Plasma Vitamin D Metabolites and Risk of Colorectal Cancer in Women

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Abstract

Objective: Experimental evidence suggests that 1,25-dihydroxyvitamin D and its precursor, 25-hydroxyvitamin D [25(OH)D], may aid in the prevention of colorectal cancer. We therefore examined risk in relation to plasma concentrations of these vitamin D metabolites. Methods: In a nested case-control study among women in the Nurses' Health Study, we identified 193 colorectal cancer cases, ages 46 to 78 years, diagnosed up to 11 years after blood collection. Two controls were matched per case on year of birth and month of blood draw. Odds ratios (OR) for risk of colorectal cancer were calculated using conditional logistic regression adjusted for body mass index, physical activity, smoking, family history, use of hormone replacement therapy, aspirin use, and dietary intakes. Results: We found a significant inverse linear association between plasma 25(OH)D and risk of colorectal cancer (P = 0.02). Among women in the highest quintile, the OR (95% confidence interval) was 0.53 (0.27–1.04). This inverse association remained strong when limited to women ≥60 years at blood collection (P = 0.006) but was not apparent among the younger women (P = 0.70). Benefit from higher 25(OH)D concentrations was observed for cancers at the distal colon and rectum (P = 0.02) but was not evident for those at the proximal colon (P = 0.81). In contrast to 25(OH)D, we did not observe an association between 1,25-dihydroxyvitamin D and colorectal cancer, although risk was elevated among the women in the highest quintile if they were also in the lower half of the 25(OH)D distribution (OR, 2.52; 95% confidence interval, 1.04–6.11). Conclusion: From these results and supporting evidence from previous studies, we conclude that higher plasma levels of 25(OH)D are associated with a lower risk of colorectal cancer in older women, particularly for cancers at the distal colon and rectum. (Cancer Epidemiol Biomarkers Prev 2004;13(9):1502–8)

Introduction

Experimental evidence suggests that vitamin D may reduce the risk of colorectal cancer through regulation of cellular proliferation and differentiation (1, 2) and inhibition of angiogenesis (3). These anticancer properties have been attributed primarily to 1,25-dihydroxyvitamin D [1,25$(OH)_2$D], the physiologically more active metabolite produced in the kidney from 25-hydroxyvitamin D [25(OH)D]. However, evidence now suggests that this conversion can be done in other tissues, including the colon (4), raising the possibility that anticancer effects may also be directly attributed to 25(OH)D (5).

The hypothesis that vitamin D may help to prevent colorectal cancer originated with the observation that colon cancer death rates were lowest in the states with the highest mean solar radiation (6), and this has been supported by population studies of disease incidence (7, 8). To investigate risk of colorectal cancer on an individual level, epidemiologic research has focused on vitamin D intake from foods and supplements. Among the prospective studies, most (9-13), but not all (14, 15), have reported an inverse association, including one from this Nurses' Health Study (NHS) group of investigators (12) in which a 67% lower risk of colorectal cancer was found among the women in the highest quintile of consistent vitamin D intake over time.

Studies of sunlight exposure and vitamin D consumption are interesting, but each explains only a portion of an individual's vitamin D status. A better indicator of status is plasma 25(OH)D (16) because it is determined not only by the amount of skin exposure to UV light and the quantity of vitamin D consumed from foods and supplements but also by the body's ability to produce cholecalciferol in the skin and to hydroxylate the cutaneous and food sources of cholecalciferol in the liver. Plasma levels of 25(OH)D usually range between 10 and 50 ng/mL (17). In contrast, 1,25$(OH)_2$D acts as a hormone to increase calcium absorption and plasma levels are regulated at ~30 pg/mL to ensure calcium homeostasis (17).

Only two studies have examined circulating vitamin D concentrations in relation to colorectal cancer (18-20). Within the Washington County, Maryland cohort, an

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initial report with 34 cases found a marginally significant lower risk of colon cancer among those with 25(OH)D ≥20 ng/mL compared with those with lower serum levels, although a linear relation was not observed (18). A subsequent report from this cohort based on 57 cases with a lag time between sample collection and diagnosis found a weaker association for 25(OH)D and none for $1,25(OH)_2D$ (19). The second study, which was nested within the male Finnish α-Tocopherol, β-Carotene Cancer Prevention Study cohort and included 146 colorectal cancer cases, reported a significant inverse association only for 25(OH)D and cancers of the distal colon and rectum and no association overall for $1,25(OH)_2D$ and colorectal cancer, although the low plasma vitamin D levels in Finland make application of these results to a U.S. population questionable. Studies of distal colorectal adenomas, precursor lesions of colorectal cancer, provide some additional support that low plasma levels of 25(OH)D (21, 22) and possibly $1,25(OH)_2D$ (23) contribute to cancer risk, although the data are inconclusive.

Given the importance of studying the role of vitamin D as a chemopreventive agent of colon and rectal cancers and the equivocal evidence from the research to date, we examined plasma 25(OH)D and $1,25(OH)_2D$ in colorectal cancer cases and controls among women in the NHS cohort. We also examined the proximal and distal colon and rectal cancers separately to compare our results with those from the α-Tocopherol, β-Carotene Study.

Materials and Methods

Study Population. The NHS is an ongoing cohort study with 121,700 female participants who were ages 30 to 55 years at study initiation in 1976. The design and follow-up procedures have been described in detail elsewhere (24, 25). Briefly, a mailed questionnaire is sent to participants every 2 years on which they are asked to report disease diagnoses and various characteristics and behaviors that are potential risk factors for cancers and other chronic diseases. Dietary information was first collected in 1980 with a food frequency questionnaire and is updated in alternate follow-up cycles. Blood samples were provided by 32,826 participants, ages 43 to 70 years, from June 1989 to 1990. The samples were collected in tubes with heparin and sent to us by overnight courier in chilled containers. On receipt, the bloods were centrifuged, aliquoted, and stored in liquid nitrogen freezers at −70°F.

Colorectal Cancer Cases and Controls. When colon or rectal cancer was reported by a participant on a NHS questionnaire, we requested medical records to confirm the report and to establish a date of diagnosis. We also identified colorectal cancer cases from death record information. For this analysis, we used the 193 confirmed incident cases from 1989 to June 2000 among the women who gave blood and had not reported any noncutaneous cancer previous to the colorectal cancer diagnosis. Most cases were confirmed by medical record (96%) and were classified by site and type. Cases for which we were unable to get medical records were reconfirmed by the participant in writing or by telephone. The mean age at diagnosis was 65.5 years (range 46.0-77.8 years). Squamous cell (n = 4), *in situ* (n = 26), and metastatic (n = 2) cancers from other sites were excluded. Reported diagnoses that were not confirmed as colon or rectal cancer after medical record review (n = 15) and those for which we could not obtain sufficient information (n = 9) were also excluded.

Two controls were randomly selected for each case from among the pool of women who provided blood samples and who had not reported any noncutaneous cancer diagnosis prior to the date of colorectal cancer of the case. Controls were matched to cases on year of birth and month of blood draw to account for seasonal variation in vitamin D blood levels. Three cases had only one matched control; one control blood sample was unsuitable for the vitamin D assays and two cases had been analyzed previously as part of a study of colorectal adenomas that required only one control per case (23).

Plasma Vitamin D Assays. 25(OH)D and $1,25(OH)_2D$ concentrations were measured by RIA (26). Each case and matched controls were assayed in the same laboratory run, although personnel were blind to the case-control status. Twenty-one masked triplets of quality control samples from pooled plasma sources were interspersed among the case and control samples. Their mean intraset coefficients of variation were 11.8% for 25(OH)D and 10.1% for $1,25(OH)_2D$.

Although all blood samples were assayed at the same laboratory, cases identified from the 1990, 1992, 1994, and 1996 NHS questionnaires (n = 102) and their matched controls were assayed in 2000 and those identified on the 1998 and 2000 questionnaires (n = 91 cases) were assayed in 2003. 25(OH)D values were lower and $1,25(OH)_2D$ values were higher in the earlier versus later assays (e.g., within 12 quality control samples from the same plasma pool, mean values for 25(OH)D were 19.2 ng/mL in the 2000 assays and 22.8 ng/mL in the 2003 assays; for $1,25(OH)_2D$, mean values were 31.3 and 25.2 pg/mL, respectively). Therefore, quantile categories for statistical analyses were determined separately for data from the 2000 and 2003 laboratory assays.

Colorectal Cancer Risk Factors. All data used in statistical analyses were collected on NHS questionnaires prior to or at the time of the blood draw. To assess long-term status, we used data from biennial questionnaires through 1990 to determine mean values for body mass index, physical activity, and dietary intakes of folate, methionine, retinol, alcohol, and red meat and to calculate total values for pack-years of smoking and duration of regular aspirin use. For menopausal status and use of hormone replacement therapy (HRT), we used the most recent (1988 or 1990) questionnaire data because current use of HRT is more strongly associated than past use with a reduced risk of colorectal cancer (27). We also used the most recent data for dietary intakes of vitamin D and calcium and for multivitamin and calcium supplement use because vitamin D and calcium intakes at the time of blood draw are most related to the plasma levels of vitamin D metabolites. Family history (parent or sibling) of colorectal cancer was positive if reported on the 1982 or 1988 NHS questionnaire. We also obtained the average daily availability of UV radiation from sun exposure by zip code of residence in 1990 (28). All nutrient intakes, except alcohol, were adjusted for total energy intake using the residual method (29).

Statistical Analyses. For the main analyses, we used conditional logistic regression models to calculate odds ratios (OR) with 95% confidence intervals (95% CI) for associations between 25(OH)D and $1,25(OH)_2D$ and colorectal cancer. These models used quintile categories of the vitamin D metabolites based on separate distributions among the controls assayed in 2000 and 2003. *P*s for linear trends in the associations were calculated from models with continuous vitamin D values.

To adjust for possible confounding, the following colorectal cancer risk factors were included as covariates in the multivariate models: continuous values of body mass index, physical activity, pack-years of smoking, and daily intakes of calcium, folate, methionine, retinol, red meat, and alcohol plus binary variables for categories of menopausal status and use of HRT (premenopausal or postmenopausal and a never, past, or current HRT user), duration of regular aspirin use (nonuser, <10 years, ≥10 years), and family history of colorectal cancer (yes or no). Although retinol is not a recognized risk factor for colorectal cancer, it was included as a covariate because it can interfere with vitamin D functioning (30). Models with continuous values for vitamin D metabolites included an indicator for year of assay (2000 or 2003). Average available UV light at zip code of residence and dietary or supplemental intakes of vitamin D were not included in the multivariate models because they are determinants of the vitamin D metabolite concentrations.

Unconditional logistic regression analyses that were further adjusted for the case-control matching factors of age and month of blood draw (grouped by season) showed very similar ORs to those from the conditional analyses. We therefore used unconditional logistic regression to examine whether associations between 25(OH)D and $1,25(OH)_2D$ and colorectal cancer differed by calcium or vitamin D intakes, HRT use, or average availability of UV light because analyses stratified by variables other than the matching factors break the case-control matching. *P*s for multiplicative interaction between the vitamin D metabolites and each stratifier were calculated by entering their product into the logistic regression model.

Case-control differences in vitamin D metabolite levels and other factors were tested for statistical significance using a paired *t* test.

Results

Mean plasma 25(OH)D was significantly lower ($P = 0.03$) in cases compared with controls among those assayed in 2003 (27.0 versus 30.3 ng/mL, respectively) but did not differ among those assayed in 2000 (23.6 versus 24.3 ng/mL, respectively), whereas $1,25(OH)_2D$ levels were similar in cases and controls for both assay years. As shown in Table 1, potential colorectal cancer risk factors did not differ significantly by case-control status but several were in the same direction as observed previously in the larger NHS cohort. Many of the risk factors differed significantly by plasma vitamin D

Table 1. Mean age and age-standardized characteristics at time of blood draw (1989–1990) among incident colorectal cancer cases (*n* = 193) and their matched controls (*n* = 383) and by lowest and highest quintiles of plasma 25(OH)D and $1,25(OH)_2D$ among both cases and controls in the NHS

	Cases	Controls	25(OH)D		$1,25(OH)_2D$	
			Quintile 1	Quintile 5	Quintile 1	Quintile 5
Age, y	60.0	60.0	60.1	60.2	60.8	60.8
Body mass index, kg/m^2	25.3	25.0	26.5	23.9*	26.3	24.1*
Physical activity, met-hr/wk	14.9	15.2	12.1	22.9*	13.2	18.1†
UV light from the sun, langleys/d‡	348	346	340	357†	352	355
Dietary intakes per day						
Calcium,§ mg	1,004	1,072	894	1,138*	1,212	1,084†
Vitamin D,§ μg	8.7	9.6	7.2	11.0*	10.3	10.0
Retinol,§ μg retinol equivalents	2,086	2,221	2,058	2,285	2,107	2,186
Folate,§ μg	402	415	373	454*	408	411
Methionine,§ g	1.8	1.8	1.8	1.8	1.9	1.8
Red meat, servings	1.1	1.1	1.2	1.1	1.2	1.1
Alcohol, g	7.6	6.4	6.1	9.3†	7.1	7.3
Family history of colorectal cancer (%)	18	15	12	22†	18	19
Calcium supplement user (%)	36	39	25	42*	47	34†
Multivitamin user (%)	39	36	29	44†	38	40
Aspirin user for ≥10 y (%)	9	10	8	7	9	15
Postmenopausal (%)	86	86	89	82	84	86
HRT user‖ (%)	29	37	21	49*	29	46†
Past smoker (%)	44	44	39	55†	42	50
Current smoker (%)	14	13	18	9†	13	11
Pack-years of smoking¶	29	25	30	25	28	25

NOTE: Values are standardized to the age distribution among cases and controls. Controls were matched to cases on year of birth and month of blood draw.

*$P < 0.01$ for cases versus controls or for quintile 5 versus quintile 1.

†$P < 0.05$ for cases versus controls or for quintile 5 versus quintile 1.

‡UV sunlight is average available at zip code of residence.

§Nutrients include intakes from foods plus supplements.

‖Percentage of women using HRT was calculated among postmenopausal women only.

¶Pack-years were calculated among past and current smokers only.

concentrations. Body mass index was negatively associated and physical activity and HRT use were positively associated with both 25(OH)D and $1,25(OH)_2D$. Associations for calcium intake and calcium supplement use were positive for 25(OH)D and negative for $1,25(OH)_2D$. Multivitamin use; intakes of vitamin D, folate, and alcohol; availability of UV light from the sun; and family history of colorectal cancer were all positively associated with 25(OH)D but unrelated to $1,25(OH)_2D$. Smokers were more likely to have low levels of 25(OH)D.

Plasma 25(OH)D was inversely associated with risk of colorectal cancer. In the top versus bottom quintile, risk was 47% lower (OR, 0.53; 95% CI, 0.27–1.04) and a significant inverse trend was detected (P = 0.02) after controlling for other risk factors for colorectal cancer (Table 2). Results from the unadjusted analyses were similar, indicating that there was little confounding by covariates. For $1,25(OH)_2D$, there was no dose-response association (P = 0.51), although risk of colorectal cancer was nonsignificantly elevated in the highest quintile (OR, 1.77; 95% CI, 0.93–3.36). HRT use was the primary confounder that raised the risk estimates from the unadjusted model. Results for both 25(OH)D and $1,25(OH)_2D$ were not notably changed when cases in the first year after blood draw (n = 15) were excluded or when vitamin D or average UV light at zip code of residence were added to the models (data not shown).

We conducted subanalyses to examine associations between the vitamin D metabolites and cancers at the colon (n = 149) and rectum (n = 44) and at the proximal (n = 78) and distal (n = 61) colon (Table 3). Due to smaller sample sizes in these subgroups, we used quartile or tertile cut points instead of quintiles. 25(OH)D exhibited a strong inverse linear association with risk of rectal cancer (P = 0.03), whereas its association with colon cancer, although still inverse, was not significant (P = 0.17). Within the colon, cancers at the distal end seemed to be more related to 25(OH)D concentrations than those at proximal sites, although case numbers were small and evidence for a distinction therefore was weak. When cancers at the rectum and distal colon were combined, a significant inverse dose-response association was observed (P = 0.02) and risk was 54% lower in the highest versus lowest quartile of 25(OH)D (OR, 0.46; 95% CI, 0.19–1.09). For $1,25(OH)_2D$, there was no evidence of an association with cancer risk at any colorectal site (data not shown).

We considered the relative contributions of plasma 25(OH)D and $1,25(OH)_2D$ to colorectal cancer risk by examining the effects of one within low and high strata of the other (defined by the median level among controls within year of assay). For 25(OH)D, we observed an inverse association in both low (P = 0.06) and high (P = 0.09) strata of $1,25(OH)_2D$. In contrast, a positive association between $1,25(OH)_2D$ and risk of colorectal cancer was evident only among women with lower 25(OH)D (Fig. 1). Compared with a reference group of women in the low 25(OH)D stratum and the lowest quintile of $1,25(OH)_2D$, those in the highest quintile of $1,25(OH)_2D$ had a significantly elevated OR (95% CI) of 2.52 (1.04–6.11) only if their 25(OH)D value was in the low stratum; high $1,25(OH)_2D$ was not associated with increased risk of colorectal cancer when the 25(OH)D value was in the high stratum (OR, 1.08; 95% CI, 0.48–2.40).

Because plasma 25(OH)D levels are largely determined by diet and sunlight, we examined the association between 25(OH)D and risk of colorectal cancer within strata of vitamin D consumption and average availability of UV light at zip code of residence. The association remained inverse among those with lower (<7.5 μg/d) or higher (≥7.5 μg/d) intakes of vitamin D. Differences were apparent by level of UV light, although a test for interaction was not significant (P = 0.16). Among the women residing in areas with ≥335 langleys/d of UV light (e.g., Florida, Texas, and California), the association remained significantly inverse (P = 0.01) and the OR (95% CI) in the highest versus lowest quintile of 25(OH)D was 0.25 (0.10-0.69), whereas no inverse association was detected among women in areas with <335 langleys/d (e.g., Massachusetts, New York, and Pennsylvania; OR, 1.24; 95% CI, 0.42–3.64 in the highest quintile).

Dairy foods are the primary dietary contributors to both vitamin D and calcium intakes and evidence

Table 2. Risk of colorectal cancer within quintiles of plasma 25(OH)D and $1,25(OH)_2D$ among women in the NHS, 1989–2000

Quintile	25(OH)D (ng/mL)					$1,25(OH)_2D$ (pg/mL)				
	Medians*		Cases/controls†	OR‡	Multivariate OR (95% CI)§	Medians*		Cases/controls†	OR‡	Multivariate OR (95% CI)§
	Lab 1	Lab 2				Lab 1	Lab 2			
1	14.9	17.4	53/77	1.00	1.00	24.6	18.7	34/77	1.00	1.00
2	19.6	24.8	47/79	0.87	0.93 (0.53–1.63)	29.4	24.2	42/78	1.24	1.45 (0.78–2.69)
3	24.1	29.6	35/75	0.70	0.79 (0.44–1.40)	33.2	29.8	33/76	1.01	0.93 (0.48–1.78)
4	27.9	34.5	29/77	0.52	0.58 (0.31–1.07)	36.8	33.8	38/76	1.17	1.21 (0.64–2.26)
5	35.3	44.5	29/75	0.53	0.53 (0.27–1.04)	43.7	42.3	46/75	1.46	1.77 (0.93–3.36)
P for trend‖				0.01	0.02				0.65	0.51

*Median 25(OH)D and 1,25(OH)2D values per quintile. Lab 1 values are from blood samples assayed in 2000. Lab 2 values are from blood samples assayed in 2003.

†No. cases/controls per quintile. Controls were matched to cases on year of birth and month of blood draw.

‡ORs from conditional logistic regression analyses without any covariates in the model.

§ORs and 95% CIs from conditional logistic regression analyses with adjustment for the following covariates: body mass index; physical activity; pack-years of smoking; menopausal status; use of HRT; duration of aspirin use; family history of colorectal cancer; and daily intakes of calcium folate, methionine, retinol, red meat, and alcohol.

‖P in test of linear trend in risk over continuous values of 25(OH)D and $1,25(OH)_2D$.

Table 3. Risk of colorectal cancer by site within quartiles or tertiles of plasma 25(OH)D, among women in the NHS, 1989–2000

Colon			Proximal colon			Distal colon		
Quartile	Cases/controls*	OR (95% CI)†	Tertile	Cases/controls*	OR (95% CI)†	Tertile	Cases/controls*	OR (95% CI)†
1	49/75	1.00	1	33/49	1.00	1	24/41	1.00
2	43/71	1.03 (0.56–1.89)	2	21/54	0.62 (0.28–1.37)	2	20/39	0.68 (0.27–1.74)
3	27/77	0.54 (0.28–1.03)	3	24/52	1.05 (0.43–2.56)	3	17/40	0.56 (0.19–1.67)
4	30/72	0.70 (0.35–1.38)						
P for trend‡		0.17			0.81			0.35

Rectum			Distal colon + rectum		
Tertile	Cases/controls*	OR (95% CI)†	Quartile	Cases/controls*	OR (95% CI)†
1	24/31	1.00	1	36/53	1.00
2	10/26	0.52 (0.14–1.93)	2	30/50	0.82 (0.38–01.78)
3	10/31	0.31 (0.08–1.31)	3	18/52	0.42 (0.19–0.91)
			4	21/53	0.46 (0.19–1.09)
P for trend‡		0.03			0.02

*No. cases/controls per quartile or tertile. Controls were matched to cases on year of birth and month of blood draw.
†ORs and 95% CIs from conditional logistic regression analyses with adjustment for the covariates listed in Table 2 (footnote §).
‡*P* in test of linear trend in risk over continuous values of 25(OH)D.

suggests that calcium, independent of vitamin D, may help to reduce the risk of colorectal cancer (13, 31, 32). Although our multivariate analyses were controlled for total calcium intake, we examined the association between 25(OH)D and risk of colorectal cancer stratified by calcium (<900 versus ≥900 mg/d) and found no evidence that the observed inverse association for 25(OH)D could be attributed to concurrent calcium intake. In both low and high calcium strata, risk of colorectal cancer decreased with higher 25(OH)D levels. The ORs (95% CIs) for the highest versus lowest quintiles were 0.38 (0.14-1.03; *P* for trend = 0.08) among those with lower calcium consumption and 0.66 (0.26-1.69; *P* for trend = 0.14) in the higher calcium group.

The association between 25(OH)D and risk of colorectal cancer seemed to be modified by age at blood collection, although a test for interaction was not statistically significant (*P* = 0.18). Among women ≥60 years, the association remained significantly inverse (*P* for trend = 0.006) and the OR (95% CI) in the highest quintile of 25(OH)D was 0.35 (0.14-0.87). Among women <60 years, there was no evidence of an inverse association (OR, 1.36; 95% CI, 0.48–3.92 in highest quintile; *P* for trend = 0.70). For postmenopausal women, risk of colorectal cancer was reduced in the highest quintile of 25(OH)D among both current HRT users (OR, 0.61; 95% CI, 0.16–2.41) and never/past users (OR, 0.41; 95% CI, 0.15–1.12).

Discussion

In this case-control analysis nested within the NHS cohort of women, we observed a statistically significant inverse dose-response relationship between plasma 25(OH)D and subsequent risk of colorectal cancer in which risk was 46% lower among the women in the highest versus lowest quintile of 25(OH)D. The benefit was significant for cancers of the rectum and distal colon, whereas there was little evidence that 25(OH)D was associated with a lower cancer risk at the proximal colon. It is plausible that effects may differ for the proximal and distal colorectal sites given differences in their molecular features and in risks associated with genetic and environmental factors (33). Within the NHS, for example, we reported previously that higher calcium intake was associated with a lower risk of cancer at the distal colon and rectum but not at the proximal colon (31). In contrast to 25(OH)D, we observed no association between $1,25(OH)_2D$ and risk of colorectal cancer. The lack of association for this more active vitamin D metabolite is not surprising given the tight regulation and low levels found in the blood and its short half-life of 4 to 6 hours (34).

Our results support the findings from the Finnish male α-Tocopherol, β-Carotene Study, which also found no

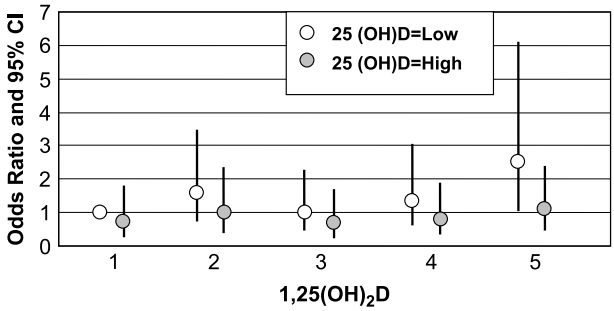

Figure 1. Risk of colorectal cancer within quintiles of plasma $1,25(OH)_2D$ by level of plasma 25(OH)D among women in the NHS, 1989–2000. The reference group are women in the lowest quintile of $1,25(OH)_2D$ and the lower stratum of 25(OH)D. ORs and 95% CIs are from an unconditional logistic regression analyses adjusted for age and season at time of blood draw plus the covariates in Table 2 (footnote §).

association between $1,25(OH)_2D$ and colorectal cancer but reported a nonsignificant 40% lower risk in the highest versus lowest quartile of 25(OH)D and a significant dose-response association ($P = 0.03$) when limited to cancers at the distal colon and rectum (20). This concurrence of results is reassuring given the difference in 25(OH)D levels in the NHS and Finnish populations (mean levels among controls were 27.1 ng/mL in NHS and 13.8 ng/mL in α-Tocopherol, β-Carotene Study). Our results differ from those reported in a previous NHS investigation of vitamin D metabolites in relation to distal colorectal adenomas (23). In that study, risk of adenoma was increased among the women with $1,25(OH)_2D$ concentrations below the level defined as clinical normal, whereas 25(OH)D exhibited a U-shaped relationship. Although adenomas are precursors of colorectal cancer, it is possible that the importance of vitamin D differs with progressive stages of carcinogenesis.

Although we did not observe a dose-response relation between $1,25(OH)_2D$ and colorectal cancer, risk was elevated among the women in the highest quintile. Further analysis revealed that risk became significantly elevated by 150% if the women in this highest quintile of $1,25(OH)_2D$ also had a low concentration of 25(OH)D. This may be an indication of early vitamin D insufficiency when circulating levels of $1,25(OH)_2D$ are mildly elevated due to secondary hyperparathyroidism (35). High $1,25(OH)_2D$ may also be an indication of low calcium intake, which in itself is thought to be a risk factor for colorectal cancer (13, 31, 32). Recent research suggests that standards for adequate 25(OH)D levels may need to be redefined to levels that sustain $1,25(OH)_2D$ concentrations, prevent an elevation in parathyroid hormone, and maintain calcium homeostasis (36). Some studies recommend a lower limit of 20 to 30 ng/mL for 25(OH)D sufficiency (37, 38), although levels <40 ng/mL have also been proposed as a definition of hypovitaminosis D (16, 35, 39).

Our observation of an inverse association between plasma 25(OH)D and risk of colorectal cancer is unlikely due to differential selection of cases and controls by exposure to UV sunlight because the mean available UV levels at their places of residence were very similar and results did not change when this variable was added to the regression model. However, availability of sunlight may not predict exposure to sunlight and plasma 25(OH)D values are likely to vary widely depending on the amount of time spent outside with skin exposed to sunlight and without sunscreen. An unexpected finding was that the inverse association between 25(OH)D and risk of colorectal cancer was evident only among the women residing in areas with higher available levels of UV light from the sun. Although plasma 25(OH)D concentrations were positively associated with the amount of UV light available, differences in 25(OH)D between low and high UV groups were too small to account for this difference in association with colorectal cancer risk.

Age seemed to modify the observed association between 25(OH)D and colorectal cancer, being significantly inverse among women ≥60 years but null among the younger women. Genetic factors in the development of colorectal cancer (40) may play a larger role in the earlier diagnoses, superceding benefits from vitamin D. It is also likely that the benefits of vitamin D are accentuated as insufficiency becomes more prevalent with age (41). In older adults, cutaneous production is reduced due to little time spent in the sun and/or use of sunscreens (42) and a reduced capacity of the skin to manufacture cholecalciferol (43). In addition, a lower consumption of dairy foods or diminished intestinal absorption of vitamin D (44) add to the likelihood of low 25(OH)D concentrations with age.

Although calcium and vitamin D work together metabolically and both are possible protective agents against colorectal cancer, it is unknown whether they interact in carcinogenesis. In a recent analysis of data from the Calcium Polyp Prevention Study randomized trial, 25(OH)D levels were associated with a reduced risk of recurrent adenoma only among the subjects receiving calcium supplements (45). Our results with colorectal cancer did not support this finding. Cancer risk decreased with higher 25(OH)D concentrations among those with total calcium intakes above or below 900 mg/d.

One strength of this study is that it assessed both 25(OH)D, a measure that integrates dietary and cutaneous sources of vitamin D, and $1,25(OH)_2D$, a measure of vitamin D hormonal status. The vitamin D metabolites were measured prior to colorectal cancer diagnosis and other major risk factors were assessed prior to blood collection to control for confounding. The absolute vitamin D values in this study should be used with caution because not all blood samples were assayed at the same time and changes in laboratory reagents may have introduced variation. Still, our results should be valid because statistical analyses used vitamin D rankings that were specific for year of assay.

Another limitation of this study is that the vitamin D metabolites were assessed from a single blood sample. Season of blood collection likely introduced some measurement error, for, although we matched controls to cases on month of blood draw, we did not have a measure of intraindividual variation in plasma vitamin D levels by season. In terms of change over time, we have found plasma vitamin D to be fairly consistent. Correlations were 0.70 ($P < 0.0001$) and 0.50 ($P < 0.0001$) for 25(OH)D and 1,25(OH)2D, respectively, from blood samples drawn ~3 years apart in a sample of 144 men of similar age to the NHS women in this study (E.L.G.).

In conclusion, older women with higher circulating levels of 25(OH)D may be at lower risk of colorectal cancer, particularly for cancers at the distal colon and rectum. Although further studies with repeated measures of vitamin D status are warranted, this study provides additional evidence for the importance of vitamin D for aging adults.

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