

A Nested Case–Control Study of Plasma 25-Hydroxyvitamin D Concentrations and Risk of Colorectal Cancer

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- Background** Low vitamin D status has long been implicated in colorectal carcinogenesis. We investigated this relationship in a nested case–control study within the Health Professionals Follow-up Study (HPFS), a large ongoing study of male health professionals living in the United States.
- Methods** Between 1993 and 2002, 179 colorectal cancer patients were diagnosed and matched to 356 control subjects by age and by month and year of blood collection. Results were also pooled with previously published results from the Nurses' Health Study (NHS) cohort, a large female cohort. Conditional logistic regression was used to analyze the association between plasma 25-hydroxyvitamin D [25(OH)D] and colorectal cancer, and pooled estimates were calculated using the method of DerSimonian and Laird. All statistical tests were two-sided.
- Results** In the HPFS, we observed a non-statistically significant inverse association between higher plasma 25(OH)D concentration and risk of colorectal cancer and a statistically significant inverse association for colon cancer (highest versus lowest quintile: odds ratio [OR] = 0.46, 95% confidence interval [CI] = 0.24 to 0.89; $P_{\text{trend}} = .005$). After pooling the results from the HPFS and NHS, higher plasma 25(OH)D concentrations were statistically significantly associated with decreased risks of both colorectal cancer (highest versus lowest quintile, OR = 0.66, 95% CI = 0.42 to 1.05; $P_{\text{trend}} = .01$) and colon cancer (highest versus lowest quintile, OR = 0.54, 95% CI = 0.34 to 0.86; $P_{\text{trend}} = .002$). Inverse associations with plasma 25(OH)D concentration did not differ by location of colon cancer (proximal versus distal), but the number of patients was small and none of the associations was statistically significant. Opposite relationships between plasma 25(OH)D levels and risk of rectal cancers were found among men (positive) and women (inverse).
- Conclusion** Our data provide additional support for the inverse association between vitamin D and colorectal and, in particular, colon cancer risk.

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Low vitamin D status has long been implicated in colorectal carcinogenesis, based primarily on findings that colorectal cancer mortality rates are lower in regions with higher mean sun exposure during the year than in regions with lower mean sun exposure during the year (1,2). In recent years, many observational studies have supported this hypothesis (3–5). The majority of observational studies have focused on the association between vitamin D intake and risk of colon or colorectal cancer (5), whereas fewer studies have examined the association with plasma or serum vitamin D concentrations (6–9). Plasma 25-hydroxyvitamin D [25(OH)D] concentrations may be a better marker of vitamin D status than vitamin D intake because vitamin D status is influenced by factors in addition to intake, such as sun exposure, the degree of conversion of 7-dehydrocholesterol to cholecalciferol in the skin, and the degree of conversion of cholecalciferol to 25(OH)D in the liver (10,11). In addition, plasma 25(OH)D has a relatively long half-life of 2–3 weeks (10,11). Hypothesized possible mechanisms through which the primary active metabolite of vitamin D, 1,25-dihydroxyvitamin D [1,25(OH)₂D], may be involved in colon

carcinogenesis include its ability to control cell growth and differentiation (12,13). Recent studies have suggested that, contrary to earlier beliefs, metabolic transformation of 25(OH)D to 1,25(OH)₂D may take place not only in the kidney but also in several other sites, including the colon (14–16).

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To investigate the association between plasma 25(OH)D concentration and the risk of colorectal cancer, we conducted a nested case-control study within the Health Professionals Follow-up Study (HPFS) cohort. To increase the statistical power for analyses by certain strata and by colon cancer subsite, we also combined our results with previously published results on the association between plasma vitamin D concentrations and colorectal cancer in a large female cohort, the Nurses' Health Study (NHS) (8).

Subjects and Methods

Study Population

The HPFS cohort has been described in detail elsewhere (17). In brief, this cohort was established in 1986 and included 51 129 male US health professionals who had provided information on a baseline questionnaire on medical history and lifestyle factors. Participants were also administered a 131-item food-frequency questionnaire (FFQ). Every 2 years, participants were asked to update their information on medical history and lifestyle factors, and every 4 years, they were also asked to complete another FFQ to update their dietary information.

Between 1993 and 1995, a subset of 18 225 health professionals from this cohort also provided a blood sample. Between the date of blood collection and January 31, 2002, 179 incident colorectal cancer case patients were diagnosed in this subset of participants (blood cohort). The vast majority of colorectal cancers (>90%) were confirmed through medical records; the remaining cases were confirmed by corroborating information on cancer diagnosis from participants (i.e., through consent forms sent by participants). Each case patient was matched to two control subjects from the blood cohort, who, at the time of the diagnosis of the case patient, had to be alive and cancer free. Case patients and control subjects were matched by age (within 2 years) and by year (same year) and month (within 1 month) of blood donation. Because one case patient could be matched to only one control subject and one control subject had to be excluded from the analysis due to laboratory error, the total number of control subjects was 356.

This study was approved by the Human Subjects Committee of the Harvard School of Public Health and the Committee on the Use of Human Subjects in Research at the Brigham and Women's Hospital, and written informed consent was obtained from all participants in the NHS and HPFS cohorts.

Laboratory Assays

All laboratory assays for plasma 25(OH)D were performed at the laboratory of Dr B. W. Hollis at the Medical University of South Carolina using a radioimmunoassay method (18). All laboratory personnel were blinded with regard to case-control status. Each triplet of samples from one case patient and his two matched control subjects was analyzed in the same batch. Fifteen quality control sets (triplets) had been randomly interspersed among the case-control samples. The mean intrapair coefficient of variation for plasma 25(OH)D based on these 15 quality control sets was 10.1%.

Questionnaire and Dietary Information

Information on lifestyle or dietary factors was obtained from the biannual questionnaires (i.e., 1986, 1988, 1990, 1992, and 1994) or

CONTEXT AND CAVEATS

Prior knowledge

A possible association has been noted between low vitamin D status and colorectal carcinogenesis.

Study design

Nested case-control study of plasma vitamin D, measured as 25(OH)D, and risk of colorectal cancer among male participants in the Health Professionals Follow-up Study (HPFS). Pooled analyses were also performed with previous results from the Nurses' Health Study.

Contribution

Higher plasma 25(OH)D concentrations were associated with a decreased risk of colon cancer among participants in the HPFS and with decreased risks of colon and colorectal cancer in the pooled analysis.

Implications

There is an inverse association between vitamin D and risk for colon cancer in these study populations.

Limitations

Their study follow-up period was only 8 years in the HPFS and 11 years in the Nurses' Health Study. Plasma vitamin D concentrations were determined from blood samples that were provided at only one time point.

FFQs (i.e., 1986, 1990, and 1994). To better approximate long-term nutrient intake, we calculated cumulatively updated intake using information from the 1986, 1990, and 1994 FFQs (19). Because plasma 25(OH)D concentrations reflect recent intake, intakes of vitamin D, calcium, and retinol were calculated using the information from the FFQ closest to the time the blood sample was provided (1994 FFQ) or, if not available, from the most recent questionnaire administered before 1994 (96% of participants in the HPFS had provided information on dietary intake in 1994). Similarly, we used information from the 1994 questionnaire or, if not available, the most recent before 1994 to estimate body mass index (BMI) and physical activity at the time of blood donation. Our questions on weight measurements and physical activity have been validated in previous studies (20,21).

Statistical Analysis

Statistical significance of differences between mean concentrations of plasma vitamin D concentrations for each case-control pair set was assessed using paired *t* test. Quantiles of plasma vitamin D concentrations were calculated based on the distribution in control subjects, and conditional logistic regression models were used to estimate the association between quantiles of plasma vitamin D concentrations and risk of colorectal cancer. We also investigated associations separately by colorectal cancer subsite (i.e., proximal, distal, and rectal cancers) and age at blood collection (i.e., <65 versus ≥65 years). In addition, associations were examined stratified by BMI, physical activity, calcium, retinol intake, and season at blood draw. With the exception of season at blood collection, cut points were calculated based on median distribution in control subjects and defined as follows: NHS: BMI ≤ 24.5 versus >24.5 kg/m², physical activity ≤ 10.2 versus >10.2 metabolic equivalents (METs)-h/wk,

calcium intake ≤ 910 versus >910 mg/day, retinol intake ≤ 2487 versus >2487 IU/day; HPFS: BMI ≤ 25.1 versus >25.1 kg/m², physical activity ≤ 27.8 versus >27.8 METs-h/wk, calcium intake ≤ 855 versus >855 mg/day, retinol intake ≤ 3469 versus >3469 IU/day. The season categories were defined as winter: November, December, January, February, March; summer: June, July, August, September; and spring/fall: all other months. When stratified analyses were conducted, we used unmatched logistic regression to assess the association between plasma 25(OH)D concentration and colorectal cancer. Final models included covariates that were considered to be known or suspected risk factors for colorectal cancer: BMI; physical activity; intakes of folate, calcium, and retinol; pack-years of smoking; intakes of meat (red and processed) and alcohol (all as continuous variables); family history of colorectal cancer (yes or no); aspirin use (≤ 2 tablets/wk versus >2 tablets/wk) in 1986 and reported on all five questionnaires (i.e., on the 1986, 1988, 1990, 1992, and 1994 questionnaires); and fasting status (1–3, 4–10, 11–12 and ≥ 13 hours since last meal). Unmatched logistic regression models included the matching variables age (in 5-year categories) and month of blood donation (winter, spring/fall, or summer), as well as all the covariates included in the multivariable models. Associations were also analyzed in the multivariable models that included region (defined according to the state of residence). Associations were also examined after excluding case patients who were diagnosed within 2 years after providing blood samples. This exclusion was made to address the possibility that 25(OH)D levels may have been lower in case patients than control subjects because preclinical cancers may have led to lower vitamin D levels. Trend tests were calculated by including the median of each quantile of plasma vitamin D concentrations as a continuous variable in addition to all above-mentioned covariates to the multivariable models. All analyses were conducted using SAS version 9.1 (SAS Institute Inc, Cary, NC). All statistical tests were two-sided, and *P* values less than .05 were considered to be statistically significant.

Pooled Analysis (Nurses' Health Study and Health Professionals Follow-up Study)

To increase the statistical power to conduct analyses by subsite and baseline characteristics, such as age, BMI, physical activity, season of blood collection, and calcium and retinol intake, we pooled results from the HPFS with those from a nested case-control study on plasma vitamin D and colorectal cancer conducted previously in a large female cohort, the NHS (8). The main results of the analysis of plasma 25(OH)D concentration and colorectal cancer risk among participants in the NHS have been reported previously (8). Some of the NHS data presented previously were reanalyzed here so that for both cohorts, covariate values were derived identically and the same quantiles of 25(OH)D concentration could be used. Pooled estimates were calculated using a method by DerSimonian and Laird (22).

Results

Mean plasma 25(OH)D concentrations were slightly higher in control subjects than case patients (*P* = .07, Table 1). A slightly higher percentage of case patients than control subjects were residents of the South and Southwest, whereas a higher percentage of

control subjects than case patients were residents of the Northeast. Case patients and control subjects did not differ considerably with regard to most risk factors; however, control subjects were more likely than case patients to have used aspirin in 1994 and to have had endoscopies before 1994 and less likely to report a family history of colorectal cancer (Table 1). In the HPFS, we observed a non-statistically significant inverse association between higher plasma 25(OH)D concentration and risk of colorectal cancer and a statistically significant inverse association for colon cancer (highest versus lowest quintile, odds ratio [OR] = 0.46, 95% confidence interval [CI] = 0.24 to 0.89; *P*_{trend} = .005; Table 2).

After pooling the results from the HPFS and NHS, higher plasma 25(OH)D concentrations were statistically significantly associated with decreased risks of both colorectal cancer (highest versus lowest quintile, OR = 0.66, 95% CI = 0.42 to 1.05; *P*_{trend} = .01; Table 2) and colon cancer (highest versus lowest quintile, OR = 0.54, 95% CI = 0.34 to 0.86; *P*_{trend} = .002; Table 2). In the pooled analysis, the inverse associations with plasma 25(OH)D concentration did not differ by location of colon cancer (proximal versus distal), but the number of patients was small and none of the associations was statistically significant. Opposite associations between plasma 25(OH)D levels and risk of rectal cancers were found among men (positive) and women (inverse). In women, a statistically significant inverse association was observed (highest versus lowest tertile, OR = 0.15, 95% CI = 0.02 to 1.05; *P*_{trend} = .03, Table 2) and in men, a positive association was suggested (highest versus lowest tertile, OR = 3.32, 95% CI = 0.87 to 12.69; *P*_{trend} = .08; Table 2; *P*_{heterogeneity} = .15). Therefore, results for rectal cancers were not pooled.

When we included region in the analyses, results were similar to those observed without region in the models in both cohorts (data not shown). Similarly, in analyses that were stratified by region, inverse associations between plasma 25(OH)D concentrations and colorectal cancers differed little by region (data not shown).

After pooling results from both cohorts, associations between plasma 25(OH)D concentration and colorectal cancer did not differ by age or after excluding case patients who were diagnosed within 2 years after providing blood samples (Table 3). The exclusion of case patients who were diagnosed within 2 years after blood collection was done to address the possibility that 25(OH)D levels may have been lower in case patients than control subjects because preclinical cancers may have led to lower vitamin D levels. To further investigate this possibility, we considered whether 25(OH)D levels among case patients was lower primarily in the earlier years of follow-up by including, in a conditional regression model, time between blood donation and diagnosis (continuous), vitamin D level (continuous), and a product term (i.e., interaction term) of both variables. No evidence for interaction was found (colorectal cancer in HPFS, *P*_{interaction} = .97 and in NHS, *P*_{interaction} = .72; colon cancer in HPFS, *P*_{interaction} = .34 and in NHS, *P*_{interaction} = .42; mean $\pm$ standard deviation follow-up in HPFS = 4.4 $\pm$ 2.3 years and NHS = 5.5 $\pm$ 3.1 years), suggesting that the lower vitamin D levels were not observed preferentially in the case patients who were diagnosed closer to the time of blood donation.

We also investigated associations by lifestyle characteristics that could influence plasma 25(OH)D status. Inverse associations

Table 1. Mean plasma 25(OH)D concentrations and baseline characteristics (1994) for case patients and control subjects in the Health Professionals Follow-up Study, 1994–2002*

Characteristic	Case patients (N = 179)	Control subjects (N = 356)
Plasma 25(OH)D, ng/mL	28.7 (9.2)	29.4 (8.6)
Age at blood donation, y	66.3 (8.2)	66.1 (8.1)
Hours since last meal, No. (%)		
1–3	47 (29.8)	80 (25.5)
4–10	38 (24.1)	75 (23.9)
11–12	38 (24.1)	80 (25.5)
≥13	35 (22.2)	79 (25.2)
Season of blood donation†, No. (%)		
Winter	50 (27.9)	99 (27.8)
Spring/fall	37 (20.7)	73 (20.5)
Summer	92 (51.4)	184 (51.7)
Region of residence‡		
South/Southwest	86 (48.0)	145 (41.1)
Midwest/Northwest	63 (35.2)	126 (35.7)
Northeast	30 (16.7)	82 (23.2)
Body mass index, kg/m ²	26.0 (3.2)	25.6 (3.0)
Physical activity, metabolic equivalents—h/wk	33.8 (33.0)	34.9 (33.1)
Aspirin use in 1994, No. (%)	62 (34.6)	152 (42.7)
Aspirin use in 1986, No. (%)	30 (16.8)	71 (19.9)
Aspirin use for 8 years, No. (%)	19 (10.6)	41 (11.5)
Pack-years of smoking§	14.8 (18.5)	14.6 (19.3)
Current smoking, No. (%)	9 (5.0)	19 (5.3)
Family history of colon or rectal cancer, No. (%)	37 (20.7)	45 (12.6)
Endoscopy before 1994, No. (%)	107 (59.8)	239 (67.1)
Alcohol intake, g/day¶	12.1 (13.5)	12.1 (14.1)
Multivitamin/vitamin D supplement use in 1994	89 (49.7)	185 (52.0)
Total folate intake, µg/day¶	503 (212)	526 (234)
Total vitamin D intake in 1994, IU/day	447 (310)	460 (303)
Vitamin D from food only in 1994, IU/day	259 (125)	261 (122)
Vitamin D from supplements only in 1994, IU/day	185 (274)	193 (256)
Calcium in 1994, mg/day	917 (419)	950 (422)
Retinol in 1994, IU/day	4700 (5520)	4913 (4636)
Total red meat intake, servings/day¶	0.60 (0.37)	0.59 (0.37)
Processed meat intake, servings/day¶	0.38 (0.45)	0.35 (0.38)
Sum of red and processed meat, servings/day¶	0.98 (0.70)	0.94 (0.64)

* Numbers in parenthesis indicate standard deviation for means and percentages for frequencies. If not noted otherwise, information was obtained from 1994 questionnaires, or, if missing, data were carried forward from most recent questionnaire before 1994. 25(OH)D = 25-hydroxyvitamin D.

† Seasons defined as winter: November, December, January, February, March; summer: June, July, August, September; and spring/fall: all other months.

‡ Region assigned according to state of residency: Midwest/Northwest: ND, SD, NE, KS, MN, IA, MO, WI, IL, MI, IN, OH, WA, OR, MT, ID, WY; South/Southwest: OK, TX, LA, AR, MS, AL, FL, GA, SC, NC, VA, TN, KY, WV, DC, MD, DE, CA, NV, UT, CO, AZ, NM; Northeast: NY, NJ, CT, RI, MA, NH, VT, ME.

§ Never smokers were assigned 0 pack-years of smoking.

|| Family history defined as family history of colorectal cancer in either parents and/or siblings.

¶ Cumulative average dietary intake during 1986–1994.

between plasma 25(OH)D concentration and colorectal cancer were limited to participants with a low BMI in both cohorts (pooled, $P_{\text{interaction}} = .17$, Table 4) and, to a lesser extent, to participants who were more physically active (Table 4). We also analyzed associations after stratification by calcium intake. In the HPFS, inverse associations between plasma 25(OH)D concentration and colorectal cancer appeared to be restricted to participants with high calcium intake; however, in the NHS, associations between plasma 25(OH)D and colorectal cancer did not appear to differ by calcium intake. When the results from both cohorts were pooled, inverse associations between plasma 25(OH)D concentration and colorectal cancer did not differ considerably by calcium intake. Pooled results were also similar across retinol intake categories but suggested a strong seasonal effect, with inverse associations between

plasma vitamin D and colorectal cancer being more pronounced among those who had provided blood during the winter months.

Discussion

In this nested case-control study among participants in the HPFS, we observed a non-statistically significant inverse association between plasma 25(OH)D concentrations and risk of colorectal cancer and a statistically significant inverse association with colon cancer. Further, after pooling these results with previously published results from the NHS, higher plasma 25(OH)D concentrations were statistically significantly associated with decreased risk of colorectal cancer, but the inverse association appeared to be restricted to colon cancer.

Table 2. Multivariable-adjusted odds ratios (ORs) and 95% confidence intervals (CIs) of colorectal cancer and its subsites by quantile of plasma 25-hydroxyvitamin D [25(OH)D] concentrations in the Health Professionals Follow-up Study (HPFS) (1994–2002) and the Nurses' Health Study (NHS) (1990–2000)*

Cancer site	Quantile of plasma 25(OH)D	HPFS		NHS		Pooled analysis
		No. of case patients/control subjects	Multivariable OR (95% CI)	No. of case patients/control subjects	Multivariable OR (95% CI)	Multivariable OR (95% CI)
Colorectal cancer	Quintile 1	45/71	1.00 (referent)	53/77	1.0 (referent)	1.0 (referent)
	Quintile 2	44/71	0.97 (0.55 to 1.70)	47/79	0.91 (0.52 to 1.59)	0.94 (0.63 to 1.39)
	Quintile 3	30/68	0.66 (0.35 to 1.24)	35/75	0.78 (0.44 to 1.39)	0.72 (0.47 to 1.11)
	Quintile 4	23/74	0.51 (0.27 to 0.97)	29/77	0.56 (0.30 to 1.04)	0.53 (0.34 to 0.84)
	Quintile 5	37/72	0.83 (0.45 to 1.52)	29/75	0.51 (0.26 to 1.00)	0.66 (0.42 to 1.05)
$P_{\text{trend}}^{\dagger}$			.24		.02	.01
Colon cancer	Quartile 1	49/66	1.0 (referent)	49/75	1.0 (referent)	1.0 (referent)
	Quartile 2	44/68	0.74 (0.42 to 1.33)	43/71	1.12 (0.62 to 2.03)	0.91 (0.60 to 1.38)
	Quartile 3	17/68	0.29 (0.14 to 0.59)	27/77	0.60 (0.32 to 1.13)	0.43 (0.27 to 0.69)
	Quartile 4	29/74	0.46 (0.24 to 0.89)	30/72	0.64 (0.33 to 1.26)	0.54 (0.34 to 0.86)
$P_{\text{trend}}^{\dagger}$			.005		.08	.002
Proximal colon cancer	Quartile 1	20/30	1.0 (referent)	26/38	1.0 (referent)	1.0 (referent)
	Quartile 2	21/36	0.84 (0.29 to 2.41)	20/38	0.87 (0.35 to 2.14)	0.86 (0.43 to 1.70)
	Quartile 3	10/35	0.35 (0.11 to 1.09)	18/42	0.93 (0.38 to 2.25)	0.64 (0.32 to 1.29)
	Quartile 4	18/35	0.69 (0.23 to 2.08)	14/37	0.67 (0.22 to 2.00)	0.68 (0.31 to 1.48)
$P_{\text{trend}}^{\dagger}$			.36		.47	.25
Distal colon cancer	Quartile 1	19/27	1.0 (referent)	18/31	1.0 (referent)	1.0 (referent)
	Quartile 2	16/22	0.93 (0.34 to 2.55)	20/26	1.27 (0.39 to 4.14)	1.06 (0.49 to 2.28)
	Quartile 3	4/21	0.31 (0.07 to 1.36)	9/33	0.23 (0.06 to 0.87)	0.26 (0.10 to 0.71)
	Quartile 4	8/24	0.71 (0.21 to 2.39)	14/30	0.67 (0.18 to 2.48)	0.69 (0.28 to 1.68)
$P_{\text{trend}}^{\dagger}$			.29		.22	.10
Rectal cancer	Tertile 1	11/30	1.00 (referent)	24/31	1.00 (referent)	N/A
	Tertile 2	15/28	1.74 (0.61 to 5.00)	10/26	0.44 (0.09 to 2.11)	
	Tertile 3	14/22	3.32 (0.87 to 12.69)	10/31	0.15 (0.02 to 1.05)	
$P_{\text{trend}}^{\dagger}$			.08		.03	

* Odds ratios were calculated using conditional logistic regression. For HPFS, multivariable models were adjusted for family history (yes versus no); aspirin use (≤ 2 tablets/wk versus > 2 tablets/wk); physical activity (metabolic equivalents [METs]-h/wk, continuous); intakes of folate (continuous), calcium (continuous), retinol (continuous); pack-years of smoking (continuous), alcohol intake (continuous), and meat intake (total red and processed meat intake) (continuous). For NHS, multivariable models were adjusted for family history (yes versus no), physical activity (< 3 , 3–8.9, 9–17.9, 18–26.9, ≥ 27 METs-h/wk), menopausal status and postmenopausal hormone use (premenopausal, post-never pmh, post-past pmh, post-current pmh, post-unknown pmh, dubious status, and missing), aspirin use (duration, < 10 years, ≥ 10 years, missing), pack-years of smoking (never smokers, < 10 , 10–24.9, 25–49.9, ≥ 50), alcohol intake (continuous), folate intake (continuous), red meat intake (continuous), retinol intake (continuous), and calcium intake (continuous). Medians and range were calculated among control subjects only. Median plasma 25(OH)D concentrations (ng/mL) by quantiles: HPFS: quintile 1: 18.4, quintile 2: 25.0, quintile 3: 29.1, quintile 4: 33.3, quintile 5: 39.4; HPFS: quartile 1: 19.3, quartile 2: 26.7, quartile 3: 32.0, quartile 4: 38.8; HPFS: tertile 1: 21.2, tertile 2: 29.3, tertile 3: 37.4. In the NHS, analyses for plasma vitamin D were performed at two different times (but by the same lab, Bruce Hollis). 1990–1996 case patient and control subject samples were analyzed together (lab 1), and 1998–2000 case patient and control subject samples were analyzed together (lab 2) (8). NHS: lab 1 (1990–1996): quintile 1: 14.1, quintile 2: 19.7, quintile 3: 24.0, quintile 4: 27.9, quintile 5: 37.0; NHS: lab 2 (1998–2000): quintile 1: 17.0, quintile 2: 24.9, quintile 3: 29.6, quintile 4: 34.6, quintile 5: 45.5; NHS: lab 1 (1990–1996): quartile 1: 15.1, quartile 2: 21.4, quartile 3: 26.2, quartile 4: 35.2; NHS: lab 2 (1998–2000): quartile 1: 18.0, quartile 2: 26.7, quartile 3: 32.8, quartile 4: 43.8; NHS: lab 1 (1990–1996): tertile 1: 16.1, tertile 2: 23.7, tertile 3: 33.3; NHS: lab 2 (1998–2000): tertile 1: 19.6, tertile 2: 29.6, tertile 3: 41.1. N/A = not applicable.

P_{trend} values (two-sided) were calculated by including the median of each quantile of plasma vitamin D concentrations as a continuous variable in addition to all above-mentioned covariates to the multivariable models.

One strength of our study is the large sample size, especially after combining the results from both cohorts (number of case patients: HPFS $n = 179$, NHS $n = 193$; number of control subjects: HPFS $n = 356$, NHS $n = 383$). The larger sample size enabled us to investigate associations by certain factors including subsite of cancer, age, season, BMI, and physical activity in detail. However, even with a combined number of 372 colorectal cancer case patients, the sample size for some subgroups (e.g., when examining associations for rectal cancers) was still small.

One limitation of our study is the short follow-up in both cohorts (HPFS up to 8 years, NHS up to 11 years). When we excluded case patients who were diagnosed up to 2 years after blood donation, inverse associations between plasma 25(OH)D

concentrations and colorectal cancer persisted. In addition, in both cohorts, plasma 25(OH)D levels were only determined based on blood samples obtained at one time point, which may not represent average long-term 25(OH)D concentrations. Therefore, we cannot exclude the possibility of misclassification of exposure. However, any misclassification resulting from this should be nondifferential and should have biased the results toward unity (23). In addition, previous data on a subsample of 143 men from our cohort, who had provided a second blood sample showed reasonable reproducibility for plasma vitamin D levels with regard to blood samples donated 3–4 years apart ($r = .70$) (24).

Recent studies have provided evidence for biologically plausible mechanisms through which vitamin D may be associated with reduced risk for colorectal cancer and other chronic

Table 3. Multivariable-adjusted odds ratios (ORs) and 95% confidence intervals (CIs) of colorectal cancer by age and time since blood donation and by quantile of plasma 25-hydroxyvitamin D [25(OH)D] concentrations in the Nurses' Health Study (NHS) (1990–2000) and in the Health Professionals Follow-up Study (HPFS) (1994–2002)*

Variable/cancer site	Quantile of plasma 25(OH)D	HPFS		NHS		Pooled analysis
		No. of case patients/control subjects	Multivariable OR (95% CI)	No. of case patients/control subjects	Multivariable OR (95% CI)	Multivariable OR (95% CI)
Age <65 y/ colorectal cancer	Quartile 1	25/34	1.00 (referent)	49/68	1.00 (referent)	1.00 (referent)
	Quartile 2	15/28	1.03 (0.36 to 2.91)	35/72	0.81 (0.44 to 1.51)	0.86 (0.51 to 1.47)
	Quartile 3	9/30	0.38 (0.12 to 1.26)	25/68	0.52 (0.27 to 0.98)	0.48 (0.27 to 0.85)
	Quartile 4	14/36	0.45 (0.15 to 1.40)	32/71	0.68 (0.34 to 1.35)	0.61 (0.34 to 1.09)
$P_{\text{trend}}^{\dagger}$			.13		.12	.04
Age ≥65 y/ colorectal cancer	Quartile 1	34/55	1.00 (referent)	18/29	1.00 (referent)	1.00 (referent)
	Quartile 2	36/61	0.97 (0.50 to 1.87)	18/23	1.77 (0.50 to 6.18)	1.10 (0.61 to 1.98)
	Quartile 3	19/58	0.56 (0.27 to 1.15)	11/28	0.79 (0.21 to 3.03)	0.60 (0.32 to 1.44)
	Quartile 4	27/54	0.83 (0.39 to 1.75)	5/24	0.21 (0.03 to 1.30)	0.68 (0.34 to 1.36)
$P_{\text{trend}}^{\dagger}$			.34		.05	.08
>2 y since blood donation/ colorectal cancer	Quintile 1	36/58	1.00 (referent)	43/60	1.00 (referent)	1.00 (referent)
	Quintile 2	38/55	1.29 (0.66 to 2.51)	37/65	0.83 (0.44 to 1.58)	1.03 (0.65 to 1.63)
	Quintile 3	22/56	0.63 (0.31 to 1.28)	29/63	0.71 (0.36 to 1.39)	0.67 (0.41 to 1.09)
	Quintile 4	20/60	0.63 (0.30 to 1.29)	24/61	0.57 (0.28 to 1.17)	0.60 (0.36 to 1.00)
	Quintile 5	29/59	0.84 (0.41 to 1.72)	26/66	0.55 (0.26 to 1.16)	0.69 (0.41 to 1.15)
$P_{\text{trend}}^{\dagger}$			.31		.05	.03
>2 y since blood donation/ colon cancer	Quartile 1	40/55	1.00 (referent)	40/62	1.00 (referent)	1.00 (referent)
	Quartile 2	37/52	0.91 (0.46 to 1.78)	36/62	1.12 (0.57 to 2.20)	1.01 (0.63 to 1.62)
	Quartile 3	16/59	0.32 (0.15 to 0.69)	23/65	0.62 (0.30 to 1.30)	0.45 (0.26 to 0.77)
	Quartile 4	20/58	0.43 (0.20 to 0.94)	28/62	0.81 (0.38 to 1.70)	0.59 (0.35 to 1.02)
$P_{\text{trend}}^{\dagger}$			.008		.26	.01

* Odds ratios were calculated using conditional logistic regression. For HPFS, multivariable models were adjusted for family history (yes versus no); aspirin use ($\leq 2/\text{wk}$ versus $>2/\text{wk}$); physical activity (metabolic equivalents [METs]-h/wk, continuous), intakes of folate (continuous), calcium (continuous), and retinol (continuous); pack-years of smoking (continuous), alcohol intake (continuous), and meat intake (total red and processed meat intake) (continuous). For NHS, multivariable models were adjusted for family history (yes versus no), physical activity (< 3 , 3–8.9, 9–17.9, 18–26.9, ≥ 27 METs-h/wk), menopausal status and postmenopausal hormone use (pmh) (premenopausal, post-never pmh, post-past pmh, post-current pmh, post-unknown pmh, dubious status and missing), aspirin use (duration, <10 years, ≥ 10 years, missing), packyears of smoking (never smokers, <10 , 10–24.9, 25–49.9, ≥ 50), alcohol intake (continuous), folate intake (continuous), red meat intake (continuous), retinol intake (continuous), calcium intake (continuous). In the NHS, analyses for plasma vitamin D were performed at two different times (but by the same lab, Bruce Hollis). 1990–1996 case patient and control subject samples were analyzed together (lab 1), and 1998–2000 case patient and control subject samples were analyzed together (lab 2) (8). Quantiles are defined as in Table 2.

$\dagger P_{\text{trend}}$ values (two-sided) were calculated by including the median of each quantile of plasma vitamin D concentrations as a continuous variable in addition to all above-mentioned covariates to the multivariable models.

diseases (13). Hypothesized mechanisms include the function of 1,25(OH)₂D to control cell growth and differentiation as well as its potential to facilitate apoptosis (12,13). However, 1,25(OH)₂D concentrations in serum are strictly controlled at approximately 30 pg/mL (10). Recently, colon tumor cells as well as neighboring normal colon cells have been shown to express 25-hydroxyvitamin D-1 α -hydroxylase (CYP27B1) and the vitamin D receptor gene (VDR), implying that the metabolic transformation of 25(OH)D to 1,25(OH)₂D can also take place in the colon (15,16,25–27). Thus, 1,25(OH)₂D concentrations in the colon may reach the high levels (in the range of ng/mL) that have been considered to be essential for a possible anticancer effect (28).

Few studies have examined the association between serum/plasma levels of 25(OH)D in relation to colon or colorectal cancer risk (6–9). Two of the studies, both conducted among participants of the Johns Hopkins University Operation Clue Cohort, were small, with 34 case patients (diagnosed between 1975 and 1983) and 57 case patients (diagnosed between 1984 and 1991) (6,9). In both studies, the observed risk pattern suggested an association

between higher 25(OH)D concentration and reduced risk of colon cancer (highest quintile versus lowest quintile: 1975–1983, OR = 0.73; 1984–1991, OR = 0.4, 95% CI = 0.1 to 1.4); however, none of the trend tests attained statistical significance (6,9). Another nested case–control study was conducted among participants in the Alpha-Tocopherol, Beta-Carotene (ATBC) Trial in Finland (7). That study, which included 146 case patients, found a 40% reduced risk of colorectal cancer among participants in the highest quartile of serum 25(OH)D concentration; however, the trend test was not statistically significant ($P_{\text{trend}} = .13$). In the ATBC study, inverse associations were observed to be strongest for rectal cancers (highest versus lowest quartile, OR = 0.4, 95% CI = 0.1 to 1.1; $P_{\text{trend}} = .06$) and for distal and rectal cancers combined (highest versus lowest quartile, OR = 0.5, 95% CI = 0.2 to 0.9; $P_{\text{trend}} = .03$) (7). The individual results for the analysis of plasma 25(OH)D and colorectal cancers among participants in the NHS have been reported in more detail in a previous publication (8). In brief, in that study, higher plasma levels of 25(OH)D were statistically significantly associated with decreased risk of colorectal cancer ($P_{\text{trend}} = .02$). As with the ATBC cohort, inverse associations observed between

Table 4. Multivariable-adjusted odds ratios (ORs) and 95% confidence intervals (CIs) of colorectal cancer by lifestyle characteristics and season and by quartile of plasma 25-hydroxyvitamin D [25(OH)D] concentrations in the Nurses' Health Study (NHS) (1990–2000) and in the Health Professionals Follow-up Study (HPFS) (1994–2002)

Variable	Quartile of Plasma 25(OH)D	HPFS		NHS		Pooled analysis
		No. of case patients/control subjects	Multivariable OR* (95% CI)	No. of case patients/control subjects	Multivariable OR* (95% CI)	Multivariable OR* (95% CI)
BMI						
Below median†	Quartile 1	24/34	1.00 (referent)	28/31	1.00 (referent)	1.00 (referent)
	Quartile 2	20/44	0.46 (0.20 to 1.07)	28/43	0.73 (0.33 to 1.62)	0.58 (0.33 to 1.05)
	Quartile 3	13/50	0.25 (0.10 to 0.61)	19/52	0.32 (0.14 to 0.73)	0.28 (0.15 to 0.52)
	Quartile 4	22/59	0.34 (0.14 to 0.82)	24/60	0.31 (0.13 to 0.72)	0.32 (0.18 to 0.60)
<i>P</i> _{trend} ‡			.008		.008	< .001
Above median†	Quartile 1	35/55	1.00 (referent)	39/66	1.00 (referent)	1.00 (referent)
	Quartile 2	31/45	1.09 (0.56 to 2.12)	25/52	1.03 (0.50 to 2.11)	1.06 (0.65 to 1.73)
	Quartile 3	15/38	0.65 (0.29 to 1.44)	17/44	0.80 (0.37 to 1.73)	0.72 (0.41 to 1.26)
	Quartile 4	19/31	1.06 (0.49 to 2.31)	13/35	0.86 (0.36 to 2.08)	0.97 (0.54 to 1.73)
<i>P</i> _{trend} ‡			.82		.28	.34
Physical activity						
Below median†	Quartile 1	38/61	1.00 (referent)	37/61	1.00 (referent)	1.00 (referent)
	Quartile 2	31/44	1.14 (0.59 to 2.21)	20/49	0.64 (0.30 to 1.36)	0.89 (0.54 to 1.46)
	Quartile 3	12/37	0.50 (0.22 to 1.16)	17/49	0.59 (0.27 to 1.27)	0.55 (0.31 to 0.96)
	Quartile 4	19/36	0.88 (0.40 to 1.96)	16/38	0.58 (0.24 to 1.38)	0.73 (0.40 to 1.31)
<i>P</i> _{trend} ‡			.46		.23	.17
Above median†	Quartile 1	21/28	1.00 (referent)	30/35	1.00 (referent)	1.00 (referent)
	Quartile 2	20/45	0.64 (0.27 to 1.48)	32/46	0.75 (0.36 to 1.55)	0.70 (0.40 to 1.21)
	Quartile 3	16/51	0.39 (0.16 to 0.95)	18/47	0.36 (0.16 to 0.79)	0.37 (0.21 to 0.67)
	Quartile 4	22/54	0.52 (0.22 to 1.26)	20/56	0.33 (0.14 to 0.74)	0.41 (0.23 to 0.74)
<i>P</i> _{trend}			.11		.002	<.001
Calcium intake						
Below median†	Quartile 1	36/57	1.00 (referent)	44/59	1.00 (referent)	1.00 (referent)
	Quartile 2	30/47	1.02 (0.53 to 1.98)	21/56	0.42 (0.20 to 0.87)	0.68 (0.42 to 1.12)
	Quartile 3	13/36	0.54 (0.23 to 1.27)	20/35	0.68 (0.31 to 1.49)	0.61 (0.34 to 1.09)
	Quartile 4	24/38	0.83 (0.38 to 1.79)	13/38	0.38 (0.16 to 0.90)	0.59 (0.33 to 1.05)
<i>P</i> _{trend} ‡			.45		.06	.06
Above median†	Quartile 1	23/32	1.00 (referent)	23/38	1.00 (referent)	1.00 (referent)
	Quartile 2	21/42	0.61 (0.26 to 1.41)	32/39	1.56 (0.71 to 3.42)	1.01 (0.57 to 1.79)
	Quartile 3	15/52	0.39 (0.16 to 0.93)	16/61	0.42 (0.19 to 0.96)	0.41 (0.22 to 0.74)
	Quartile 4	17/52	0.41 (0.17 to 0.99)	24/57	0.68 (0.30 to 1.54)	0.54 (0.29 to 0.98)
<i>P</i> _{trend} ‡			.03		.04	.003
Retinol intake						
Below median†	Quartile 1	34/54	1.00 (referent)	45/55	1.00 (referent)	1.00 (referent)
	Quartile 2	37/45	1.64 (0.84 to 3.22)	30/52	0.72 (0.36 to 1.41)	1.09 (0.67 to 1.75)
	Quartile 3	11/40	0.49 (0.20 to 1.17)	16/44	0.38 (0.17 to 0.84)	0.42 (0.24 to 0.77)
	Quartile 4	14/39	0.58 (0.25 to 1.39)	15/33	0.44 (0.19 to 1.05)	0.51 (0.28 to 0.93)
<i>P</i> _{trend} ‡			.10		.07	.01
Above median†	Quartile 1	25/35	1.00 (referent)	22/42	1.00 (referent)	1.00 (referent)
	Quartile 2	14/44	0.45 (0.19 to 1.06)	23/43	1.09 (0.48 to 2.48)	0.71 (0.39 to 1.30)
	Quartile 3	17/48	0.48 (0.21 to 1.09)	20/52	0.73 (0.32 to 1.65)	0.59 (0.33 to 1.06)
	Quartile 4	27/51	0.79 (0.35 to 1.77)	22/62	0.61 (0.26 to 1.43)	0.70 (0.39 to 1.25)
<i>P</i> _{trend} ‡			.59		.06	.07
Season						
Winter§	Quartile 1	26 /28	1.00 (referent)	32/49	1.00 (referent)	1.00 (referent)
	Quartile 2	15 /33	0.41 (0.16 to 1.07)	20/31	0.99 (0.41 to 2.38)	0.66 (0.35 to 1.27)
	Quartile 3	7/22	0.26 (0.08 to 0.85)	13/43	0.41 (0.17 to 0.97)	0.35 (0.18 to 0.70)
	Quartile 4	2/16	0.09 (0.02 to 0.53)	10/29	0.48 (0.18 to 1.30)	0.32 (0.13 to 0.76)
<i>P</i> _{trend} ‡			.002		.04	<.001
Summer§	Quartile 1	20/39	1.00 (referent)	18/30	1.00 (referent)	1.00 (referent)
	Quartile 2	24/38	1.35 (0.58 to 3.11)	23/36	1.29 (0.49 to 3.38)	1.32 (0.70 to 2.48)
	Quartile 3	15/47	0.56 (0.24 to 1.31)	15/36	0.72 (0.27 to 1.94)	0.62 (0.33 to 1.19)
	Quartile 4	33/60	1.05 (0.48 to 2.27)	17/45	0.68 (0.24 to 1.87)	0.89 (0.48 to 1.65)
<i>P</i> _{trend} ‡			.70		.22	.26

(Table continues)

Table 4 (continued).

- * Odds ratios calculated using unmatched logistic regression. For HPFS, multivariable models were adjusted for age at blood donation (5-year categories), hours since last meal (1–3, 4–10, 11–12, ≥ 13 hours), family history of colorectal cancer (yes versus no), pack-years of smoking (continuous), aspirin use (≤ 2 /wk versus >2 /wk), retinol (continuous), folate (continuous), calcium (continuous), alcohol (continuous), and meat intake (continuous) as well as BMI (continuous), physical activity (continuous), and season of blood draw (winter: November, December, January, February, March; summer: June, July, August, September; spring/fall: all other months) (if not stratification variable). For NHS, multivariable models were adjusted for family history (yes versus no), physical activity (<3 , 3–8.9, 9–17.9, 18–26.9, ≥ 27 metabolic equivalents [METs]-h/wk), menopausal status and postmenopausal hormone use (pmh) (premenopausal, post-never pmh, post-past pmh, post-current pmh, post-unknown pmh, dubious status, and missing), aspirin use (duration, <10 years, ≥ 10 years, missing), pack-years of smoking (never smokers, <10 , 10–24.9, 25–49.9, ≥ 50), alcohol intake (continuous), folate intake (continuous), red meat intake (continuous), retinol intake (continuous), calcium intake (continuous), age at blood donation (continuous), season of blood draw (winter: December, January, February, March; summer: June, July, August, September; spring/fall: all other months) (if not stratification variable).
- † Median cutoffs were calculated among control subjects only and defined as follows: NHS: BMI: 24.5 kg/m², physical activity: 10.2 METs-h/wk, calcium intake: 910 mg/day, retinol intake: 2487 IU/day, HPFS: BMI: 25.1 kg/m², physical activity: 27.8 METs-h/wk, calcium intake: 855 mg/day, retinol intake: 3469 IU/day.
- ‡ P_{trend} values (two-sided) were calculated by including the median of each quantile of plasma vitamin D concentrations as a continuous variable in addition to all above-mentioned covariates to the multivariable models.
- § Season defined as—HPFS: winter: November, December, January, February, March; summer: June, July, August, September; NHS: winter: December, January, February, March; summer: June, July, August, September.

colorectal cancers and plasma 25(OH)D appeared to be limited to distal and rectal cancer combined, whereas proximal cancers did not appear to be related to 25(OH)D levels (8). However, after pooling the results from the HPFS and NHS cohorts, inverse associations observed between colorectal cancers and plasma 25(OH)D levels appeared to be restricted to colon cancers. In a recently published systematic review, Gorham et al. (5) reviewed 18 published studies on vitamin D (intake and serum studies) and colon or colorectal cancer and also assessed the dose–response relationships between vitamin D and risk of colon or colorectal cancer by plotting them as trend lines. The review included the four aforementioned serum studies as well as 14 other epidemiologic studies of vitamin D intake and colon or colorectal cancer. The authors concluded that, in general, vitamin D intake of 1000 IU/day (compared with a reference intake of <100 IU/day) or blood serum levels of 33 ng/mL (compared with a reference serum level of <13 ng/mL) may be associated with an approximately 50% decreased risk of colorectal cancer (5). These findings suggest that the current recommendations for vitamin D intake, which varies between 200 IU (adults younger than age 50 years), 400 IU (adults between ages 50–70 years), and 600 IU per day (adults older than age 70 years) (29), may be too low to reduce colorectal cancer risk (5).

One recently published large trial of 36282 postmenopausal women, the Women's Health Initiative (WHI) trial, investigated the effect of vitamin D and calcium supplementation on the risk of colorectal cancer (30). The treatment group included 18176 women who received supplements containing 1000 mg calcium and 400 IU vitamin D each day for an average of 7 years. In this trial, daily supplementation with vitamin D and calcium did not appear to be associated with risk of colorectal cancer. Using a nested case–control study design, the authors also examined associations between baseline serum 25(OH)D concentrations and risk of colorectal cancer. Consistent with our findings, participants with lower baseline 25(OH)D concentrations appeared to have an increased risk of developing colorectal cancer (<31 versus ≥ 58.4 nmol/L, OR = 2.53, 95% CI = 1.49 to 4.32; $P_{\text{trend}} = .02$). However, their data did not support an interaction between serum vitamin D levels at baseline and intervention (30). One possible explanation for the overall null effect and lack of interaction between serum 25(OH)D levels and intervention in the WHI trial could be that participants were allowed to take additional calcium and/or vitamin D supplements

on their own, which could have attenuated associations. Before the start of the trial, participants already had higher mean intakes of calcium and vitamin D than the reported national average (31), and while they were participating in the trial, consumption of these two micronutrients increased even further (30). Another possible explanation for the overall null effect is that compliance in the WHI trial was only approximately 65% (30). Other possible explanations for the null finding include the short follow-up of 7 years and that the dose of vitamin D required to achieve a protective effect against colorectal cancer may be higher than the dose recommended by the Institute of Medicine (400 IU) (29), which was the dose administered in the WHI (5).

It should be noted that 400 IU vitamin D is expected to increase 25(OH)D levels by only about 3 ng/mL (5), which is far lower than the range between the median of the lowest and highest quintiles in our study (HPFS: 21 ng/mL, NHS: lab 1, 22.9 ng/mL; lab 2, 28.5 ng/mL). Therefore, the observed association between higher vitamin D levels and reduced colorectal cancer risk on colorectal cancer might be expected to be even stronger in populations among whom low vitamin D levels are more commonly observed, such as African Americans and the elderly (32–34).

Vitamin D metabolism is closely tied to calcium metabolism (35). However, the pooled results in our study provided no evidence for an interaction between calcium and vitamin D with regard to colorectal cancer risk. Results from a randomized trial on calcium supplementation and the risk of adenoma recurrence (36) also suggest that higher serum vitamin D levels may protect against recurrence of adenomas, but the reduced risk was seen mainly among participants who were given calcium supplements. In addition, calcium supplements mainly exhibited a protective effect on adenoma recurrence among participants with higher serum vitamin D levels, suggesting a synergistic effect of calcium and vitamin D on adenoma recurrence (36).

Retinol may also modify the possible association between vitamin D and colorectal cancers (37). It has been suggested that increased consumption of retinol may result in impaired function of 1,25(OH)₂D, and a recent study in rats provided some support for this hypothesis, but the mechanisms through which increased retinol consumption may impair vitamin D function are unclear. It is possible that retinol can compete with vitamin D for binding to the retinoid X receptor (38). In another study from our

group conducted among participants in the NHS (39), higher intake of retinol did seem to modify observed associations between vitamin D intake and colorectal adenoma (39). However, in our study, inverse associations between plasma 25(OH)D and colorectal cancer did not seem to differ by retinol intake.

Results from our study also suggested a strong seasonal effect, with inverse associations between plasma 25(OH)D concentrations and colorectal cancer being more pronounced among those whose blood was collected during the winter months than during the summer months. Participants who exhibited higher vitamin D levels in the summer months could have either high or low vitamin D levels in the winter. However, participants with high plasma levels of vitamin D during the winter months are unlikely to have low plasma levels during the summer and are probably those with consistently higher plasma vitamin D levels, which may partly explain why inverse associations in our study appeared to be restricted to those who donated blood in the winter months.

We found that the inverse association with 25(OH)D concentrations was much stronger for both lean and physically active men and women but much weaker in overweight and inactive individuals. This finding was unexpected, but the magnitude and consistency in both cohorts suggest that it may not have been due to chance. Inactive individuals with a high BMI tend to be insulin resistant, and hyperinsulinemia may be associated with risk for colon cancer (40). In a prospective study, obese individuals or those with high C-peptide levels (a marker for hyperinsulinemia) appeared to be at high risk of colon cancer regardless of their insulin-like growth factor (IGF)-1 levels, or IGF/insulin-like growth factor-binding protein (IGFBP)-3 ratio, whereas among leaner, less insulin-resistant individuals, IGF-1 and IGF/IGFBP-3 ratio are strong risk factors (41). We speculate that vitamin D may be associated with decreased risk of colorectal cancers that are associated with a high IGF-1 or IGF/IGFBP-3 ratio, i.e., in leaner and more physically active individuals. This theory is further supported by a recent study suggesting that 1,25(OH)₂D may induce expression of IGFBPs, including IGFBP-3 (42).

In conclusion, our data provide additional support for an inverse association between vitamin D and colorectal and in particular colon cancer risk. Future studies should examine possible interactions between vitamin D and certain factors, including calcium and retinol intake and adiposity with regard to colorectal cancer risk. In addition, possible interactions between vitamin D status and polymorphisms in genes involved in vitamin D metabolism and function, such as VDR (43), should also be taken into consideration when examining this association.

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