6)	33.4 (25.3–41.6)
Total	830	992	5.5 (2.3–10.5)	49.4 (34.6–66.4)	50.8 (36.7–67.1)

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] A 1:2 case:control ratio was used for the NHS.

Conversion of 25-hydroxyvitamin D (25(OH)D) to 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), the active vitamin D metabolite, occurs in the endometrium (11, 12), although the main site of conversion is the kidney (4). In addition, endometrial tissue expresses the vitamin D receptor (12, 13), a $1,25(OH)_2D$-activated nuclear transcription factor that regulates the production of proteins involved in cell proliferation and differentiation (14). These data support the hypothesis that vitamin D plays a role in the etiology of endometrial cancer. 25(OH)D is considered the best indicator of vitamin D status, because it measures vitamin D resulting from both ultraviolet B exposure and dietary/supplement intake and because it has a longer half-life (2–3 weeks) than $1,25(OH)_2D$ (4–6 hours) (15–17). Because no epidemiologic study to date has examined the hypothesis that circulating 25(OH)D is inversely related to risk of endometrial cancer, a case-control study nested within 6 cohorts in the United States and 1 in Shanghai, China, was conducted to examine this hypothesis as part of the Cohort Consortium Vitamin D Pooling Project of Rarer Cancers (VDPP).

MATERIALS AND METHODS

Study design and population

A detailed description of the overall methods of the VDPP and participating cohorts is provided elsewhere in this issue (18). All VDPP cohorts that included women (7 out of 10) participated in the nested case-control study of endometrial cancer: CLUE; the Cancer Prevention Study II Nutrition Cohort (CPS-II); the Multiethnic Cohort Study (MEC); the Nurses' Health Study (NHS); 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) (Table 1).

Incident cases included *International Classification of Diseases for Oncology* (ICD-O) codes 8010, 8140, 8210, 8260, 8310, 8323, 8380, 8382, 8441, 8460, 8461, 8480, 8481, 8560, and 8570. In situ tumors, as well as Mullerian tumors, stromal tumors, and sarcomas, were excluded. Case ascertainment methods for each cohort are summarized (18).

Control selection was done by using incidence density sampling. Individually matched controls were selected from the parent cohort among women who were free of cancer at the date of diagnosis of the case (index date) and who had not had a hysterectomy at the index date (except for the CLUE cohort that did not collect data on hysterectomy). Matching factors included age at blood donation ($\pm$1 year, except for CLUE and the Shanghai Women's Health Study ($\pm$2 years)), date of blood donation ($\pm$1 month, except for the NYU-WHS ($\pm$3 months)), and race (white, black, Asian, other). All cohorts also matched on menopausal status at blood donation.

Out of the total of 843 cases initially identified, 11 were excluded because of ineligible histology and 2 because the date of diagnosis was before the date of blood donation. Thus, this analysis includes 830 cases and 992 controls (a 1:1 case:control ratio was used, except for the Nurses' Health Study, which used a 1:2 ratio). Histologic confirmation was obtained for 97% of the cases.

Measurement of circulating 25(OH)D

As reported elsewhere (18), all serum or plasma samples were assayed for 25(OH)D at Heartland Assays, Inc. (Ames, Iowa) by a direct, competitive chemiluminescence immunoassay by using the DiaSorin LIAISON 25 OH Vitamin D TOTAL Assay (19, 20). Quality control procedures are described (18). The inter- and intrabatch coefficients of variation using masked National Institute of Standards and Technology samples were 12.7% and 9.3% for samples at level 1 (~60 nmol/L) and 13.6% and 11.0% for samples at

level 2 (~35 nmol/L), respectively. Based on the masked quality control samples provided by each cohort, the median interbatch coefficient of variation was 13.2% (range: 4.8%–17.0%), and the median intrabatch coefficient of variation was 9.9% (range: 3.8%–16.4%).

Statistical analysis

The statistical analysis was conducted following the general approach and specific methods approved by the VDPP Steering Committee (18). Aspects of the analysis specific to the endometrial cancer study are described here. For the main analysis, 25(OH)D concentrations were grouped in a priori defined, clinically relevant, categories (<25.0, 25.0–<37.5, 37.5–<50.0, 50.0–<75.0, 75.0–<100.0 and ≥100 nmol/L). The 50–<75 nmol/L category was used as the referent category. Because of the small number of subjects in the top category (≥100 nmol/L), the two top categories were combined (≥75 nmol/L) for stratified and subgroup analyses. Analyses were also conducted after classifying women into 25(OH)D quartiles using cohort- and season-specific cutpoints, with the lowest quartile as referent category. In these analyses, seasons were defined as winter (December to May) or summer (June to November). Circulating 25(OH)D was also analyzed as a natural log-transformed continuous variable.

The seasonal variations in concentrations of 25(OH)D were taken into account in various ways: 1) by matching on date of blood draw and taking the matching into consideration in the statistical analysis; 2) by conducting analyses on quartiles using season-specific cutpoints; and 3) by conducting analyses using residuals from regression of 25(OH)D on week of the year, in an attempt to take into account the gradual nature of changes in concentrations of 25(OH)D over the year better than by adjusting for season (18).

The conditional logistic regression model was used for the main analysis to take into account the matched design. Because a comparison of conditional and unconditional logistic regression models using the full dataset showed that the odds ratios obtained by the two methods were nearly identical, stratified and subgroup analyses were conducted using the unconditional logistic regression model and adjusting for the matching factors (cohort, race, age (log-transformed) and season at blood draw). The use of unconditional logistic regression prevented loss of data in analyses stratifying by factors not used in the matching.

Data on potential confounders were collected from each cohort and standardized as described in (18). For most variables, data collected at, or close to, blood donation were used. For oral contraceptive and hormone replacement therapy use, data up to the index date were used for the Nurses' Health Study and the NYU-WHS, whereas data collected at, or close to, blood donation were used for the other cohorts. Both conditional and unconditional logistic regression models based on the full dataset are presented adjusting for the following known endometrial cancer risk factors: education, menopausal status, age at menarche, parity, oral contraceptive use, hormone replacement therapy use, body mass index, smoking, history of high blood pressure, and history of diabetes. To assess which factors contributed to confounding, the change in the 25(OH)D regression coefficient in the conditional logistic regression model upon addition of each risk factor, one at a time, was examined. Because only body mass index changed the 25(OH)D coefficient by more than 10%, stratified/subgroup analyses are presented adjusting for this factor only. Analyses adjusting for body mass index on the continuous, log-transformed, scale led to odds ratios qualitatively similar to those of the analysis adjusting for body mass index as a categorical variable (<25, 25–<30, ≥30 kg/m^2, missing), but resulted in a smaller sample size since subjects missing body mass index data were excluded from these analyses. Therefore, results are presented adjusting for body mass index as a categorical variable.

Analyses stratifying by known endometrial cancer risk factors were also conducted and possible effect modification of the 25(OH)D-endometrial cancer association by these factors was assessed by conducting interaction tests (18). Because of the known biological interactions between vitamin D and calcium, we were also interested in conducting an analysis stratifying by calcium supplement use; however, because of the small number of calcium supplement users, we were only able to conduct an analysis among non users of calcium supplements. An analysis limited to whites was also conducted. Finally, an analysis excluding cases with ICD-O codes other than 8010, 8140, 8380, 8382 was conducted to assess a possible effect of vitamin D limited to endometrioid tumors.

A meta-analysis assuming a random effects model was conducted to assess between-cohort heterogeneity and to compute overall odds ratios for endometrial cancer associated with the low (<25 nmol/L) and high (≥75 nmol/L) 25(OH)D categories, as compared to the 50–<75 nmol/L category (21). Cohort-specific odds ratios were computed using the conditional logistic regression model and adjusting for body mass index. Heterogeneity of cohort-specific estimates was measured using the DerSimonian and Laird Q statistic (22) and data are presented as forest plots. Finally, to explore the influence of each cohort on our results, the main analysis (using conditional logistic regression and adjusting for all risk factors for endometrial cancer listed above) was repeated excluding one cohort at a time.

RESULTS

Table 1 describes the number of cases and controls from each cohort and the median time between blood donation and diagnosis for the cases, which varied from 1.4 year (Multiethnic Cohort Study) to 10.7 years (NYU-WHS). Among controls, a two-fold variation in median 25(OH)D concentrations was seen across cohorts with the lowest median observed in Shanghai Women's Health Study (33.4 nmol/L) and the highest in the Cancer Prevention Study II Nutrition Cohort (63.5 nmol/L).

Table 2 describes characteristics of the cases and controls. The median age at blood donation was 58 years and at diagnosis 64 years. Most subjects were white (79.2% of cases). Compared to controls, cases had younger age

at menarche ($P = 0.02$) and older age at menopause ($P = 0.09$). Cases were also more likely than controls to be obese ($P \leq 0.0001$), to be never smokers ($P = 0.04$) and to have a history of high blood pressure ($P = 0.0005$) or diabetes ($P = 0.003$). Cases were less likely than controls to report oral contraceptive use ($P = 0.09$). Overall, the median concentration of 25(OH)D was slightly lower in cases than in controls (49.4 nmol/L and 50.8 nmol/L, respectively, $P = 0.08$), and the proportions of women with vitamin D concentrations less than 25 nmol/L or 37.5 nmol/L were slightly higher among cases than controls.

Table 3 presents results using the conditional logistic regression model. In the crude analysis, there was some suggestion that lower concentrations (<25 nmol/L) were associated with a small increased risk of endometrial cancer compared with the referent category of 50–<75 nmol/L (odds ratio = 1.20, 95% confidence interval (CI): 0.83, 1.72) and that higher concentrations (≥100 nmol/L) were associated with a lower risk (odds ratio = 0.78, 95% CI: 0.45, 1.34); however, the test for trend was not statistically significant ($P = 0.12$). After adjusting for body mass index, odds ratios were attenuated, 1.08 (95% CI: 0.73, 1.57) for the <25 nmol/L 25(OH)D category and 0.90 (95% CI: 0.51, 1.58) for the ≥100 nmol/L 25(OH)D category, and there was no longer any evidence of a trend ($P = 0.99$). As compared to the body mass index-adjusted odds ratios, odds ratios varied only slightly when adjusted for additional known endometrial cancer risk factors. Similarly, analyses using cohort- and season-specific quartiles, residuals, or log-transformed 25(OH)D showed no evidence of association with endometrial cancer risk after adjusting for body mass index (data not shown).

There was no evidence of an association between concentrations of circulating 25(OH)D and endometrial cancer risk in strata defined according to season of blood draw, age at diagnosis, lag-time between blood donation and diagnosis, body mass index, oral contraceptive use or hormone replacement therapy use (Table 4). There was no evidence of interaction for any of these factors, except hormone replacement therapy ($P = 0.04$). However no consistent trend was observed in either users ($P = 0.24$) or non users of hormone replacement therapy ($P = 0.36$). No associations were observed when analyses were limited to women who did not use calcium supplements, white women, or women with endometrioid tumors.

No association between circulating 25(OH)D and endometrial cancer risk was observed in the meta-analysis (Figure 1). The overall, body mass index-adjusted odds ratio comparing the lowest concentration (<25 nmol/L) to the referent category (50–75 nmol/L) was 1.21 (95% CI: 0.75, 1.98) while the odds ratio associated with concentrations ≥75 nmol/L was 0.98 (95% CI: 0.71, 1.35). There was no evidence of heterogeneity between cohorts ($P = 0.23$ for the comparison of the <25 nmol/L and 50–75 nmol/L categories and 0.92 for the comparison of the ≥75 nmol/L and 50–75 nmol/L categories). Finally, results from analyses that excluded cohorts one at a time were consistent, showing no statistically significant trend in risk across categories of 25(OH)D concentrations.

DISCUSSION

Circulating concentrations of 25(OH)D were not associated with risk of endometrial cancer in this nested case-control study based on seven cohorts. Though there was some indication of a trend of decreasing risk with increasing concentrations of 25(OH)D in crude analyses, no trend was observed after adjusting for body mass index. Obesity plays an important role in endometrial cancer etiology because it is associated with increased exposure to estrogen unopposed by progesterone, leading to increased mitotic activity of endometrial cells and greater opportunity for the occurrence of DNA replication errors (3). Consistent with results from other studies (23, 24), the prevalence of obesity was greater among cases than among controls (Table 2). Body mass index was also inversely associated with 25(OH)D (cohort-adjusted Spearman correlation coefficient = −0.16 in controls and −0.28 in cases), as was observed in the overall population of controls included in VDPP (25); this was expected since vitamin D tends to be sequestered in adipose tissue (26). These associations led to negative confounding of the 25(OH)D - endometrial cancer risk relationship by body mass index. Such negative confounding, if ignored, will lead to a spurious association as was observed in our study prior to adjusting for body mass index.

This study is the first to examine the association of endometrial cancer risk with circulating 25(OH)D, which is considered the best marker of vitamin D status (15–17). The results of this study are in agreement with a review of the literature on vitamin D intake in relation to endometrial cancer which concluded that the limited evidence available regarding vitamin D did not support an association (10). These studies, though, did not take into account vitamin D obtained from ultraviolet B exposure. An ecological study of 107 countries reported that endometrial cancer incidence rates were higher at higher latitudes and concluded that low ultraviolet B irradiance, which is associated with lower vitamin D exposure, was associated with endometrial cancer risk (6). However, although the authors adjusted for the proportion of the population who were overweight as well as for some other risk factors, the observed association could be due to ecological fallacy since control for body mass index and other risk factors at the individual level was not possible.

A strength of the present study was the availability of individual data, collected prospectively, on known risk factors for endometrial cancer. For most of these risk factors, differences between cases and controls were as expected. Hormone replacement therapy use, though, was less common in cases than in controls. This result appears inconsistent with the known positive association between estrogen-only replacement therapy and endometrial cancer risk. However, our study did not have the ability to assess the association of this variable with disease risk because most participating cohorts matched on use of hormone replacement therapy at entry. In addition, we were not able to distinguish between estrogen-only and estrogen plus progestin formulations, nor to take into account the recency of use, both factors which impact the association of hormone replacement therapy with endometrial cancer risk (23, 27–29).

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

Characteristic	Cases (N = 830)			Controls (N = 992)			P Value[a]
	No.	%	Median (Interquartile Range)	No.	%	Median (Interquartile Range)	
Age at blood draw, years			58 (50–65)			58 (50–64)	Matched
Age at diagnosis, years			64 (58–70)				
Race[b]							Matched
White	657	79.2		819	82.6		
Black	18	2.2		23	2.3		
Asian	120	14.5		118	11.9		
Other	23	2.8		26	2.6		
Education[b]							0.22
Less than high school	152	18.3		164	16.5		
Completed high school	203	24.5		183	18.4		
Vocational school	32	3.9		45	4.5		
Some college	232	28.0		333	33.6		
College graduate	102	12.3		136	13.7		
Graduate studies	82	9.9		122	12.3		
Height, cm			162.6 (157.5–167.6)			163 (157–168)	0.76
Missing	73	8.8		104	10.5		
Weight, kg			70.5 (61.4–83.9)			65.8 (59–75)	<0.0001
Missing	104	12.5		108	10.9		
Body mass index, kg/m^2							<0.0001
<25	255	30.7		459	46.3		
25–<30	229	27.6		291	29.3		
≥30	239	28.8		132	13.3		
Missing	107	12.9		110	11.1		
Age at menarche, years			12.5 (12–14)			13 (12–14)	0.02
Missing	131	15.8		138	13.9		
History of full-term pregnancy							0.12
Yes	561	67.6		722	72.8		
No	98	11.8		94	9.5		
Missing	171	20.6		176	17.7		
Ever used an oral contraceptive							0.09
Yes	254	30.6		363	36.6		
No	465	56.0		522	52.6		
Missing	111	13.4		107	10.8		
Menopausal status[b]							Matched
Premenopause	235	28.36		273	27.5		
Perimenopause	29	3.5		36	3.6		
Postmenopause	558	67.2		680	68.5		
Age at menopause, years			52 (49–53)			51 (47–53)	0.09
Missing	295	35.5		328	33.1		
Ever used HRT							–[c]
Yes	302	36.4		409	41.2		
No	420	50.6		475	47.9		
Missing	108	13.0		108	10.9		
Smoking status[b]							0.04
Never	512	61.7		543	54.7		

Table continues

Table 2. Continued

Characteristic	Cases (*N* = 830)			Controls (*N* = 992)			*P* Value[a]
	No.	%	Median (Interquartile Range)	No.	%	Median (Interquartile Range)	
Former	232	28.0		325	32.8		
Current	74	8.9		116	11.7		
Physical activity							0.69
Sedentary	228	27.5		264	26.6		
Light	163	19.6		197	19.9		
Moderate	140	16.9		188	19.0		
Vigorous	145	17.5		196	19.8		
Missing	154	18.6		147	14.8		
History of high blood pressure[b]							0.0005
Yes	270	32.5		248	25.0		
No	547	65.9		735	74.1		
History of diabetes[b]							0.003
Yes	50	6.0		30	3.0		
No	760	91.6		948	95.6		
Caloric intake, kcal/day			1,613 (1,276–1,993)			1,634 (1,286–2,027)	0.52
Missing	140	16.9		140	14.1		
Vitamin D, IU/day			161 (98–237)			179 (105–305)	0.86
Missing	140	16.9		140	14.1		
Energy-adjusted vitamin D, IU/day			179 (117–252)			197 (126–311)	0.76
Missing	140	16.9		140	14.1		
Current use of multivitamins[b]							0.83
Yes	243	29.3		297	29.9		
No	547	65.9		652	65.7		
Current use of vitamin D supplements							0.06
Yes	32	3.9		54	5.4		
No	297	35.8		440	44.4		
Missing	501	60.4		498	50.2		
Current use of calcium supplement							0.55
Yes	170	20.5		223	22.5		
No	437	52.7		514	51.8		
Missing	223	26.9		255	25.7		
Season of blood draw							–[d]
Winter	134	16.1		167	16.8		
Spring	192	23.1		236	23.8		
Summer	262	31.6		295	29.7		
Fall	242	29.2		294	29.6		
25(OH)D, nmol/L			49.4 (34.6–66.4)			50.8 (36.7–67.1)	0.08
25(OH)D, <25 nmol/L	93	11.1		88	8.9		0.12
25(OH)D, <37.5 nmol/L	255	30.4		263	26.5		0.07

Abbreviations: HRT, hormone replacement therapy; MEC, Multiethnic Cohort Study; NHS, Nurses' Health Study; 25(OH)D, 25-hydroxyvitamin D; NYU-WHS, New York University Women's Health Study; PLCO, Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial.

[a] Wald test from conditional logistic regression, excluding subjects with missing data.

[b] Data were missing for <5% of subjects.

[c] –, matching factor for CLUE, MEC, NHS, and PLCO.

[d] –, matching on date of blood draw (±1 month except for NYU-WHS, ±3 months).

Because sun exposure is the main source of vitamin D, circulating concentrations of 25(OH)D are lower in the winter than in the summer months and it is important to take into account such variations to avoid bias (30). In addition to matching on date of blood draw, this issue was addressed using various statistical methods. There

was no evidence of a protective effect of vitamin D in any of these analyses.

Interactions of vitamin D and vitamin D analogs with estrogens have been reviewed (31, 32). Although, to our knowledge, there are no data specific to the endometrium, it has been proposed, based on an animal model of breast cancer, that $1,25(OH)_2D$ opposes estrogen-driven proliferation (33). In this study, the test for interaction by hormone replacement therapy use was statistically significant (P for heterogeneity = 0.04); however, there was no evidence of a protective effect of vitamin D in either ever or never users of hormone replacement therapy (Table 4). In addition, no association was observed between circulating 25(OH)D and risk of endometrioid endometrial cancer, a subtype strongly associated with estrogen (34). Because of the data collection procedures of some of the cohorts and in order to have sufficient numbers of cases, non-endometrioid subtypes (mucinous, serous, clear cell, squamous-cell, mixed) were excluded in this analysis but adenocarcinomas not-otherwise -specified (ICD-O codes 8140 and 8010) (n = 462) were combined with endometrioid tumors (n = 223). It is therefore likely that some tumors of non-endometrioid subtype were included. However, since endometrioid tumors represent about 80% of all endometrial carcinomas, it is unlikely that an association was missed in this subgroup. Because of small numbers, the association of 25(OH)D with other subtypes of endometrial cancer could not be examined.

The concentrations of circulating 25(OH)D observed in this study were similar to concentrations observed in women in the United States (35). Few women, though, had high concentrations and the highest category that could be studied was was ≥100 nmol/L, and for stratified and subgroup analyses, ≥75 nmol/L. Therefore, conclusions cannot be drawn regarding the potential protective effect of higher concentrations of 25(OH)D. However, although a threshold effect is possible, the complete lack of a dose-response relationship in this study argues against a protective role of vitamin D.

Strengths of this study include the prospective assessment of vitamin D status and possible confounders, the inclusion of women living in a wide range of latitudes, a large number of cases and the use of the same laboratory to assay all samples. Only one serum/plasma sample was used for each participant which leads to some measurement error regarding the exposure of interest, i.e. the long-term average circulating level of 25(OH)D. However, circulating concentrations of 25(OH)D appear relatively stable when collected during the same season. A pilot study conducted in the NYU-WHS using the same assay in the same laboratory found an intraclass correlation coefficient of 0.78 (95% CI: 0.64, 0.88) in 30 healthy women who contributed three samples each at yearly intervals (unpublished data). Likewise, in the Nurses' Health Study, the intraclass correlation coefficient for 25(OH)D was 0.72 (95% CI: 0.62, 0.80) in 71 women over a 2–3 year period using a similar assay (unpublished data). These results are comparable to those observed in 144 middle-aged men for whom the Pearson correlation between samples collected 4 years apart was 0.70 (36). Such temporal reliability compares favorably to

Table 3. Odds Ratios and 95% Confidence Intervals for the Association Between Circulating 25(OH)D and Risk of Endometrial Cancer Within the Cohort Consortium Vitamin D Pooling Project of Rarer Cancers

	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	No. of Cases	No. of Controls	OR	95% CI	
All	93	88			162	170			163	224			293	349			94	126			25	35			
Crude[a]			1.20	0.83, 1.72			1.10	0.83, 1.46			0.86	0.66, 1.11			1.0	Referent			0.88	0.64, 1.20			0.78	0.45, 1.34	0.12
Body mass index, adjusted[b]			1.08	0.73, 1.57			0.97	0.72, 1.30			0.82	0.62, 1.07			1.0	Referent			1.02	0.74, 1.42			0.90	0.51, 1.58	0.99
Multivariate adjusted[c]			1.02	0.68, 1.53			0.91	0.67, 1.24			0.79	0.60, 1.05			1.0	Referent			1.00	0.71, 1.42			0.85	0.47, 1.53	0.81

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

[a] Conditional logistic regression model, unadjusted.

[b] Conditional logistic regression model, adjusted for body mass index (<25, 25–<30, ≥30 kg/m^2, missing).

[c] Conditional logistic regression model, adjusted for education (less than high school, completed high school, vocational school, some college, college graduate, graduate studies, missing), menopausal status (pre-, peri-, post-, missing), age at menarche (<13, ≥13 years of age, missing), parity (0, 1, 2, 3, ≥4, missing), oral contraceptive use (never, ever, missing), HRT use (never, ever, missing), smoking (never, former, current, missing), history of high blood pressure (yes, no, missing), history of diabetes (yes, no, missing), and body mass index (<25, 25–<30, ≥30 kg/m^2, missing).

Table 4. Odds Ratios and 95% Confidence Intervals for the Association Between Circulating 25(OH)D and Risk of Endometrial Cancer Within the Cohort Consortium Vitamin D Pooling Project of Rarer Cancers With Stratified/Subgroup Analyses[a]

	Circulating 25(OH)D, nmol/L																				P_{trend}
	<25.0				25.0–<37.5				37.5–<50.0				50.0–<75.0				≥75.0				
	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	93	88			162	170			163	224			293	349			119	161			
Crude[b]			1.22	0.86, 1.75			1.11	0.84, 1.46			0.85	0.66, 1.11			1.0	Referent			0.85	0.64, 1.14	0.11
Adjusted[c]			1.02	0.70, 1.47			0.93	0.70, 1.24			0.79	0.61, 1.04			1.0	Referent			0.93	0.69, 1.24	0.89
Adjusted[d]			0.98	0.67, 1.44			0.91	0.67, 1.22			0.80	0.61, 1.05			1.0	Referent			0.93	0.69, 1.26	0.73
Season[c]																					
December–May	54	55	0.85	0.49, 1.48	77	87	0.82	0.52, 1.31	55	77	0.81	0.50, 1.30	83	107	1.0	Referent	27	49	0.67	0.37, 1.21	0.99
June–November	39	33	1.16	0.68, 1.98	85	83	1.01	0.69, 1.47	108	147	0.79	0.57, 1.08	210	242	1.0	Referent	92	112	1.02	0.72, 1.44	0.92
Age at diagnosis, years[c]																					
≤58	31	27	0.94	0.46, 1.91	43	48	0.70	0.38, 1.28	38	55	0.68	0.39, 1.21	71	71	1.0	Referent	25	43	0.72	0.38, 1.37	0.87
>58–64	28	24	1.38	0.67, 2.84	34	45	0.89	0.49, 1.60	34	50	0.71	0.40, 1.27	63	82	1.0	Referent	32	29	1.50	0.80, 2.81	0.61
>64–70	17	19	0.86	0.38, 1.97	41	43	0.85	0.48, 1.52	46	58	0.90	0.53, 1.51	77	96	1.0	Referent	31	48	0.81	0.45, 1.45	0.91
>70	17	18	0.92	0.41, 2.09	44	34	1.45	0.81, 2.59	45	61	0.86	0.52, 1.42	82	100	1.0	Referent	31	41	0.86	0.48, 1.55	0.45
Lagtime, years[c]																					
≤5	44	42	0.98	0.57, 1.68	72	77	0.82	0.53, 1.26	73	88	0.78	0.52, 1.17	145	151	1.0	Referent	53	78	0.77	0.50, 1.20	0.97
>5	49	46	1.07	0.64, 1.79	90	93	1.04	0.70, 1.54	90	136	0.81	0.57, 1.15	148	198	1.0	Referent	66	83	1.08	0.72, 1.62	0.84
Body mass index, kg/m^2 [c]																					
<25	24	47	0.81	0.44, 1.48	31	66	0.76	0.45, 1.29	48	94	0.81	0.52, 1.26	98	162	1.0	Referent	54	90	0.98	0.63, 1.52	0.31
25–<30	27	20	1.42	0.70, 2.86	51	61	0.98	0.59, 1.64	49	66	0.99	0.61, 1.61	76	102	1.0	Referent	26	42	0.85	0.47, 1.53	0.38
≥30	38	15	1.05	0.46, 2.39	63	28	1.13	0.60, 2.12	44	37	0.62	0.34, 1.16	72	38	1.0	Referent	22	14	0.79	0.35, 1.79	0.59
Oral contraceptive use[c]																					
Never	60	50	1.09	0.67, 1.77	90	101	0.75	0.50, 1.11	96	116	0.84	0.59, 1.21	159	174	1.0	Referent	60	81	0.83	0.55, 1.27	0.96
Ever	27	32	0.88	0.45, 1.72	52	55	1.15	0.69, 1.91	44	81	0.75	0.47, 1.22	87	131	1.0	Referent	44	64	1.10	0.67, 1.80	0.71
HRT use[c]																					
Never	76	57	1.27	0.78, 2.07	86	98	0.81	0.53, 1.25	93	102	1.10	0.74, 1.64	118	145	1.0	Referent	47	73	0.79	0.49, 1.28	0.36
Ever	13	25	0.65	0.31, 1.37	54	59	0.97	0.61, 1.55	48	98	0.55	0.35, 0.84	133	157	1.0	Referent	54	70	0.95	0.61, 1.47	0.24
No calcium supplements use[c]	41	35	1.21	0.69, 2.10	76	90	0.85	0.57, 1.26	94	114	0.89	0.62, 1.27	165	192	1.0	Referent	61	83	0.87	0.58, 1.30	0.81
White race[c]	46	53	0.96	0.61, 1.51	116	114	1.09	0.79, 1.50	133	190	0.79	0.60, 1.06	255	317	1.0	Referent	107	145	0.93	0.68, 1.27	0.91
Endometrioid tumors[c]	93	88	1.01	0.70, 1.47	162	170	0.93	0.70, 1.24	163	224	0.79	0.61, 1.04	293	349	1.0	Referent	119	161	0.93	0.69, 1.24	0.89

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

[a] All P values for heterogeneity > 0.14, except for HRT ($P = 0.04$).

[b] Unconditional logistic regression model, adjusted for matching factors.

[c] Unconditional logistic regression model, adjusted for matching factors and body mass index (<25, 25–<30, ≥30 kg/m^2, missing).

[d] Unconditional logistic regression model, adjusted for education (less than high school, completed high school, vocational school, some college, college graduate, graduate studies, missing), menopausal status (pre-, peri-, postmenopause, missing), age at menarche (<13, ≥13 years of age, missing), parity (0, 1, 2, 3, ≥4, missing), oral contraceptive use (never, ever, missing), HRT use (never, ever, missing), smoking (never, former, current, missing), history of high blood pressure (yes, no, missing), history of diabetes (yes, no, missing), and body mass index (<25, 25–<30, ≥30 kg/m^2, missing).

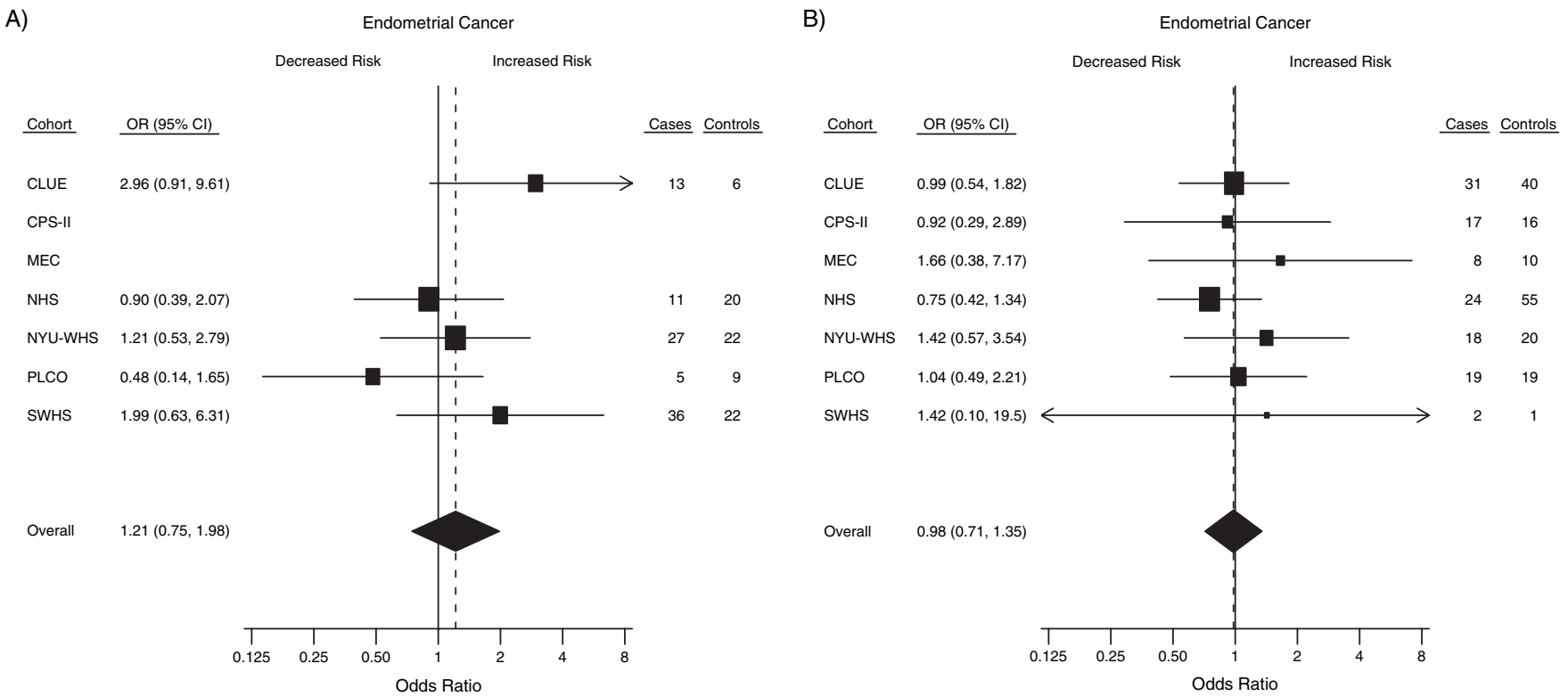

Figure 1. Forest plots for the meta-analysis of the association between circulating 25(OH)D and risk of endometrial cancer within the Cohort Consortium Vitamin D Pooling Project of Rarer Cancers. Risk estimates, by cohort, are shown for subjects with circulating 25(OH)D concentrations of $<$25 nmol/L (A) and $\geq$75 nmol/L (B) compared with the referent group (50–$<$75 nmol/L). Odds ratios and 95% confidence intervals were derived from conditional logistic regression models adjusted for body mass index. The boxes show the odds ratios, the bars show the 95% confidence intervals, and the size of each box is inversely proportional to the variance of the log odds ratio estimate in each cohort. The overall estimates (diamonds) come from a meta-analysis with random-effects modeling. CPS-II and MEC data are not included in the low versus referent category forest plot (A) because of highly unstable risk estimates. 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; 25(OH)D, 25-hydroxyvitamin D; OR, odds ratio; PLCO, Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial; SWHS, Shanghai Women's Health Study.

that of other biomarkers that have been found to be associated with disease, such as circulating estrogens (e.g., intraclass correlation coefficient in the 0.50–0.70 range for estradiol over a 2–3 year period (37, 38)), which have been consistently found to be associated with breast cancer risk (39).

In conclusion, after taking into account the effect of body mass index, circulating concentrations of 25(OH)D do not appear to be associated with risk of endometrial cancer.

ACKNOWLEDGMENTS

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This work was supported by the Extramural Research Program of the National Institutes of Health, Division of Cancer Control and Population Sciences, National Cancer Institute (NCI) (Bethesda, Maryland), and the Intramural Research Program of the National Institutes of Health, Division of Cancer Epidemiology and Genetics, NCI. The New York University Women's Health Study was supported by the NCI (grant R01 CA098661). The Nurses' Health Study was supported by the NCI (grants P01 CA055075, P01 CA87969, R01 CA49449, and R01 CA082838). The Multiethnic Cohort Study was supported by the NCI (grants R37 CA54281, P01 CA33619, R01 CA063464, and N01-PC35137). The Shanghai Women's Health Study was supported by the NCI (grants R37 CA70867 and N02-CP-11010-66). The Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial was supported by contracts from the NCI to the University of Colorado, Denver, Colorado (grant N01-CN-25514); Georgetown University, Washington, DC (grant N01-CN-25522); the Pacific Health Research Institute, Honolulu, Hawaii (grant N01-CN-25515); the Henry Ford Health System, Detroit, Michigan (grant N01-CN-25512); the University of Minnesota, Minneapolis, Minnesota (grant N01-CN-25513); Washington University, St. Louis, Missouri (grant NO1-CN-25516); the University of Pittsburgh, Pittsburgh, Pennsylvania (grant N01-CN-25511); the University of Utah, Salt Lake City, Utah (grant N01-CN-25524); the Marshfield Clinic Research Foundation, Marshfield, Wisconsin (grant N01-CN-25518); the University of Alabama, Birmingham, Alabama (grant NO1-CN-75022); Westat, Inc., Rockville, Maryland (grant N01-CN-25476); and the University of California, Los Angeles, California (grant NO1-CN-25404). CLUE was supported by the National Institute on Aging (grant U01 AG018033) and the National Cancer Institute (grants R01 CA105069 and K07 CA73790). The participation of CLUE investigators was also supported by an NCI contract awarded to Mercy Medical Center through the University of Hawaii (Honolulu, Hawaii). The Cancer Prevention Study II Nutrition Cohort was supported by the American Cancer Society (Atlanta, Georgia).

The authors thank Dr. Karen Phinney of the National Institute of Standards and Technology for providing the vitamin D in human serum (SRM 972) used in this work.

Members of the VDPP Endometrial Cancer Writing Committee: Anne Zeleniuch-Jacquotte, Lisa Gallicchio, Virginia Hartmuller, Kathy J. Helzlsouer, Marjorie L. McCullough, V. Wendy Setiawan, Xiao-Ou Shu, Stephanie J. Weinstein, Jocelyn M. Weiss, and Susan E. Hankinson.

This report is based at least in part on information provided by the Maryland Cancer Registry, Maryland Department of Health and Mental Health.

Dr. Ronald L. Horst is the President and Chief Executive Officer of Heartland Assays, Inc.

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