Plasma 25-Hydroxyvitamin D and 1,25-Dihydroxyvitamin D and Risk of Incident Ovarian Cancer

Shelley S. Tworoger,1,3 I-Min Lee,2,3 Julie E. Buring,2,3 Bernard Rosner,1,4 Bruce W. Hollis,5 and Susan E. Hankinson1,3

1Channing Laboratory, Department of Medicine and 2Division of Preventive Medicine, Department of Medicine, Brigham and Women’s Hospital and Harvard Medical School; Departments of 3Epidemiology and 4Biostatistics, Harvard School of Public Health, Boston, Massachusetts; and 5Department of Pediatrics, Medical University of South Carolina, Charleston, South Carolina

Abstract

Few modifiable factors are known to reduce ovarian cancer risk. Ecologic studies and experimental data suggest that vitamin D may reduce ovarian cancer risk. Therefore, we examined whether plasma concentrations of 25-hydroxyvitamin D (a measure of overall vitamin D status) and 1,25-dihydroxyvitamin D (biologically active form) were associated with risk of epithelial ovarian cancer in a nested-case control study using data from three prospective cohorts: the Nurses’ Health Study (NHS), NHSII, and the Women’s Health Study ( WHS). The analysis had 224 cases (161 from NHS/NHSII and 63 from WHS) and 603 controls (matching ratio, 1:3 for NHS/NHSII and 1:2 for WHS). Women ranged in age from 34 to 73 years (mean, 56 years). We did not observe significant associations between 25-hydroxyvitamin D [top versus bottom quartile: relative risk (RR), 0.83; 95% confidence interval (95% CI), 0.49-1.39; \( P_{\text{trend}} = 0.57 \)] or 1,25-dihydroxyvitamin D levels (RR, 1.14; 95% CI, 0.70-1.85, \( P_{\text{trend}} = 0.93 \)) and ovarian cancer risk. Study-specific associations were not statistically significant and no statistical heterogeneity existed between studies (\( P = 0.66 \), 25-hydroxyvitamin D; \( P = 0.40 \), 1,25-dihydroxyvitamin D). However, there was a significant inverse association among overweight and obese women for 25-hydroxyvitamin D levels (RR, 0.39; 95% CI, 0.16-0.93; \( P_{\text{trend}} = 0.04 \)). Further, those with adequate (≥32 ng/mL) versus inadequate 25-hydroxyvitamin D levels had a modestly decreased risk of serous ovarian cancer (RR, 0.64; 95% CI, 0.39-1.05). Overall, our results do not suggest that plasma vitamin D levels are associated with risk of ovarian cancer. However, we observed significant associations in some subgroups, which should be evaluated further in other studies because increasing vitamin D intake is an easy preventive measure to adopt. (Cancer Epidemiol Biomarkers Prev 2007;16(4):783–8)

Introduction

Increasing evidence suggests a role for vitamin D in reducing cancer risk by decreasing proliferation and increasing apoptosis (1). Experimental and epidemiologic studies have suggested that vitamin D may be specifically associated with a reduced risk of ovarian cancer. For example, animal data suggest that vitamin D is involved in ovarian development and fertility (2, 3). Further, the vitamin D receptor is weakly to moderately expressed in normal ovarian cells, with stronger expression in ovarian cancer cell lines and tumor tissue (4-8). In normal Chinese hamster ovarian cells and in normal human mammary cells, vitamin D administration in vitro inhibited cell growth in a dose-dependent manner (9, 10); there are no similar studies of normal human ovarian cells. Additional in vitro and in vivo evidence indicates that 1,25-dihydroxyvitamin D can inhibit cell growth and induce apoptosis in ovarian cancer cell lines by reducing the number of cells in the S phase of the cell cycle (5, 6, 11-16).

Several ecological studies reported that ovarian cancer mortality was inversely associated with UV-B exposure, which initiates vitamin D production in the skin, based on location of residence or occupation (17-21). In addition, some studies of dietary intake of vitamin D observed an inverse association with ovarian cancer risk (22, 23), but others did not (24, 25). It is difficult to study dietary intake of vitamin D because it is strongly correlated with lactose intake, which may increase risk of ovarian cancer (24, 26). Thus, plasma vitamin D levels may be more useful in investigating a potential relationship with ovarian cancer. However, to our knowledge, no study has examined the association between circulating vitamin D levels and risk of ovarian cancer. Therefore, we examined whether plasma concentrations of 25-hydroxyvitamin D (a measure of overall vitamin D status) and 1,25-dihydroxyvitamin D (the biologically active form) were associated with risk of epithelial ovarian cancer in a prospective, nested-case control study among three cohort studies: the Nurses’ Health Study (NHS), NHSII, and the Women’s Health Study (WHS). The three cohorts were combined to provide sufficient sample size to evaluate these associations.

Materials and Methods

Study Population

NHS/NHSII. The NHS cohort was established in 1976 among 121,700 U.S. female registered nurses, ages 30 to 55 years, and the NHSII was established in 1989 among 116,609 female registered nurses, ages 25 to 42 years. Women in both cohorts completed an initial questionnaire and have been followed biennially by questionnaire to update exposure status and disease diagnoses. The racial/ethnic breakdown of the NHS is 96% Caucasian, 2% African American, 1% Asian, and 1% Hispanic, and of NHSII is 94% Caucasian, 2% Asian, 2% African American, and 2% Hispanic.

In 1989 to 1990, 32,826 NHS participants (ages 43-69 years) provided blood samples and completed a short questionnaire (27). Briefly, women arranged to have their blood drawn and shipped with an icepack via overnight courier to our laboratory where it was processed and separated into plasma...
(heparin), RBC, and WBC components. Follow-up of the NHS blood study cohort was 98% in 2004.

Between 1996 and 1999, 29,611 NHSII participants (ages 32-54 years) provided blood samples and completed a short questionnaire (28). Briefly, premenopausal women (n = 18,521) who had not taken hormones, been pregnant, or lactated within 6 months provided blood samples drawn on the 3rd to 5th day of their menstrual cycle (follicular draw) and 7 to 9 days before the anticipated start of their next cycle (luteal draw, called timed samples). Follicular plasma was aliquoted by the participant and frozen. Other women (n = 11,090) provided a single 30-mL untimed blood sample. Luteal and untimed samples were shipped and processed identically to the NHS samples. Follow-up of the NHSII blood study cohort was 98% in 2003.

All samples have been stored in liquid nitrogen freezers since collection. These studies were approved by the Committee on the Use of Human Subjects in Research at the Brigham and Women’s Hospital.

Cases were diagnosed with ovarian cancer after blood collection but before June 1, 2004 (NHS) or June 1, 2003 (NHSII). Overall, 161 epithelial ovarian or peritoneal cancer cases were confirmed by medical record review (141 from NHS and 20 from NHSII). Mean time from blood draw to diagnosis was 80 months (range, 1-174 months). Cases were matched to 20 from NHSII. Overall, 161 epithelial ovarian or peritoneal cancer cases were identified; the mean time from blood draw to diagnosis with at least one ovary remaining and was ≥47 (nonsmokers) or ≥45 (smokers) years old (32). Regardless of postmenopausal hormone use status, we considered a woman to be postmenopausal if (a) her natural menstrual periods had ceased permanently or (b) she had a hysterectomy with at least one ovary remaining and was ≥56 (nonsmokers) or ≥54 (smokers) years old (for the NHS and NHSII) or ≥60 years old (for the WHS). The remaining women, most of whom had a simple hysterectomy or were unsure of their menopausal status, were considered to be of unknown menopausal status.

**Laboratory Assays.** Vitamin D analytes (25-hydroxy and 1,25-dihydroxy) were assayed byRIA, as previously described (33), in four batches (two for NHS, one for NHSII, and one for WHS) at the laboratory of one of the authors (B.W.H.). Briefly, the RIA used radiiodinated tracers, with a prior acetonitrile extraction. Case-control sets and samples from the same study were assayed together, ordered randomly, and labeled to mask case-control status and quality control status. The intra-assay coefficient of variation from blinded, replicate, quality control samples (10% of the total sample number) ranged from 8.0% to 9.6% for 25-hydroxyvitamin D and from 8.6% to 14.1% for 1,25-dihydroxyvitamin D. The correlation of 25-hydroxyvitamin D in whole blood processed immediately versus processed 24 or 48 h after collection was 0.97 and 0.95, respectively.

**Statistical Analysis.** Outliers (34) were identified separately by sample type because samples collected in citrate tend to have lower values due to dilution by the preservative (35). For heparin plasma, those with 25-hydroxyvitamin D >60 ng/mL were set to missing (n = 1); there were no outliers for 1,25-dihydroxyvitamin D. For citrate plasma, those with 25-hydroxyvitamin D <5.0 ng/mL (n = 2) or 1,25-dihydroxyvitamin D <10.1 pg/mL (n = 3) were set to missing. Overall, 224 cases and 606 controls were available for analysis. Relative risks (RR) and 95% confidence intervals (95% CI) were determined using conditional logistic regression comparing quartiles of vitamin D concentrations, with cutoff points based on the control distribution within each plasma type (36). We also considered season-specific quartiles for three seasons: winter to early spring (January, February, March, April); summer to early fall (July, August, September, October); and late-spring/late fall (May, June, November, December). However, results did not differ from using overall cutoff points and thus only the latter are presented. Further, we examined whether having inadequate 25-hydroxyvitamin D levels (<32 ng/mL; ref. 37) was associated with ovarian cancer risk; individuals with levels below this cutoff point have secondary hyperparathyroidism, impaired calcium absorption, and low bone mineral density (37). To correct for the dilution factor in citrate samples, we multiplied 25-hydroxyvitamin D levels by 1.163 (35) before classification of having inadequate levels.

Because vitamin D levels differed by sample type, we included probit scores of vitamin D levels as a continuous variable for all trend tests (using the Wald test) to standardize for between-sample variability (38); probit scores, unlike z scores, have a normal distribution even if the original data are skewed. We considered multiple prior potential confounders including ever use of postmenopausal hormone, body mass index (BMI) at blood draw, parity, lactose intake, duration of oral contraceptive use, season of blood draw, age at menopause, tubal ligation, family history of ovarian cancer, physical activity, and duration of multivitamin use. The following confounders were included in the final model: ever use of postmenopausal hormone (yes, no), BMI (continuous), parity (continuous), lactose intake (quintiles, missing), duration of oral contraceptive use (never, <3, 3-<5, 5+ years), and season of...
blood draw (as noted above). Because there was some difference in the confounding by duration of oral contraceptive use and BMI at blood draw between studies, we included an interaction term between study and these two variables in models including these variables.

In secondary analyses, we excluded cases diagnosed within 2 years of blood collection and evaluation associations among only invasive or serious cases because etiologic factors may vary by histologic type; we only had enough serious cases to evaluate separately. We also stratified by season (summer, other), age at diagnosis (<55, ≥55 years), menopausal status at blood draw or diagnosis (premenopausal, postmenopausal), BMI (<25, ≥25 kg/m²), and multivitamin use (ever, never) because these factors may alter vitamin D levels. Interaction terms using the above cutoff points crossed with continuous vitamin D probit scores were used to determine the $P_{het}$-heterogeneity. These analyses used unconditional logistic regression adjusting for matching factors, including age at blood draw (continuous), fasting status (yes, no), time of blood draw (1-8 am, 9 am-noon, 1 pm-midnight), month of blood draw (continuous), menopausal status at blood draw and diagnosis (premenopausal, postmenopausal, unknown), and study (NHS, NHSII, WHS).

## Results

Women in the NHS/NHSII ranged in age from 34 to 69 years (mean, 56 years) and women in the WHS ranged from 45 to 73 years (mean, 56 years) at blood collection (Table 1). With the exception of family history of ovarian cancer, tubal ligation, postmenopausal hormone/oral contraceptive use, and multivitamin use, characteristics of the participants were similar between the studies. In the NHS/NHSII, cases were more likely to have a family history of ovarian cancer (9.3%) than controls (3.5%); the opposite trend was observed in the WHS based on only two cases and seven controls. In general, controls were less likely to have used postmenopausal hormones and more likely to have used oral contraceptives than cases. Vitamin D levels were lower for the WHS participants, likely because citrate plasma tubes dilute the sample (35). Neither 25-hydroxyvitamin D nor 1,25-dihydroxyvitamin D levels differed between cases and controls within each study ($P ≥ 0.22$, NHS/NHSII or WHS).

Overall, we did not observe significant associations between quartiles of 25-hydroxyvitamin D (top versus bottom quartile: RR, 0.83; 95% CI, 0.49-1.39; $P_{trend} = 0.57$) or 1,25-dihydroxyvitamin D levels (corresponding RR, 1.14; 95% CI, 0.70-1.85; $P_{trend} = 0.93$) and ovarian cancer risk (Table 2). Study-specific associations were not statistically significant and there was no statistical heterogeneity between studies ($P = 0.66$, 25-hydroxyvitamin D; $P = 0.40$, 1,25-dihydroxyvitamin D); therefore, data were combined for the remaining analyses. Inclusion of both 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D in the model simultaneously did not alter the observed associations (data not shown). Results were similar when excluding cases diagnosed within 2 years of blood draw (25-hydroxyvitamin D: corresponding RR, 0.74; 95% CI, 0.43-1.29; $P_{trend} = 0.43$; 1,25-dihydroxyvitamin D: RR, 1.17; 95% CI, 0.69-1.97; $P_{trend} = 0.91$) and when including only invasive or serious cases (data not shown).

We observed a significant inverse association between 25-hydroxyvitamin D and risk of ovarian cancer among women with a BMI ≥25 kg/m² ($P_{trend} = 0.04$ and $P_{het}$-heterogeneity = 0.06, versus BMI <25 kg/m²; Table 3). Among overweight and obese women, the RR comparing the top versus bottom quartile of 25-hydroxyvitamin D levels was 0.39 (95% CI, 0.16-0.93), whereas among lean women, no association was observed (RR, 1.17; 95% CI, 0.61-2.23). The association with 1,25-dihydroxyvitamin D did not differ by BMI (≥25 kg/m²: RR, 0.95; ≤25 kg/m²: RR, 1.08). The associations for 25-hydroxy and 1,25-dihydroxyvitamin D did not differ by age at diagnosis, multivitamin use, menopausal status at blood collection or diagnosis, or season of blood collection (data not shown).

After excluding cases diagnosed within 2 years of blood collection, women with adequate (≥32 ng/mL; ref. 37) versus inadequate 25-hydroxyvitamin D levels had a modestly decreased risk of ovarian cancer (RR, 0.67; 95% CI, 0.43-1.05); adequate vitamin D levels also were associated with a reduced risk of serous tumors (RR, 0.64; 95% CI, 0.39-1.05; Table 4). The association seemed to be stronger for women who were overweight or obese (RR, 0.47).

## Discussion

To our knowledge, this is the first prospective study of the association between plasma 25-hydroxy and 1,25-dihydroxyvitamin D levels and risk of ovarian cancer. Our overall results suggest that increasing plasma 25-hydroxyvitamin D levels are not associated with ovarian cancer risk, although we

| Table 1. Characteristics at blood collection of cases and their matched controls from NHS/NHSII and WHS |
|-------------------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Sample size | NHS/NHSII | WHS |
| Age [mean (SD)], y | 55.9 (7.8) | 55.9 (7.8) | 55.7 (7.2) | 55.5 (7.0) |
| Age at menarche [mean (SD)], y | 12.5 (1.4) | 12.6 (1.4) | 12.6 (1.1) | 12.5 (1.4) |
| Parity* [mean (SD)] | 3.2 (1.7) | 3.3 (1.5) | 3.0 (1.3) | 3.0 (1.3) |
| BMI at blood draw [mean (SD)], kg/m² | 25.4 (5.5) | 25.1 (4.4) | 24.6 (3.9) | 25.0 (4.4) |
| Physical activity [mean (SD)], MET-h/wk | 16.9 (17.0) | 16.2 (18.1) | 14.5 (15.0) | 16.7 (16.7) |
| Premenopausal at blood draw, % | 28.6 | 29.1 | 25.4 | 30.0 |
| Family history of ovarian cancer, % | 9.3 | 3.5 | 3.2 | 5.7 |
| Tubal ligation, 7 % | 14.9 | 17.1 | 19.1 | 27.1 |
| Ever used postmenopausal hormones, 7 % | 59.6 | 56.3 | 82.9 | 82.6 |
| Ever used oral contraceptives, % | 46.0 | 49.9 | 65.1 | 71.3 |
| Ever used multivitamins, % | 70.8 | 65.7 | 87.3 | 89.3 |
| Median 25-hydroxyvitamin D 7, 8 (10th-90th percentile), ng/mL | 25.9 (13.8-36.9) | 26.5 (16.2-37.2) | 23.2 (13.9-29.7) | 22.0 (12.1-32.7) |
| Median 1,25-dihydroxyvitamin D 7, 8 (10th-90th percentile), pg/mL | 56.1 (25.6-49.5) | 56.1 (27.0-47.5) | 31.6 (20.4-39.5) | 30.8 (20.6-43.4) |

*Among parous women only.
1 Among postmenopausal women.
7 In the NHS and NHSII, vitamin D levels were measured in heparin plasma; in the WHS, levels were measured in citrate plasma.
8 values comparing levels between cases and controls in the NHS/NHSII were 0.22 for 25-hydroxyvitamin D and 0.69 for 1,25-dihydroxyvitamin D. The corresponding P values for WHS were 0.90 and 0.52, respectively.
observed an inverse association among the subgroup of overweight/obese women. Women with inadequate 25-hydroxyvitamin D levels may have a modestly increased risk of serious ovarian cancer, but the results were not significant. We did not observe any clear association between 1,25-dihydroxyvitamin D levels and risk of ovarian cancer in this population.

Our results generally are consistent with those observed in ecologic studies of UV-B exposure and ovarian cancer mortality (17-21), as well as with similar prospective studies of breast (39) and colon (40) cancers. For both of these cancer types and in our data, only 25-hydroxyvitamin D levels were suggestively associated with a decreased risk, whereas no association was observed for 1,25-dihydroxyvitamin D. This pattern suggests that 25-hydroxyvitamin D levels may be a better reflection of overall vitamin D exposure than 1,25-dihydroxyvitamin D levels (39), although 1,25-dihydroxyvitamin D is the active metabolite that binds to the vitamin D receptor (41). Interestingly, 1,25-dihydroxyvitamin D concentrations are tightly regulated, whereas 25-hydroxyvitamin D levels are more responsive to sun exposure or intake of vitamin D rich foods or supplements (41). Recent data suggest that 25-hydroxyvitamin D levels may have independent effects at various target tissues due to extrarenal conversion to 1,25-dihydroxyvitamin D, including in ovarian tissue (6, 7). Ovarian cancer cells and tissue contain measurable levels of 1α-hydroxylase and 24-hydroxylase (4, 7, 15), which can convert 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D. It is possible that 1,25-dihydroxyvitamin D formed through this process only acts intracellularly or as an autocrine/paracrine factor, and therefore is not measured in circulating levels (42).

If the proportion of 1,25-dihydroxyvitamin D from this latter mechanism is high, it is reasonable to then assume that circulating levels of 25-hydroxyvitamin D may better reflect exposure at the tissue level. Given that the correlation between the two analytes was relatively low in our study (Spearman \( r = 0.29 \)), it is possible that only 25-hydroxyvitamin D may have an effect on ovarian cancer risk. However, this hypothesis should be explored in more detail in other studies.

Experimental data support a role of vitamin D analogs in ovarian cancer risk. A number of studies have observed that 1,25-dihydroxyvitamin D inhibits ovarian cancer cell growth (5, 6, 11, 13-16) and increases apoptosis (12). High 1,25-dihydroxyvitamin D levels also can increase vitamin D receptor expression in ovarian cancer cell lines (12). One study reported that 25-hydroxyvitamin D slightly increased cell growth of ovarian cancer cells; however, this effect was reduced on exposure to higher concentrations of 25-hydroxyvitamin D (15).

Interestingly, we observed that the association between vitamin D and ovarian cancer risk was stronger among overweight and obese women. The amount of adipose tissue in humans is inversely related to circulating 25-hydroxyvitamin D levels, likely due to uptake of vitamin D by fat cells (43-46). It is possible that this may lead to more bioavailable vitamin D at the tissue level or that circulating levels are better reflective of long-term tissue exposure in this subgroup. A study of rats fed vitamin D indicated that adipose tissue levels of vitamin D increased significantly and that vitamin D was slowly released from adipose tissue, particularly during fasting (47). This suggests that adipose tissue may be an important factor in determining long-term vitamin D status.

### Table 2. RRs (95% CIs) of ovarian cancer by quartile of plasma vitamin D concentrations among women in NHS/NHSII and WHS

<table>
<thead>
<tr>
<th></th>
<th>25-Hydroxyvitamin D concentrations, ng/mL</th>
<th>1,25-Dihydroxyvitamin D concentrations, pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25-OH D Quartile 1</td>
<td>25-OH D Quartile 2</td>
</tr>
<tr>
<td>NHS/NHSII Quartile 1</td>
<td>&lt;20.6</td>
<td>20.6-&lt;26.5</td>
</tr>
<tr>
<td>Unadjusted</td>
<td>1.0 (reference)</td>
<td>0.83 (0.51-1.35)</td>
</tr>
<tr>
<td>Adjusted</td>
<td>1.0 (reference)</td>
<td>0.95 (0.56-1.55)</td>
</tr>
<tr>
<td>WHS Quartile 1</td>
<td>&lt;17.4</td>
<td>17.4-&lt;22.0</td>
</tr>
<tr>
<td>Unadjusted</td>
<td>1.0 (reference)</td>
<td>1.37 (0.53-3.35)</td>
</tr>
<tr>
<td>Adjusted</td>
<td>1.0 (reference)</td>
<td>1.45 (0.52-4.05)</td>
</tr>
<tr>
<td>Adjusted</td>
<td>1.0 (reference)</td>
<td>0.97 (0.61-1.52)</td>
</tr>
</tbody>
</table>

*P*<sub>trend</sub> = Determined using study-specific probit rankings of vitamin D concentrations.

*Adjusted for ever use of postmenopausal hormones, BMI at blood draw, parity, lactose intake, duration of oral contraceptive use, season of blood draw, and the interaction between study with both duration of oral contraceptive use and BMI at blood draw.

<table>
<thead>
<tr>
<th></th>
<th>25-OH D Quartile 1</th>
<th>25-OH D Quartile 2</th>
<th>25-OH D Quartile 3</th>
<th>25-OH D Quartile 4</th>
<th>1,25-DH D Quartile 1</th>
<th>1,25-DH D Quartile 2</th>
<th>1,25-DH D Quartile 3</th>
<th>1,25-DH D Quartile 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>NHS/NHSII Quartile 1</td>
<td>&lt;31.2</td>
<td>31.2-&lt;36.1</td>
<td>36.1-&lt;42.3</td>
<td>≥42.3</td>
<td>&lt;24.8</td>
<td>24.8-&lt;30.8</td>
<td>30.8-&lt;37.5</td>
<td>≥37.5</td>
</tr>
<tr>
<td>Unadjusted</td>
<td>1.0 (reference)</td>
<td>1.17 (0.70-1.96)</td>
<td>1.05 (0.62-1.77)</td>
<td>1.20 (0.72-2.02)</td>
<td>1.0 (reference)</td>
<td>0.84 (0.34-2.05)</td>
<td>1.32 (0.59-2.96)</td>
<td>0.50 (0.18-1.38)</td>
</tr>
<tr>
<td>Adjusted</td>
<td>1.0 (reference)</td>
<td>1.26 (0.73-2.19)</td>
<td>1.16 (0.67-2.01)</td>
<td>1.33 (0.77-2.29)</td>
<td>1.0 (reference)</td>
<td>0.84 (0.31-2.27)</td>
<td>1.47 (0.60-3.59)</td>
<td>0.50 (0.15-1.67)</td>
</tr>
<tr>
<td>WHS Quartile 1</td>
<td>&lt;24.8</td>
<td>24.8-&lt;30.8</td>
<td>30.8-&lt;37.5</td>
<td>≥37.5</td>
<td>1.0 (reference)</td>
<td>1.06 (0.68-1.66)</td>
<td>1.13 (0.73-1.74)</td>
<td>1.02 (0.65-1.60)</td>
</tr>
<tr>
<td>Unadjusted</td>
<td>1.0 (reference)</td>
<td>1.15 (0.71-1.85)</td>
<td>1.25 (0.79-1.97)</td>
<td>1.14 (0.70-1.85)</td>
<td>1.0 (reference)</td>
<td>1.15 (0.71-1.85)</td>
<td>1.25 (0.79-1.97)</td>
<td>1.14 (0.70-1.85)</td>
</tr>
</tbody>
</table>

*P*<sub>trend</sub> = Determined using study-specific probit rankings of vitamin D concentrations.

*Adjusted for ever use of postmenopausal hormones, BMI at blood draw, parity, lactose intake, duration of oral contraceptive use, season of blood draw, and the interaction between study with both duration of oral contraceptive use and BMI at blood draw.

* adjusted for heterogeneity comparing NHS/NHSII versus WHS is 0.66 for 25-hydroxyvitamin D and 0.40 for 1,25-dihydroxyvitamin D.
NOTE: Data were adjusted for ever use of postmenopausal hormones, BMI at blood draw, parity, lactose intake, duration of oral contraceptive use, season of blood draw, the interaction between study with both duration of oral contraceptive use and BMI at blood draw, and matching factors, including age at blood draw, fasting status, time of blood draw, month of blood draw, menopausal status at baseline and diagnosis, recent postmenopausal hormone use before blood draw, and study.

* Determined using study-specific probit rankings of vitamin D concentrations.

† P_{heterogeneity} between stratification groups compares the slope of the probit rankings of vitamin D levels.

(47). More research is needed to better understand these potential interrelationships.

Our study has several limitations and strengths. One limitation is that the NHS/NHSII and WHS collected different sample types (heparin and citrate plasma, respectively). In addition, citrate plasma can dilute specimens, thus lowering the measured concentrations (35), although the levels were similar between the two studies when adjusting for the dilution factor. Vitamin D levels also vary across the year; therefore, we matched cases and controls on date of the blood draw and also examined season-specific quartile cutoff points. Although there may be some general measurement error in our assay of vitamin D levels, using plasma levels provides an integrated measure of dietary intake, sunlight exposure, and genetic variability for vitamin D exposure. In addition, we only had one blood sample per person, which may not reflect exposure over the 14 years of follow-up. However, the intraclass correlation over 3 years was 0.70 for 25-hydroxyvitamin D and 0.50 for 1,25-dihydroxyvitamin D (48), suggesting that one sample is fairly representative at least over a 3-year period. The strength of this study was its prospective nature, with blood samples being collected before disease diagnosis. We also had >200 ovarian cancer cases, thus providing reasonable power to detect an association among all women and for serous tumors, but not for other ovarian cancer subtypes.

Overall, we observed no clear association of plasma vitamin D levels and ovarian cancer risk; however, our study is suggestive that inadequate levels of 25-hydroxyvitamin D may be associated with an increased risk of ovarian cancer of the serous subtype in overweight/obese women. These findings should be evaluated in future prospective studies because, if an association exists, increasing vitamin D intake/production could provide a simple and important mechanism for preventing ovarian cancer.

Acknowledgments

We thank the women in the NHS, NHSII, and WHS studies for their valuable participation; Marilyn Chown and Jeanne Sparrow for data management support; and Dr. Walter C. Willett for helpful comments on the manuscript.

Table 4. RRs (95% CIs) of ovarian cancer for women with adequate versus inadequate plasma 25-hydroxyvitamin D concentrations among women in NHS/NHSII and WHS combined

<table>
<thead>
<tr>
<th>n, cases/control</th>
<th>Inadequate levels (&lt;32 ng/mL)</th>
<th>Adequate levels (≥32 ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI at blood, kg/m²</td>
<td>25-Hydroxyvitamin D concentrations, ng/mL</td>
<td>1,25-Dihydroxyvitamin D concentrations, ng/mL</td>
</tr>
<tr>
<td>&lt;25</td>
<td>136/356</td>
<td>1.0 (reference)</td>
</tr>
<tr>
<td>≥25</td>
<td>86/240</td>
<td>1.0 (reference)</td>
</tr>
<tr>
<td>Age at diagnosis/reference date, y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;55</td>
<td>58/149</td>
<td>1.0 (reference)</td>
</tr>
<tr>
<td>≥55</td>
<td>164/447</td>
<td>1.0 (reference)</td>
</tr>
<tr>
<td>Multivitamin use</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never user</td>
<td>55/178</td>
<td>1.0 (reference)</td>
</tr>
<tr>
<td>Current/past user</td>
<td>165/420</td>
<td>1.0 (reference)</td>
</tr>
</tbody>
</table>

NOTE: Data were adjusted for ever use of postmenopausal hormones, BMI at blood draw, parity, lactose intake, duration of oral contraceptive use, season of blood draw, the interaction between study with both duration of oral contraceptive use and BMI at blood draw, and matching factors, including age at blood draw, fasting status, time of blood draw, month of blood draw, menopausal status at baseline and diagnosis, recent postmenopausal hormone use before blood draw, and study.

* P_{interaction} for BMI is 0.21 compared with BMI <25 kg/m².
References


7. Anderson MG, Nakane M, Ruan X, Kroeger PE, Wu-Wong JR. Expression of


9. Anderson MG, Nakane M, Ruan X, Kroeger PE, Wu-Wong JR. Expression of


Plasma 25-Hydroxyvitamin D and 1,25-Dihydroxyvitamin D and Risk of Incident Ovarian Cancer

Shelley S. Tworoger, I-Min Lee, Julie E. Buring, et al.


Updated version
Access the most recent version of this article at:
http://cebp.aacrjournals.org/content/16/4/783

Cited articles
This article cites 46 articles, 19 of which you can access for free at:
http://cebp.aacrjournals.org/content/16/4/783.full.html#ref-list-1

Citing articles
This article has been cited by 19 HighWire-hosted articles. Access the articles at:
/content/16/4/783.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.