Plasma 1,25-Dihydroxy- and 25-Hydroxyvitamin D and Adenomatous Polyps of the Distal Colorectum[1]

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Abstract

1,25-dihydroxyvitamin D [1,25(OH)$_2$D] inhibits proliferation and promotes differentiation of human colon cancer cell lines. Epidemiological findings, although not entirely consistent, suggest an inverse relationship between vitamin D intake and colorectal cancer and adenoma, colorectal cancer precursor lesions. We evaluated the relationship of plasma 1,25(OH)$_2$D and 25-hydroxyvitamin D [25(OH)D] with distal colorectal adenoma among 326 matched case and control pairs (nested in the prospective Nurses' Health Study), who provided blood in 1989–1990 and who underwent endoscopy in 1989–1996. Plasma vitamin D metabolite concentrations were determined blindly by RIA. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated from multiple conditional logistic regression models. Mean plasma 1,25(OH)$_2$D and 25(OH)D levels did not significantly differ ($P = 0.3$ and 0.7, respectively) between cases (31.6 ± 8.4 pg/ml and 26.4 ± 10.6 ng/ml, respectively) and controls (32.2 ± 8.6 pg/ml and 26.8 ± 10.2 ng/ml, respectively). However, women whose plasma 1,25(OH)$_2$D concentration was below 26.0 pg/ml (a level typically considered to be below normal) were at increased risk of distal colorectal adenoma (OR, 1.58; 95% CI, 1.03–2.40). Compared with the lowest 1,25(OH)$_2$D quartile, women in the second (OR, 0.64; 95% CI, 0.41–1.02), third (OR, 0.80; 95% CI, 0.50–1.30), or upper (OR, 0.71; 95% CI, 0.43–1.15) quartiles were at a statistically nonsignificant lower risk of adenoma. The relationship was stronger for large/villous adenoma and among those with consistent vitamin D intake over the 10 years prior to blood draw. Compared with women in the lowest quartile, for plasma 25(OH)D, women in the second (OR, 0.64; 95% CI, 0.41–1.00) and third (OR, 0.58; 95% CI, 0.36–0.95) quartiles were at a statistically significantly lower risk of distal colorectal adenoma, but there was no difference in risk in the top quartile (OR, 1.04; 95% CI, 0.66–1.66). We conclude that women who have low levels of circulating 1,25(OH)$_2$D may be at higher risk of distal colorectal adenomas, but additional study is warranted.

Introduction

Previously we observed in the Nurses' Health Study a weak inverse association between vitamin D intake from the diet and supplements and colorectal cancer risk (1), consistent with three other prospective studies (2–4). Experimental studies have demonstrated that the hormonally active form of vitamin D, 1,25(OH)$_2$D[3] inhibits human colonic cell and colon cancer cell line proliferation (5–7), whereas diets deficient in vitamin D increase colonic crypt hyperplasia and hyperproliferation (8). Taken together, this evidence suggests that vitamin D may affect the incidence of colorectal adenomas, colon cancer precursor lesions that are thought to arise 10 or more years before malignancy (9). However, in the Nurses' Health Study, a cohort of women, as well as in a cohort of 50,000 men, no association between vitamin D intake from the diet and supplements and colorectal adenoma was found, except for an inverse relationship for vitamin D and rectal adenoma in the women (10).

The immediate precursor of 1,25(OH)$_2$D is 25(OH)D, which is formed in the liver from cholecalciferol (vitamin D) and which is considered to be the best indicator of nutritional vitamin D status (11). The major dietary sources of vitamin D are fish, eggs, butter, fortified milk products, and multivitamins and specific vitamin D supplements. The conversion of 7-dehydrocholesterol to vitamin D in the skin by UV light contributes to vitamin D stores (12). Production of 1,25(OH)$_2$D, which is present in plasma at a concentration of about 30 pg/ml, is tightly regulated (13) and typically does not substantially change with alterations in dietary vitamin D intake and sunlight exposure. Conversely, 25(OH)D level is not tightly homeostatically controlled in adults (14) and, thus, reflects both dietary and sunlight sources of vitamin D. 25(OH)D circulates bound to vitamin D-binding protein at a concentration of 10–50 ng/ml (13). The half-lives of 25(OH)D and 1,25(OH)$_2$D are 3 weeks and 4–6 h, respectively (15).

Epidemiological studies of dietary intake of vitamin D and colorectal cancer or adenoma have not considered the vitamin D contribution from sunlight. Three studies (16–18), two of which were conducted in the same cohort (16, 17), have evaluated the relationship of blood levels of vitamin D metabolites and colorectal cancer. Two (16, 18), but not the third (17),

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[3] The abbreviations used are: 1,25(OH)$_2$D, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D; OR, odds ratio; CI, confidence interval; RR, relative risk.

showed an inverse relationship for 25(OH)D. The studies (17, 18) that measured $1,25(OH)_2D$ concentration noted no relationship with colorectal cancer.

In this report, we present our findings from a case-control study nested in the prospective Nurses' Health Study on the relationship of distal colorectal adenoma to plasma 25(OH)D and $1,25(OH)_2D$. This analysis thus integrates dietary and sunlight sources of vitamin D.

Materials and Methods

Study Population. Cases and controls were drawn from among participants in the Nurses' Health Study, an ongoing prospective study of 121,700 United States female registered nurses. Details of the design and follow-up of this cohort have been described previously (19). Briefly, at enrollment in 1976, the women, who were 30–55 years old, completed a mailed questionnaire providing information on risk factors for cancer and cardiovascular disease. A semiquantitative food frequency questionnaire was added in 1980. Biennially, updated exposure and disease information was collected by mail, including reports of endoscopy and polyp diagnosis, and dietary information was updated in 1984, 1986, 1990, and 1994. In May 1989 through June 1991, 32,826 participants provided a blood specimen. After receipt by overnight courier, the chilled heparinized blood was centrifuged, aliquoted into plasma, erythrocytes, and buffy coat, and stored in liquid nitrogen freezers.

Selection of Cases and Controls. Cases and controls were chosen from among women who supplied a blood sample in 1989–1991, had a sigmoidoscopy or colonoscopy after providing a blood sample (1989–1996), and were free from diagnosed cancer (except nonmelanoma skin cancer) and polyps before endoscopy. Newly diagnosed polyps (in individuals who before the date of blood draw never had a polyp diagnosed) were reported on the 1990, 1992, 1994, or 1996 questionnaires. These polyps were confirmed to be adenomatous by review of histopathological reports and were classified by location (proximal or distal colon, or rectum), size (<1 cm, ≥ 1 cm), and histology (villous, tubulovillous, tubular) by study investigators blinded to exposure information. Hyperplastic and other nonadenomatous polyps were excluded. We were able to obtain medical records for 93% of the women reporting a polyp diagnosis.

Because most of the endoscopies were limited to the sigmoid colon and, thus, could not detect proximal colon adenomas, we included as cases only women with adenomas of the distal colon and rectum. Because endoscopied controls who had a sigmoidoscopy may also have had an undetected proximal adenoma, we included women with detected proximal colon adenomas in the pool of individuals who were eligible to be selected as a control. Controls were matched to cases based on endoscopy during the same 2-year period, year of birth, indication(s) for endoscopy (58% gastrointestinal symptoms, 48% routine screening, 19% because of family history), time period of first or most recent endoscopy excluding the one in the current time period, and date of blood draw. For all of the cases, a matched control was successfully identified. A total of 326 matched pairs were included in the analysis. Of the cases, 36.8% and of the controls, 31.9% had had a negative endoscopy prior to the blood collection period, with 50% of those endoscopies having taken place 10 or more years before blood draw.

Plasma $1,25(OH)_2D$ and 25(OH)D Assays. Plasma $1,25(OH)_2D$ and 25(OH)D concentrations were determined by RIA as described previously (11, 20). The mean intrapair coefficients of variation calculated from blinded quality control samples were 9.9% for $1,25(OH)_2D$ and 7.5% for 25(OH)D.

Assessment of Other Factors. Mean values for anthropometric, dietary, and other covariates were computed from the 1980-through-1990 questionnaires, including body mass index (weight in kg/height in m^2), physical activity [metabolic equivalent tasks (MET)-h/week], regular aspirin use (2+ days/week), cigarette smoking (pack-years), alcohol consumption (g/day), red meat intake (servings/day), and dietary intake of methionine (g/day) adjusted for total energy intake by residual analysis (21). Mean values were used to obtain measures of consistent exposure over time to these factors. We also assessed current (1990) use of postmenopausal hormones, and vitamin D and calcium-containing multivitamins and supplements. In this cohort, current postmenopausal hormone use rather than past use is more strongly related to risk of colorectal cancer and adenoma (22), and the relatively short half-lives of the two vitamin D metabolites suggest that plasma concentrations would reflect current vitamin D and calcium intakes. We used 1980 energy-adjusted dietary intake of folate (μg/day) plus intake from multivitamins and supplements to represent past exposure level; only long-term use of multivitamins and supplements for more than 15 years appears to be inversely associated with risk of colorectal neoplasia in this cohort (23) and intake of folic acid has increased in the recent years. We obtained mean daily solar UV irradiation (24) for the UV monitoring station closest to the postal code of each woman's residence in 1990.

Data Analysis. We estimated ORs and corresponding 95% CIs for the relationship between plasma $1,25(OH)_2D$ and 25(OH)D and adenomatous polyps of the distal colon and rectum from conditional logistic regression models with the vitamin D metabolites entered in quartiles with cutpoints based on the distribution among controls. We also estimated the OR for having a plasma $1,25(OH)_2D$ level below 26.0 pg/ml (25) or for having a plasma 25(OH)D level below 15 ng/ml (26), levels typically considered to be clinically below normal. We considered distal colorectal adenoma, and separately distal colon adenoma, as well as adenoma size (small, <1 cm; large, ≥ 1 cm) and potential for progression to colorectal cancer (lower risk: small and tubular histology; higher risk: large or villous/tubulovillous histology). To adjust for possible confounding, we entered body mass index, physical activity, regular aspirin use, cigarette pack-years smoked, and intake of alcohol, red meat, folate, and methionine, and current postmenopausal hormone use as covariates in conditional logistic regression models. Covariates chosen for inclusion in the multivariate models were based on *a priori* hypotheses for colorectal cancer and adenoma risk factors. To increase efficiency, we modeled all of the covariates as continuous terms, except aspirin and postmenopausal hormone use, which we modeled as binary. We tested for trend by entering continuous terms for $1,25(OH)_2D$ or 25(OH)D into the conditional logistic regression model. We used stratified analysis to examine whether the association between distal colorectal adenoma and clinically low plasma $1,25(OH)_2D$ level varied by use of vitamin D supplements in 1990 (binary), consistent total vitamin D intake (within 1 decile in 1980 and 1990, binary) or calcium supplement use in 1990 (binary), mean daily solar UV irradiation (cutpoint at median, binary), or season of blood draw (winter/early spring: January, February, March, April; summer/early fall: July, August, September, October; late spring/late fall: May, June, November, December). To test for statistical multiplicative interaction, the product of clinically low $1,25(OH)_2D$ and each of the above factors ex-

Table 1 Characteristics[a] of distal colorectal adenoma cases (326) and matched controls (326) nested in the Nurses' Health Study, 1989–1996

	Case	Control	P^b
Plasma 1,25(OH)$_2$D in 1989–1991 (pg/ml)			
Mean ± SD	31.6 ± 8.4	32.2 ± 8.6	0.3[c]
Median	31.1	31.4	
90th percentile	42.6	42.4	
10th percentile	21.4	21.6	
<26.0 pg/ml (%)	27.6	21.2	0.03
Plasma 25(OH)D in 1989–1991 (ng/ml)			
Mean ± SD	26.4 ± 10.6	26.8 ± 10.2	0.7[c]
Median	25.3	25.6	
90th percentile	40.1	39.5	
10th percentile	13.9	14.9	
Plasma 1,25(OH)$_2$D/25(OH)D ($\times 10^3$)	1.4 ± 0.77	1.4 ± 7.4	0.6
Mean age in 1990 (yr)	58.5 ± 6.6	58.5 ± 6.6	Matched
Mean solar radiation[d] in 1990 (Langleys/day)	354 ± 61	350 ± 61	0.4
Mean vitamin D intake (IU/day)	313 ± 187	333 ± 216	0.2
Mean calcium intake (mg/day)	977 ± 334	992 ± 333	0.6
Vitamin D supplement use in 1990 (%)	36.5	38.3	0.6
Calcium supplement use in 1990 (%)	49.1	48.5	0.9
Mean phosphorus intake (mg/day)	1153 ± 173	1160 ± 188	0.6
Mean protein intake (g/day)	75.2 ± 9.8	75.1 ± 10.9	0.9
Mean body mass index (kg/m^2)	25.0 ± 4.0	24.9 ± 3.8	0.8
Mean physical activity (MET[e]-h/week)	14.6 ± 19.2	16.1 ± 19.2	0.3
Regular aspirin use (%)	22.7	29.4	0.05
Mean alcohol intake (g/day)	7.3 ± 10.6	6.5 ± 9.4	0.3
Mean cigarette pack-years smoked	18.1 ± 22.6	15.2 ± 21.5	0.08
Mean red meat intake (servings/day)	0.29 ± 0.20	0.29 ± 0.21	0.9
Mean folate intake in 1980 (μg/day)	356 ± 217	367 ± 253	0.6
Mean methionine intake (g/day)	1.79 ± 0.29	1.79 ± 0.31	0.9
Postmenopausal hormone use in 1990 (%)	32.2	38.7	0.07

[a] Unless otherwise indicated, values for characteristics are the average exposure calculated from the 1980–1990 questionnaires.
[b] For the hypothesis test of no difference in means (paired *t* test) or distribution (McNemar's test) between adenoma cases and controls.
[c] Wilcoxon (nonparametric) sign-rank test: $P = 0.4$ and 0.8 for 1,25(OH)$_2$D and 25(OH)D, respectively.
[d] Mean daily solar UV irradiation to the postal code area of residence in 1990.
[e] MET, metabolic equivalent tasks.

pressed as a continuous term (season as an ordinal variable) was entered along with each covariate into the conditional logistic regression model; we evaluated the statistical significance of the interaction terms by the Wald test. All of the analyses were done in SAS version 6.12 (SAS Institute, Cary, NC).

Results

Adenoma cases and controls did not differ in intake of vitamin D or calcium from the diet and supplements, mean daily solar UV irradiation in the postal code in which the nurse resided in 1990, or on other dietary factors that might influence vitamin D metabolite levels or calcium balance (Table 1). Cases tended to be less likely to use aspirin and postmenopausal hormones regularly and to be more likely to smoke more (Table 1).

Mean plasma 1,25(OH)$_2$D and 25(OH)D did not significantly differ between cases and controls (Table 1). However, women whose plasma concentration was below the typical lower cutpoint for clinically normal, 1,25(OH)$_2$D level of 26.0 pg/ml (cases, $n = 90$; controls, $n = 69$), had an increased risk of distal colorectal adenoma of 1.58 (95% CI, 1.03–2.40) after controlling for the dietary and life-style covariates. The OR for distal colorectal adenoma among women whose 25(OH)D level was below 15 ng/ml (cases, $n = 42$, controls, $n = 33$) was 1.29 (95% CI, 0.75–2.20). Only 10 cases and 8 controls had low levels of both vitamin D metabolites.

Compared with the lowest 1,25(OH)$_2$D quartile, women in the second (OR, 0.64), third (OR, 0.80), and upper (OR, 0.71) quartiles appeared to have a slight, but not significant, downward trend (*P*-trend, 0.4) in multivariate models (Table 2). For plasma 25(OH)D level, women in the second (OR, 0.64) and third (OR, 0.58) quartiles were at significantly lower risk of distal colorectal adenoma compared with the first quartile. There was no difference in risk in the top quartile (OR, 1.04; Table 2). The associations were essentially unchanged before and after controlling for body mass index, physical activity, aspirin use, cigarette pack-years smoked, alcohol consumption, intake of red meat, folate, and methionine, and postmenopausal hormone use (Table 2). The associations also were similar before and after mutually controlling for 1,25(OH)$_2$D and 25(OH)D (not shown). Adjusting for intake of vitamin D and calcium overall and from dairy and nondairy sources, or adjusting for milk consumption did not attenuate these associations. There was no association between the ratio of 1,25(OH)$_2$D:25(OH)D and distal colorectal adenoma (ORs for quartiles 1–4: 1.00, 0.83, 0.90, and 0.91; *P*-trend, 0.8).

Adenoma cases were diagnosed >0–1 year ($n = 46$), >1–2 years ($n = 53$), >2–3 years ($n = 57$), >3–4 years ($n = 53$), >4–5 years ($n = 53$), >5–6 years ($n = 42$), and >6–7 years ($n = 22$) after the date of blood draw. Although all of the cases included in the analysis were those who never had a polyp diagnosed prior to the date of blood draw, only about one-third had had a negative endoscopy prior to blood draw, and one-half of these took place at least 10 years before blood draw. Thus, some may have had an undetected polyp present at the time of blood draw. To limit the number of prevalent adenoma cases, we restricted the analysis to the 227 women whose adenomas were diagnosed more than 2 years after providing a blood sample and to their matched pairs. The observed ORs for distal colorectal adenoma did not notably differ from those overall [1,25(OH)$_2$D ORs for quartiles 1–4: 1.00, 0.64, 0.75, 0.68; 25(OH)D ORs for quartiles 1 to 4: 1.00, 0.73, 0.85, 0.96].

Because season of the year in which blood was collected likely influences levels of 25(OH)D, and possibly 1,25(OH)$_2$D in individuals who have low circulating levels of 25(OH)D, we repeated the multivariable analysis using separate cutpoints for quartiles by season of blood collection. The observed associations between distal colorectal adenoma and 1,25(OH)$_2$D (ORs for quartiles 1–4: 1.00, 0.59, 0.72, 0.68) and 25(OH)D (ORs for quartiles 1–4: 1.00, 0.51, 0.71, 0.94) were comparable with the overall findings.

There was no association between rectal adenoma and 1,25(OH)$_2$D level, although the U-shaped relationship for 25(OH)D that was seen overall was also present for rectal adenoma (Table 3). As would be expected, restricting the cases to those of the distal colon (80% of cases) resulted in a modest increase in strength of the inverse association for the top three quartiles (Table 3). The OR comparing women below the typical normal cutpoint for 1,25(OH)$_2$D level increased to 1.86 (95% CI, 1.16–2.98) for distal colon adenoma (cases, $n = 75$; controls, $n = 53$). Women whose 25(OH)D level was below the typical normal cutpoint were not at increased risk of distal colon adenoma (OR, 1.12; 95% CI, 0.62–2.02). The U-shaped relationship for 25(OH)D level was observed irrespective of adenoma size (large: ≥1 cm, small: <1 cm) or histology (villous/tubulovillous, tubular). The lower risk of adenoma among women in the top three quartiles of 1,25(OH)$_2$D was stronger for larger or villous/tubulovillous adenoma than for

Table 2 Relationship between plasma $1,25(OH)_2D$ and 25(OH)D and distal colorectal adenoma among 326 matched pairs nested in the Nurses' Health Study, 1989–1996

	Quartile				*P*-trend[a]
	1	2	3	4	
Median $1,25(OH)_2D$ (pg/ml)	22.3	29.0	33.9	40.9	
No. of cases	97	71	84	74	
No. of controls	80	84	80	82	
OR[b]	1.00	0.68	0.83	0.71	0.3
95% CI		0.44–1.06	0.53–1.31	0.44–1.13	
OR[c]	1.00	0.64	0.80	0.71	0.4
95% CI		0.41–1.02	0.50–1.30	0.43–1.15	
Median 25(OH)D (ng/ml)	16.3	22.6	28.3	38.0	
No. of cases	103	62	61	100	
No. of controls	82	80	82	82	
OR[b]	1.00	0.63	0.59	0.96	0.7
95% CI		0.41–0.97	0.38–0.93	0.63–1.47	
OR[c]	1.00	0.64	0.58	1.04	1.0
95% CI		0.41–1.00	0.36–0.95	0.66–1.66	

[a] Test for trend calculated by entering a continuous term in the conditional logistic regression model.
[b] OR and 95% CI estimated from conditional logistic regression model.
[c] OR and 95% CI estimated from conditional logistic regression model after adjusting for mean values (1980–1990) for body mass index, physical activity, aspirin use, cigarette pack-years smoked, alcohol consumption, intake of red meat and methionine, 1980 folic acid intake, and 1990 postmenopausal hormone use.

small and tubular adenoma (Table 3). For women whose plasma $1,25(OH)_2D$ level was below 26.0 pg/ml, the OR for large or villous/tubulovillous was 1.62 (95% CI, 0.87–3.03) and for small and tubular adenoma was 1.45 (95% CI, 0.84–2.50).

We examined whether the association between $1,25(OH)_2D$ and adenoma varied by levels of possible determinants of its precursor, 25(OH)D. There was no obvious variation in risk of adenoma associated with clinically low plasma $1,25(OH)_2D$ levels by vitamin D supplement use in 1990 (*P*-interaction, 0.8), calcium supplement use in 1990 (*P*-interaction, 0.9), higher mean daily UV irradiation for the postal code of 1990 residence (*P*-interaction, 0.3), or season of the year in which blood was drawn (*P*-interaction, 1.0). Women whose total intake of vitamin D was consistent between 1980 and 1990, and thus who may have had more stable plasma 25(OH)D and $1,25(OH)_2D$ levels over time, had a higher risk of distal colorectal adenoma associated with clinically low plasma $1,25(OH)_2D$ levels (OR, 2.46; 95% CI, 1.30–4.67) than women whose intake was not consistent (OR, 1.25; 95% CI, 0.70–2.23), although the interaction was not statistically significant (*P*-interaction, 0.19). A notably inverse association for adenoma and plasma $1,25(OH)_2D$ among women with consistent vitamin D intake was observed (*P*-trend, 0.01), whereas there was no association among those whose intake was not consistent (*P*-trend, 0.7; *P*-interaction, 0.08). Among women with consistent vitamin D intake and compared with quartile 1, the ORs for quartiles 2–4 were 0.64 (95% CI, 0.31–1.30), 0.41 (95% CI, 0.19–0.91), and 0.34 (95% CI, 0.16–0.75).

Discussion

We examined the relationship of plasma $1,25(OH)_2D$ and 25(OH)D concentrations with adenomatous polyps of the distal colon and rectum in a nested case-control study of women, ages 44–69 years, in 1990 and observed that women whose plasma concentrations of $1,25(OH)_2D$ were below the level typically considered to be clinically normal had an increased risk of adenoma. Although the association between plasma $1,25(OH)_2D$ and adenoma was nonlinear, women in the top three quartiles were at lower risk. The relationship of 25(OH)D with adenoma was U-shaped; those with the lowest and the highest plasma concentrations had a higher risk of distal colorectal adenomas than did those with intermediate concentrations. These relationships were essentially unaltered when we controlled for known or suspected risk factors for colorectal cancer and adenoma.

Because risk factors for cancer and adenoma have been seen to differ between the colon and rectum [*e.g.*, physical activity and adenoma (27)], we presented the results combined, and separately for the rectum and distal colon. The reduced risk of adenoma associated with higher plasma $1,25(OH)_2D$ was apparent for the distal colon, but not for the rectum, although the U-shaped relationship of 25(OH)D with adenoma was present for both sites. Because the progression to malignancy is thought to proceed from small to large adenoma and with increasingly less differentiated histology, and because vitamin D metabolites may play a role in control over cellular proliferation and differentiation, we also explored whether the relationship of plasma levels of $1,25(OH)_2D$ and 25(OH)D with distal colorectal adenoma differed by the combination of size and histology. A reduced risk associated with higher $1,25(OH)_2D$ levels was present for adenomas that were large ($\geq$1 cm) or villous/tubulovillous, but not small and tubular. This finding possibly suggests that $1,25(OH)_2D$ may not act on the development of adenoma but may play a role in the control of progression to larger and more dysplastic adenoma phenotypes. The U-shaped relationship between 25(OH)D and adenoma was present for both of the size/histology combinations.

We used plasma concentration of two vitamin D metabolites as measures of vitamin D nutritional [25(OH)D] and hormonal [$1,25(OH)_2D$] status. $1,25(OH)_2D$ is generated in the proximal renal tubules of the kidney by hydroxylation of 25(OH)D and is regulated in part by parathyroid hormone in response to low plasma calcium (12). 25(OH)D is generated from hydroxylation by liver microsomal mixed function oxidase of cholecalciferol ingested in the diet or obtained as a skin photoproduct of 7-dehydrocholesterol (12). Unlike $1,25(OH)_2D$, the production of 25(OH)D is not tightly regulated in adults.

Like other steroid hormones, $1,25(OH)_2D$ transactivates

Table 3 Relationship between plasma 1,25(OH)$_2$D and 25(OH)D and distal colorectal adenoma by site and size/histology among 326 matched pairs nested in the Nurses' Health Study, 1989–1996

	Quartile[a]				*P*-trend[b]
	1	2	3	4	
Rectum[c]					
1,25(OH)$_2$D					
No. cases/controls	16/17	18/14	13/16	18/18	
OR[d]	1.00	1.35	0.58	1.06	0.9
95% CI		0.41–4.43	0.16–2.09	0.30–3.73	
25(OH)D					
No. cases/controls	24/18	7/16	10/13	24/18	
OR[d]	1.00	0.38	0.34	1.59	0.9
95% CI		0.12–1.19	0.08–1.42	0.50–5.03	
Distal colon[c]					
1,25(OH)$_2$D					
No. cases/controls	81/63	53/70	71/64	56/64	
OR[d]	1.00	0.51	0.78	0.60	0.2
95% CI		0.30–0.86	0.45–1.34	0.34–1.04	
25(OH)D					
No. cases/controls	79/64	55/64	51/69	76/64	
OR[d]	1.00	0.71	0.60	1.02	1.0
95% CI		0.43–1.18	0.35–1.02	0.60–1.73	
Large, villous, or tubulovillous[c]					
1,25(OH)$_2$D					
No. cases/controls	41/31	36/38	35/32	30/41	
OR[d]	1.00	0.62	0.73	0.48	0.1
95% CI		0.29–1.32	0.33–1.61	0.21–1.08	
25(OH)D					
No. cases/controls	45/36	24/37	26/35	47/34	
OR[d]	1.00	0.54	0.67	1.30	0.9
95% CI		0.25–1.16	0.31–1.44	0.59–2.86	
Small and tubular[c]					
1,25(OH)$_2$D					
No. cases/controls	51/45	33/44	47/47	43/38	
OR[d]	1.00	0.63	0.86	0.94	0.6
95% CI		0.32–1.24	0.44–1.68	0.48–1.86	
25(OH)D					
No. cases/controls	55/43	36/40	33/45	50/46	
OR[d]	1.00	0.64	0.54	0.93	0.6
95% CI		0.34–1.19	0.27–1.07	0.49–1.75	

[a] Quartile medians: 22.3, 29.0, 33.9, and 40.9 pg/ml for 1,25(OH)$_2$D and 16.3, 22.6, 28.3, 38.0 ng/ml for 25(OH)D.
[b] Test for trend calculated by entering a continuous term in the conditional logistic regression model.
[c] Numbers do not sum to total because of missing information on site, size, or histology.
[d] OR and 95% CI were estimated from conditional logistic regression model after adjusting for mean values (1980–1990) for body mass index, physical activity, aspirin use, cigarette pack-years smoked, alcohol consumption, intake of red meat and methionine, 1980 folic acid intake, and 1990 postmenopausal hormone use.

the transcription of target genes, of which one result is the stimulation of calcium transport across the small intestinal epithelium (12). Whether related to its effect on calcium or independent of it, 1,25(OH)$_2$D has been shown: (*a*) to inhibit proliferation and to induce differentiation of human colon cancer cell lines (5, 6) as well as other malignant tumor lines (28, 29); (*b*) to suppress growth of solid tumor xenografts *in vivo* (30); and (*c*) to reduce the incidence of carcinogen-induced colon tumors in rodents (31–33). Receptors for 1,25(OH)$_2$D have been detected in colon cancer cell lines (6, 34), and expression of the receptor is greater in well-differentiated cell lines than in those that are less well differentiated (5, 35, 36).

Because colorectal cancer mortality rates were higher in areas of the United States with the lowest exposure to sunlight, Garland and Garland (37) proposed in 1980 that vitamin D may play a preventive role in this cancer. Three additional ecological studies (38–40) that have evaluated the solar radiation and colorectal cancer relationship supported this hypothesis. Several cohort studies (1–4) and case-control studies (41–46) have evaluated the relationship between dietary intake of vitamin D and colorectal cancer. Some of these studies suggested weak to moderate inverse associations (1–4, 41, 42, 45), ranging from a RR for colorectal cancer of 0.88, comparing extreme quintiles of combined intake of vitamin D from the diet and supplements in the Nurses' Health Study of 501 cases among 89,448 women followed for twelve years (1), to a RR of colorectal cancer of 0.5, comparing extreme quartiles of vitamin D intake (*P*-trend < 0.05) in the Western Electric study of 47 cases among 1,924 men followed for 19 years (4).

Three studies have evaluated the relationship of plasma levels of vitamin D metabolites and colorectal cancer, two of which were conducted in the Washington County, Maryland cohort (16, 17), and the other nested in the Finnish α-Tocopherol, β-Carotene Cancer Prevention Study (18). The study by Garland *et al.* (16), which included 34 cases with a range from several months to 8 years between blood donation and colorectal cancer diagnosis and 67 controls, showed an inverse relationship between 25(OH)D and colorectal cancer, although in the highest quintile, the inverse association was not as strong as in the middle three quintiles. In contrast, the study by Braun

et al. (17), which included 57 cases with 10–17 years between donation and diagnosis and 114 controls, and which measured both 25(OH)D and $1,25(OH)_2D$, did not detect any notable relationships. In the Finnish study, no relationship was observed between serum $1,25(OH)_2D$ concentration and colorectal cancer among the 91 colon and 55 rectal cancer cases and 290 controls, but an inverse relation was suggested for 25(OH)D level, particularly for the rectum (18).

One case-control study (44) and two cohort studies (10) have reported on the relationship between vitamin D intake and colorectal adenoma. Findings were not consistent across gender or on the location within the colon. In the case-control study consisting of 154 small and 208 large adenoma cases, an inverse association with vitamin D intake was seen for small adenomas in women (extreme quintiles: OR, 0.4; *P*-trend, 0.04; Ref. 44), whereas in the cohort studies, an inverse relationship was seen only for rectal adenomas in women (extreme quintiles: RR, 0.30; *P*-trend, 0.005; Ref. 10).

There are several issues in the current analysis that require consideration. We cannot exclude the possibility that the biologically relevant vitamin D exposure to the target tissue is misclassified when using either of the plasma vitamin D metabolites as markers. Measurement error in a woman's typical circulating vitamin D metabolite concentrations may have occurred because we measured these levels at a single point in time. However, the extent of measurement error in the plasma levels is unlikely to differ between the cases and controls because of the study design and assay controls. Evidence for some attenuation of the $1,25(OH)_2D$ and adenoma relationship attributable to measurement error comes from the observation of a stronger increased risk of adenoma for clinically low plasma $1,25(OH)_2D$ and a stronger trend of decreasing risk of adenoma with increasing levels of $1,25(OH)_2D$ among those with consistent vitamin D intake from 1980 to 1990. Because plasma 25(OH)D level, as a precursor for $1,25(OH)_2D$, and low plasma $1,25(OH)_2D$ level in part, may be determined by dietary and supplemental intake of vitamin D, those with consistent vitamin D intake would more likely have vitamin D metabolite concentrations measured in blood collected in 1989/1990 that are representative of circulating levels in the previous 10 years. In any case, this earlier period may be more relevant for adenoma development and progression in women whose polyps were detected between 1989 and 1996.

We used 26 pg/ml as a definition of clinically low $1,25(OH)_2D$, which we selected *a priori.* Nearly 28% of the cases and 21% of the controls had plasma $1,25(OH)_2D$ concentrations that measured below 26 pg/ml When we reanalyzed the data using a second arbitrary cutpoint dependent on the distribution of $1,25(OH)_2D$ among the controls, below the bottom quartile of $1,25(OH)_2D$ ($<$26.9 pg/ml), the OR for distal colorectal adenoma was 1.41 (95% CI, 0.95–2.09) and the OR for distal colon adenoma was 1.64 (95% CI, 1.05–2.56). Whether the women in this nested case-control study who have $1,25(OH)_2D$ levels in the bottom quartile or $<$26.9 pg/ml are truly deficient is unknown. Estimated mean solar UV irradiation ($P = 0.05$) was significantly lower among the controls with low $1,25(OH)_2D$ than among the controls with normal range levels. However, current age or current or 1980–1990 average intake of vitamin D, calcium, or phosphorus, was not associated with $1,25(OH)_2D$ level.

Among users of multivitamins, intake of vitamin D may be confounded by folic acid in the multivitamins. Folic acid is inversely associated with colorectal cancer and adenoma in this cohort (47). We, therefore, stratified the analysis by users of multivitamins and supplements containing vitamin D. Among nonusers, who are thus less likely to be users of multivitamin and supplements containing folate, the relationship between plasma $1,25(OH)_2D$ and 25(OH)D was similar to the overall relationships.

We also examined whether the direct association of low $1,25(OH)_2D$ with adenoma varied by calcium-supplement use, because the use of such a supplement during the same period as the blood draw could result in a down-regulation of $1,25(OH)_2D$ level. However, no difference in risk of adenoma associated with low $1,25(OH)_2D$ was observed between users and nonusers of supplemental calcium. No variation in the risk of adenoma with clinically low $1,25(OH)_2D$ was observed in regard either to the estimated residential UV irradiation levels at the time of blood draw or to the season of the year, which both may be determinants of plasma 25(OH)D level and low $1,25(OH)_2D$ concentration.

Although blood was obtained before the detection of adenoma, because we did not require that participants undergo endoscopy at the time of blood draw to ensure that they were polyp-free, it is unknown what proportion of these adenomas were present at the time of blood draw. For adenomas that developed before blood was provided in 1989–1991, earlier plasma vitamin D metabolite concentrations may be more relevant. Nevertheless, when we limited the analysis to cases diagnosed more than 2 years after blood was obtained, the results were comparable with those observed overall.

To minimize confounding, we controlled for suspected colorectal neoplasia risk factors. The cases and controls in this sample of women in the Nurses' Health Study did not differ on some of these suspected colorectal neoplasia risk factors that were observed previously in the entire cohort (Table 1). A possible explanation for the lack of a difference is that these factors are more strongly associated with large adenoma ($\geq$1 cm) and cancer rather than with adenoma overall. To increase efficiency, particularly for subgroup analyses, we adjusted for the suspected colorectal neoplasia risk factors using continuous or binary terms. Although residual confounding is possible because of inadequate expression of these factors in the models, we believe that these factors are unlikely to be strongly related to vitamin D metabolite concentrations, and, thus, the degree of residual confounding is likely small.

We conclude that women who have low levels of circulating $1,25(OH)_2D$ concentrations may be at higher risk of distal colorectal adenoma. Nevertheless, additional studies in populations with differing ranges of circulating vitamin D metabolites along with measurement of plasma levels at several points in time are warranted.

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