

Vitamin D, Calcium, and Vitamin D Receptor Polymorphism in Colorectal Adenomas

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Abstract

Experimental studies suggest that vitamin D and calcium protect against cancer by reducing proliferation and inducing differentiation. The effects of vitamin D and calcium may be mediated by the vitamin D receptor (VDR), which is encoded by the VDR gene. The present study investigated whether calcium intake and serum vitamin D, as an integrated measure of intake and endogenous production, were associated with risk of colorectal adenoma, known precursors of invasive colorectal cancer. In addition, the interrelation among vitamin D, calcium, and *FokI* polymorphism of the VDR gene was investigated. Persons (239) with histologically confirmed colorectal adenomas and 228 control individuals without colorectal adenomas confirmed by sigmoidoscopy were enrolled in this case control study conducted at the National Naval Medical Center, Bethesda, MD. We observed an inverse association of serum 25-OH vitamin D [25-(OH)D] with colorectal adenoma. With each 10 ng/ml increase of serum 25-(OH)D, the risk of colorectal adenoma decreased by 26% (odds ratio 0.74, 95% confidence interval 0.60–0.92). The results provided limited evidence for a weak association between calcium intake and colorectal adenoma (odds ratio 0.97, 95% confidence interval 0.93–1.01 per each 100-mg calcium intake). However, the inverse association of serum 25-(OH)D with colorectal adenoma is suggested to be stronger in subjects with calcium intake above the median (*P* for multiplicative interaction 0.13). The VDR *FokI* polymorphism was not significantly associated with colorectal adenoma and did not modify the effect of vitamin D or calcium. In conclusion, the study results suggested a protective effect for vitamin D on colorectal adenoma.

Introduction

Endogenous vitamin D status is the sum of dietary intake and endogenous synthesis. Up to 95% is attributable to synthesis from cholesterol in the skin with sunlight exposure (1). Garland and Garland (2) hypothesized 2 decades ago that higher incidence rates for colorectal cancer in areas with lower sunlight exposure might be attributable to lower levels of vitamin D. Since then, several cell culture and experimental animal studies have suggested a protective effect of vitamin D on colorectal cancer. Vitamin D is suggested to reduce epithelial cell proliferation and to promote differentiation in various cell cultures, including colon-derived cells, as well as in experimental animal studies (3–5). More recent studies indicate, further, that vitamin D induces apoptosis in colorectal tumor cell lines and premalignant adenoma cell lines (6). Inhibition of spontaneous metastases by a vitamin D analog in an animal colon carcinoma model has been described (7). These promising results have led to Phase I and Phase II clinical trials of vitamin D and vitamin D analogs but with limited success (8–10). We have to consider that these clinical trials investigated cancer treatment and not prevention of cancer.

Calcium may have an antiproliferative effect by binding secondary bile acids (11). The antiproliferative effect has been supported by several cultured malignant colonic cell line, animal, and human intervention studies (5, 11–13). Recently, the induction of apoptosis in murine colonic epithelium has been reported (14).

Despite the promising results of several experimental studies, the results from clinical trials and epidemiological studies have been less consistent, and the evidence of a protective effect of vitamin D and calcium on colorectal neoplasia remains ambiguous. Most reported epidemiological studies have only assessed dietary vitamin D intake but not endogenous vitamin D production. In the present study, we investigated serum 25-(OH)D² levels to capture endogenous production, as well as exogenous intake of vitamin D. Additionally, we measured sun exposure and dietary vitamin D intake to correlate these measures with serum 25-(OH)D. Because vitamin D affects calcium by maintaining calcium homeostasis, we investigated the interaction between vitamin D and calcium. As vitamin D mediates its effect through the VDR, known to be genetically polymorphic, we investigated the association of a VDR polymorphism with colorectal adenoma and the interactions with vitamin D and calcium.

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² The abbreviations used are: 25-(OH)D, 25-OH vitamin D; VDR, vitamin D receptor; OR, odds ratio; CI, confidence interval; 1,25-(OH)₂D, 1,25-OH₂ vitamin D; CV, coefficient of variance; VIF, variance inflation factor; BMI, body mass index; NSAID, nonsteroidal anti-inflammatory drug; LRT, likelihood ratio test.

Materials and Methods

The study has been described in detail elsewhere (15). Briefly, the study was conducted as a case control study at the National Naval Medical Center (Bethesda, MD). Between April 1994 and September 1996, individuals with colorectal adenomas, diagnosed by colonoscopy or sigmoidoscopy and histologically confirmed, were enrolled in the study. During the same time period, controls that received a negative screening sigmoidoscopy were frequency matched by age (± 5 years) and gender. All study subjects were between the ages of 18 and 74, residents of the study area, and had never been diagnosed with Crohn's disease, ulcerative colitis, or cancer, except nonmelanoma cancer of the skin.

Dietary intake was assessed with a validated self-administered food frequency questionnaire (16). The questionnaire was mailed to the participants and checked for completeness by a trained interviewer during a home visit. At this home visit, participants were also interviewed to obtain detailed information about sun exposure, use of vitamin and mineral supplements, socio-demographic data, medical history, medication, physical activity, alcohol and tobacco consumption, and occupational history. Blood was drawn from the participants during the return clinical visit.

All together, 289 eligible cases and 314 eligible controls were identified. The participation rate in the case group was 84% ($n = 244$) and in the control group, 74% ($n = 231$). Reasons for nonparticipation were refusal (cases 12%, controls 21%), illness (cases 3%, controls 4%), or others (cases 1%, controls 1%). Because of implausible dietary information, two cases and three controls were excluded. Three cases with familiarly polyposis syndrome were excluded. In addition, 3 cases and 10 controls had missing data for serum 25-(OH)D level, and 31 cases and 44 controls had missing data for VDR *FokI* genotyping, because not enough biological material was available from all subjects.

All 228 control subjects were verified by sigmoidoscopy. Of the 239 cases, 206 (86.2%) underwent colonoscopic examination, and 33 (13.8%) underwent sigmoidoscopic examination. The time between colorectal examination and blood draw was 3 days for cases and 23 days for controls, and the time between colorectal examination and home visit was 2.4 months for cases and 3.3 months for controls.

A modified version of the validated 100-item Health Habits and History Questionnaire was used to assess usual diet over the 12 months before diagnoses or enrollment in the study (16). Except dietary vitamin D intake, nutrient values were calculated based on the database in the analysis software HHHQ-DIETSYS (17). The database for vitamin D content in food is limited and based on a provisional table from the United States Drug Administration (18). For supplements, we assigned the following values per tablet: One-A-Day-type multivitamin: 400 IU vitamin D and 130 mg of calcium; Stresstab-type: 0 IU vitamin D and 0 mg of calcium; Theragran-type: 400 IU vitamin D and 40 mg of calcium, special vitamin D, and calcium supplements (particular amount per tablet was asked); and cod oil: 1500 IU vitamin D per spoon. Total vitamin D and calcium intake was defined as dietary plus supplemental intake.

25-(OH)D was extracted from serum samples and measured in a radioimmunoassay by using a 25-(OH)D-specific antibody (Diasorin, Inc., Springfield, MN). The limit of detection was 3 ng/ml. A pooled plasma sample was used as blinded quality control samples ($n = 68$) and interspersed among the samples. The CV for the quality control was calculated by incorporating the variation associated within and between

batches (19). The CV was 11.5%. The batch means were not significantly different from the overall mean, but a marginal significant time trend was observed ($P = 0.05$).

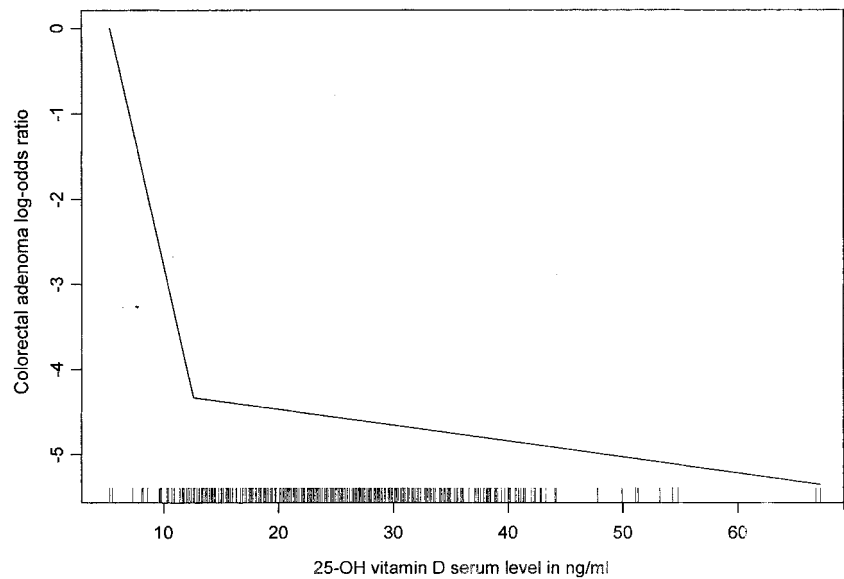
The *FokI* restriction site polymorphism in the 5' end of the VDR gene was detected using the methods described by Gross *et al.* (20). We included in each batch samples of all three known genotypes and water lanes. Additionally, we used multiple blinded DNA samples ($n = 38$) from two different individuals as quality controls and found a reliability of 100% to identify *FokI* genotype.

Approval for the study was given from the institutional review board of the National Cancer Institute, and National Naval Medical Center. Informed consent was obtained from all participants.

Data Analysis. To estimate the correlation of serum 25-(OH)D levels with sunlight exposure and total vitamin D intake, we used the multiple R^2 coefficient from a linear regression model with serum 25-(OH)D as a dependent variable with the following independent variables: hours spent outside during summer (log transformed), hours spent outside during the rest of the year (log transformed), wearing protective clothing (never, rarely, occasionally, frequently, and always), using sunscreen (never, only first few times, sometimes but not consistently, each time once, each time repeated, and never in strong sunlight), frequency of sunbaths (never, rarely, occasionally, and frequently), and total vitamin D intake (log transformed; all variables were assessed for the last 12 months before enrollment in the study). We limited this analysis to non-Hispanic Caucasian controls, because the pigmentation of the skin affects the production of vitamin D in the skin (21, 22). We used logistic regression analysis to investigate the association between the different exposures and colorectal adenoma risk. To investigate the association of sun exposure and adenoma risk, we used the time participants spent outside in the summer and during the rest of the year (continuous). We investigated the association of serum 25-(OH)D and total calcium intake with risk of colorectal adenoma in both a continuous and a categorical fashion. Additionally, serum 25-(OH)D was investigated with piecewise linear logistic modeling, which allowed different associations of serum 25-(OH)D and adenoma risk below and above a prespecified node. To choose the best point for the node, we calculated the P of the -2 log-likelihood for different values of the node and chose the one that minimized the value. The model which fit best was found by adding a node at 12.6 ng/ml (Fig. 1, appendix 1). The P corresponding to the likelihood ratio statistic that compared the model with a node at 12.6 ng/ml to the simpler logistic regression model was 0.008.

Since the controls were matched on gender and age, we controlled for these two variables in all analyses. Analyses for serum 25-(OH)D were additionally controlled for the season when the blood was drawn (summer/fall *versus* winter/spring). The following potential confounders were taken into account: family history of colorectal cancer (yes and no), family history of cancer (yes and no), reason for screening (routine and not routine), smoking status (ever and never), pack of cigarettes smoked per year (continuous), education attainment (≤ 12 years school, 1 to ≤ 3 years college, 4 years college, graduate, or higher degree), ethnicity (non-Hispanic Caucasian and others), current BMI (continuous and continuous categorical), BMI at ages 15, 25, 40, and 60 (continuous), current physical activity (hours of vigorous and moderate activity per week), and use of NSAIDs (nonregular use *versus* regular use defined as ≥ 1 /week for ≥ 1 month in the last 3 months before the interview). The following dietary variables were coded as continuous vari-

Fig. 1. Relation between serum 25-OH vitamin D and colorectal adenoma. Fitted values come from logistic regression model, which included vitamin D as a linear term with a node at 12.6 ng/ml and age, gender, ethnicity, and season when blood was drawn. Graphical display along the horizontal axis shows the distribution of the study subjects.



ables: red meat, well and very well done red meat, fruits and fruit juice, vegetables, dietary fiber, energy, total fat, saturated fatty acids, alcohol, low-fat dairy products, total vitamin A, total β -carotene, total vitamin B1, total folate, dietary niacin, dietary riboflavin, total vitamin B6, total vitamin C, total vitamin E, total zinc, and total iron intake.

We compared cases with previous adenoma ($n = 93$) and cases without previous adenoma ($n = 146$) to test whether the mean of the main exposures and listed potential confounder differed significantly by applying the t test. The variables calcium and red meat intake showed significant differences. For these two variables, we included only cases without previous adenoma in the analysis.

Variables that changed the risk estimate of the main exposure of serum 25-(OH)D, total calcium intake, or VDR polymorphism by $\geq 10\%$ were considered to be confounders and included in the final analysis. For each main exposure, the two other main exposures were treated as confounders. Collinearity between the variables was evaluated by calculating the VIF ($VIF = 1/R^2$). For variables with a VIF value >10 , the coding was changed from continuous to categorical continuous (23). To account for ethnic differences in the VDR *FokI* polymorphism, we stratified or added an interaction term for ethnicity and VDR *FokI* polymorphism.

To evaluate the interaction between serum 25-(OH)D, total calcium intake, and VDR *FokI* polymorphism, we coded serum 25-(OH)D and total calcium intake as continuous variables and VDR *FokI* polymorphisms as categorical variables.

Results

Association of Vitamin D, Calcium, and VDR *FokI* Polymorphism with Colorectal Adenoma. All together, 239 cases of colorectal adenoma and 228 controls were included in the study. The median age of cases and controls was 60 and 57 years, respectively. Fewer females than males were enrolled (cases 22.6%, controls 36.8%). The cases (88.3%) and 89% of the controls were non-Hispanic Caucasian, whereas 8.4% of the cases and 5.3% of the controls were non-Hispanic African-American. As published earlier (15), family history of colorectal cancer was more frequent in participants with a colorectal

adenoma. BMI was not a strong risk factor, whereas use of NSAIDs showed an inverse association with colorectal adenoma. Cases were more likely to be smokers. Intake of red meat and well and very well done red meat were positively associated with risk of colorectal adenomas (15). The potential dietary risk factors, energy intake, fat intake, and alcohol ingestion tended to be higher in the case group, whereas fruit and vegetable intake tended to be lower in the case group.

The correlation coefficient in controls for dietary vitamin D intake and dietary calcium was $r = 0.79$ ($P < 0.001$), for total vitamin D intake and total calcium intake was $r = 0.36$ ($P < 0.001$), and for serum 25-(OH)D and total calcium intake was $r = 0.08$ ($P > 0.05$).

To investigate the correlation of serum vitamin D and vitamin D intake and endogenous vitamin D production, we measured the correlation coefficient of serum 25-(OH)D and total vitamin D intake and the variables of sun exposure/protection as listed in "Materials and Methods." Among non-Hispanic Caucasian controls, the coefficient was 0.34 ($P < 0.001$).

As shown in Table 1, serum 25-(OH)D levels were higher in samples drawn in summer/fall than winter/spring ($P < 0.001$) and in non-Hispanic Caucasians than in subjects of other ethnicities ($P < 0.001$) in both cases and controls. Serum 25-(OH)D was inversely associated with colorectal adenomas showing a 26% decrease in the rate of colorectal adenomas with each 10 ng/ml increase in serum 25-(OH)D (Table 1). More nonparametric approaches suggested that the inverse association of serum 25-(OH)D with colorectal adenomas was stronger in the deficiency range (Fig. 1): The range of serum 25-(OH)D was 5.3–67.2 ng/ml. Applying piecewise regression method showed that in the interval of 5.3–12.6 ng/ml 25-(OH)D, the risk of colorectal adenomas decreased by 45% with each 1 ng/ml increase of 25-(OH)D (OR 0.55, 95% CI 0.32, 0.95; Fig. 1). In the interval >12.6 –67.2 ng/ml 25-(OH)D, the risk decreased by only 2% with each 1 ng/ml increase of 25-(OH)D (OR 0.98, 95% CI 0.95, 1.00). Per each 10 ng/ml increase of 25-(OH)D, the risk decreased by 22% (OR 0.78, 95% CI 0.62, 0.99; Fig. 1). In summary, we found not only that 25-(OH)D was inversely associated with adenoma risk but also that sub-

Table 1 Distribution and age- and gender-adjusted OR and 95% CI for colorectal adenomas for serum 25-OHD, sun exposure, intake of vitamin D, intake of calcium, and VDR *FokI* polymorphism

	No. of subjects		Median (10 th and 90 th percentile)		OR	95% CI
	Cases	Controls	Cases	Controls		
Serum vitamin D (ng/ml)						
Overall ^a	236	218	24.7 (12.7, 36.6)	26.5 (15.2, 39.4)	0.74 ^b	0.60, 0.92
Samples drawn in summer/fall ^a	133	124	26.9 (15.0, 41.1)	29.0 (18.4, 40.2)	0.77 ^b	0.58, 1.01
Samples drawn in winter/spring ^a	103	94	20.9 (11.8, 32.2)	23.1 (34.9, 13.4)	0.70 ^b	0.70, 1.01
Non-Hispanic Caucasians ^a	211	197	25.5 (14.3, 36.6)	27.1 (16.3, 39.7)	0.74 ^b	0.58, 0.95
Other than non-Hispanic Caucasians ^a	25	21	15.4 (9.7, 28.7)	17.4 (11.8, 31.3)	0.59 ^b	0.28, 1.23
Sun exposure (h/yr)						
Over the whole yr	239	228	325 (52, 1092)	325 (39, 1001)	1.00 ^c	0.96, 1.04
In the summer	239	228	91 (26, 364)	91 (13, 325)	1.05 ^c	0.92, 1.19
Vitamin D intake (IU/day)						
Total vitamin D	239	228	254 (53, 669)	385 (68, 692)	0.98 ^c	0.93, 1.03
Dietary vitamin D	239	228	140 (56, 266)	142 (53, 292)	0.87 ^c	0.71, 1.07
Supplement vitamin D	239	228	0 (0, 400)	114 (0, 400)	0.99 ^c	0.94, 1.04
Calcium intake (mg/day) ^d						
Total calcium	146	228	712 (332, 1511)	804 (359, 1645)	0.97 ^c	0.93, 1.01
Dietary calcium	146	228	549 (283, 1033)	607 (276, 1213)	0.93 ^c	0.88, 1.00
Supplement calcium	146	228	112 (0, 730)	130 (0, 1000)	0.99 ^c	0.94, 1.05
VDR <i>FokI</i> Genotype ^e						
ff	21	16			1.00	
Ff	115	95			0.92	0.45, 1.89
FF	72	73			0.75	0.36, 1.58

^a Additional adjusted for season when blood was drawn (summer or fall vs. winter or spring).

^b OR and 95% CI per 10 units increase.

^c OR and 95% CI per 100 units increase.

^d Limited to cases without previous adenoma.

^e Additional adjusted for ethnicity.

jects with 25-(OH)D serum levels in the deficiency range had a particular high risk to develop colorectal adenoma. For the categorical analysis of serum 25-(OH)D, the ORs were (2nd to 5th quintile compared with lowest quintile) 0.40 (95% CI 0.22, 0.74), 0.67 (95% CI 0.38, 1.19), 0.47 (95% CI 0.26, 0.85), and 0.43 (95% CI 0.23, 0.81; range of quintiles was 1st quintile: 5.3 to <19.4 ng/ml, 2nd quintile: 19.4 to <23.5 ng/ml, 3rd quintile: 23.5 to <28.9 ng/ml, 4th quintile: 28.9 to <33.7 ng/ml, and 5th quintile: 33.7–67.2 ng/ml). Stratification by adenoma location showed very similar risk estimates for rectum, distal colon (splenic flexure, descending, and sigmoid rectum), and proximal colon (hepatic flexure, cecum, ascending colon, and descending colon): per 10 ng/ml increase in 25-OH vitamin D, the risk for developing adenoma decreased by 0.73 (95% CI: 0.50–1.07), 0.72 (95% CI: 0.57–0.91), and 0.77 (95% CI: 0.59–1.03), respectively.

Mean calcium intake differed significantly between cases with and without a previous adenoma. Compared with cases without a previous adenoma (median values, see Table 1), cases with previous adenoma had higher total calcium intake (median 785.7 mg/day), higher dietary calcium intake (median 650 mg/day), and lower calcium intake from supplements (median 0 mg/day). Because knowledge about an increased risk of colorectal cancer may have changed the calcium intake of those with a previous adenoma, the analysis was limited to cases without previous adenoma. A small marginal, significant inverse association with colorectal adenoma was found for both total and dietary calcium intake (Table 1). However, categorical analysis showed no appreciable association of total calcium intake with colorectal adenoma: compared with the lowest quintile, the ORs were (from 2nd to 5th quintile) 1.26 (95% CI 0.68, 2.33), 0.86 (95% CI 0.44, 1.67), 0.62 (95% CI 0.31, 1.26), and 0.86 (95% CI 0.42, 1.75; range of quintiles was 1st quintile: 133 to <463 mg/day, 2nd quintile:

463–730 mg/day; 3rd quintile: >730 to <942 mg/day, 4th quintile: 942–1321 mg/day, and 5th quintile: >1321–3607 mg/day).

The VDR *FokI* polymorphism was not significantly associated with colorectal adenomas (Table 1).

None of the potential risk factors listed in the “Materials and Methods” section appeared to confound the risk estimates of the three main exposures (serum 25-(OH)D, total calcium intake, and VDR polymorphism *FokI*) and were, therefore, not included in the analysis.

Interaction between Vitamin D and Calcium. Even though the model containing a node at 12.6 ng/ml 25-(OH)D fit the association of serum 25-(OH)D with colorectal adenoma better, we used the linear model without a node to analyze interaction since the number of participants with values ≤ 12.6 ng/ml was too small (23 cases and nine controls) for stratification. Stratification by calcium intake showed that the inverse association of serum 25-(OH)D with colorectal adenoma was stronger in subjects with high calcium intake (Table 2). In subjects with total calcium intake above and below the median, for each 10 ng/ml increase of 25-(OH)D, the rate of colorectal adenoma was reduced by 44 and 16%, respectively. However, LRT for multiplicative interaction was not significant ($P = 0.13$). A similar trend was seen when we stratified the association between total calcium intake and colorectal adenoma by serum 25-(OH)D levels, but the difference was less considerable because the overall association of calcium intake with adenoma was weak (Table 2).

Interaction of Vitamin D and Calcium with VDR Polymorphism. Stratification by VDR *FokI* polymorphism suggested differences in the association of serum 25-(OH)D level with adenoma rate for the different VDR *FokI* genotypes (Table 2). Whereas the inverse association between serum 25-(OH)D and

Table 2 Age- and gender-adjusted ORs and 95% CI for colorectal adenomas of serum 25-(OH)D and calcium intake stratified by each other and by VDR *FokI* polymorphism

	Cases	Controls	OR	95% CI	<i>P</i> for multiplicative interaction
Serum 25-(OH)D (per 10 ng/ml) ^a					
Overall	236	218	0.74	0.60, 0.92	
Total calcium intake ^b					
<Median	82	108	0.84	0.60, 1.17	
≥Median	62	110	0.56	0.37, 0.85	0.13
VDR <i>FokI</i> polymorphism ^c					
ff	21	16	0.96	0.40, 2.34	
Ff	114	95	0.64	0.45, 0.91	
FF	71	73	0.62	0.40, 0.97	0.50
Total calcium intake (per 100 mg/day) ^b					
Overall ^a	146	228	0.97	0.93, 1.01	
Serum 25-(OH)D ^a					
≤Median	92	109	1.00	0.95, 1.05	
>Median	53	109	0.93	0.86, 1.00	0.12
VDR <i>FokI</i> polymorphism ^c					
ff	12	16	0.91	0.75, 1.10	
Ff	70	95	0.94	0.94, 1.01	
FF	46	73	1.01	0.93, 1.10	0.87

^a Additionally adjusted for season when blood was drawn (summer/fall vs. winter/spring).

^b Limited to cases without previous adenoma.

^c Additionally adjusted for ethnicity (non-Hispanic Caucasian vs. other ethnicity).

colorectal adenoma was weak in subjects with the VDR *FokI* genotype ff, in subjects with *FokI* genotype FF or Ff, the inverse association was stronger with a 33% decrease in adenoma occurrence with each 10 ng/ml increase in serum 25-(OH)D (Table 2). However, no multiplicative interaction appeared to be present (LRT for multiplicative interaction $P = 0.50$).

For the association of total calcium intake with colorectal adenoma, no appreciable difference between strata of VDR *FokI* polymorphism was observed (Table 2).

Discussion

The present study found an inverse association of serum vitamin D with colorectal adenoma. The inverse association between calcium intake and colorectal adenoma was weak. The results provided no evidence for an overall effect of the VDR *FokI* polymorphism and colorectal adenoma nor interaction with either serum vitamin D or calcium intake.

Although several epidemiological studies have investigated the association between dietary vitamin D intake and colorectal neoplasia, only a few studies have investigated the association of serum vitamin D levels with colorectal neoplasia. Similar to our findings, four nested case control studies found an inverse association of 25-(OH)D with colorectal neoplasia (24–27), although two of these studies indicated a U-shaped association. Only one case control study showed higher serum 25-(OH)D in cases with colonic adenoma and cancer (28). Except for the recent study of Platz *et al.* (27), the studies were small and had little power to detect a significant association.

In addition to examining serum 25-(OH)D, three studies also examined levels of serum 1,25-(OH)D (25–27). One of the studies (27) found a weak inverse association, whereas two of the studies did not find appreciative associations with colorectal cancer (25, 26). We decided to analyze 25-(OH)D because it may be a more relevant biomarker, according to the following findings: 1,25-(OH)₂D is the active hormonal form of vitamin D, which regulates calcium homeostasis and is under tight homeostatic control by the renal expression of 1- α -hydroxylase

(29). The half-life of serum 1,25-(OH)₂D is 3 to 8 h, whereas the half-life of serum 25-(OH)D is ~1 month (30, 31). Serum 25-(OH)D is not maintained homeostatically and is affected by vitamin D intake and endogenous production (21, 22). Further, 1- α -hydroxylase and VDR are expressed extrarenally in different cell types, such as bone cells, spleen cells, macrophages, including normal and malignant colon cells (32, 33). The activity of 1- α -hydroxylase in extrarenal tissues is not thought to be involved in maintaining serum 1,25-(OH)₂D level homeostatically and introduces the idea of a paracrine role for 1,25-(OH)₂D by reducing cell proliferation and inducing differentiation locally (32, 34). Recently, Barreto *et al.* (35) showed that 1- α -hydroxylase is expressed in prostatic human cells and that 25-(OH)D inhibited clonal growth in a dose- and time-dependent manner not significantly different from 1,25-(OH)₂D. These findings suggest that serum 25-(OH)D may be a more meaningful biomarker to measure potential anticarcinogenic effects of vitamin D. Additionally, our analysis found a significant positive correlation between serum 25-(OH)D and vitamin D exposure (intake and endogenous production). However, the correlation coefficient of 0.34 was relatively low and likely reflects measurement error in assessing sun exposure and vitamin D intake by questionnaires. Furthermore, serum 25-(OH)D levels were measured at only one time point, and dietary intake and sun exposure were assessed retrospectively for the last 12 months.

For dietary and supplemental vitamin D intake, our study suggested only a weak association with colorectal adenoma. The majority of epidemiological studies (36–44) showed an inverse but mostly nonsignificant (37–41, 44) association between vitamin D intake and colorectal adenoma, as well as cancer, although not all studies were in favor of a protective effect of vitamin D intake (45–49). However, assessment of vitamin D intake may be inaccurate because of limited data on vitamin D contents in foods (18). Furthermore, these studies did not measure an important source of vitamin D: endogenous vitamin D synthesis by UV B radiation of cholesterol in the skin (21). Kampman *et al.* (50) recently investigated the association

of sun exposure and vitamin D intake with colon cancer. Only supplemental vitamin D appeared to be inversely associated with the risk of colon cancer, although the association was nonsignificant. However, the approach of Kampman *et al.* (50) separately examined the effect of the different sources of vitamin D, whereas serum 25-(OH)D level captures both vitamin D intake and endogenous vitamin D production.

Our results suggested that the inverse association of serum 25-(OH)D with colorectal adenoma was particularly strong in the range of vitamin D deficiency (Fig. 1). The choice of the cut point for the node was data driven, but the same trend was apparent regardless of where the node was placed in the range of deficiency. The deficiency range is between 5 and 15 ng/ml 25-(OH)D (51, 52) for rickets prevention but recently theorized to be higher for cancer prevention (21, 22). A particularly high risk for colorectal adenoma in women with low vitamin D levels was also described in an earlier study but was much stronger for serum 1,25-(OH)₂D (using a cut point of 26 pg/ml) than for 25-(OH)D (using cut point of 15 ng/ml serum; Ref. 27). Although it could be biologically plausible that people with serum 25-(OH)D in the range of deficiency (10–20 ng/ml; Refs. 21 and 51) are at a particularly high risk for colorectal adenoma, the small number of cases ($n = 23$) and controls ($n = 9$) at levels <12.6 ng/ml preclude this conclusion. It would be very interesting to investigate this hypothesis in populations where food is not fortified with vitamin D and sun exposure is low, such as north European countries.

The effect of calcium on colorectal adenoma and cancer has been intensively investigated over the last decade in epidemiological studies. A meta-analysis of 24 studies found considerable heterogeneity among the risk estimates for the effect of calcium on colorectal neoplasia (53). The authors of the meta-analysis concluded that the summarized relative risk estimate for colorectal cancer comparing highest *versus* lowest category of calcium intake of 0.89 (95% CI 0.79, 1.01) and adenomas of 1.13 (95% CI 0.91, 1.39) did not support the hypothesis of a protective effect of calcium. This conclusion runs counter to two recently published intervention trials, which showed a lower risk of recurrent adenoma for individuals taking calcium supplements (54, 55). The present study indicated only limited evidence of a weak inverse association between calcium and colorectal adenoma. Considering the high correlation of calcium intake and vitamin D intake in countries fortifying dairy products with vitamin D, the weak association of calcium intake with adenoma might also be explained by vitamin D intake in these countries.

Because calcium and vitamin D are metabolically related, we looked for an interaction between the two. The results suggested only a weak effect modification.

We analyzed the 5' translational start-site polymorphism in the VDR gene for which functional differences have been described (56). We found neither a considerable direct association of *FokI* genotype with colorectal adenoma nor a multiplicative interaction with either serum 25-(OH)D or calcium intake. The limited number of individuals for whom genotype data were available and further restriction to non-Hispanic Caucasians reduced the power to detect effects, and in particular, to examine interactions. We found a relative low frequency for the *FokI* genotype ff in controls compared with other studies investigating Caucasian populations (57–60) also indicated by a low P of the Hardy-Weinberg equilibrium ($P = 0.05$). Because we were worried that analytical problems were the reason for this low P , we went back and reexamined the genotype analysis and quality control samples, which did not indicate any analytical problems. However, we have to keep in

mind that the discrepancy in genotype frequency can affect the interpretation of the association between the *FokI* polymorphism and the genotype.

Studies of the effect of VDR polymorphisms on different cancer sites, mainly prostate and breast, have been inconclusive (61–68). Since these studies analyzed mainly the 3' VDR polymorphisms, which are not linked to 5' polymorphism, we can not directly compare the studies. Only two other studies examined the effect of VDR polymorphisms on colorectal neoplasia (69, 70). Kim *et al.* (69) examined the 3' polymorphism (*BsmI*), which was not associated with colorectal adenoma, but it did modify the association of vitamin D intake (diet and supplements) and calcium intake with colorectal adenoma. In contrast, Slattery *et al.* (70) found that the variants of the 3' VDR polymorphisms (*BsmI*, *TaqI*, and polyA), but not the 5' VDR polymorphism (*FokI*), were associated with a significant reduced risk of colon cancer. The sparse data and uncertainties about functional differences in VDR polymorphisms leave the interpretation of the effect of VDR polymorphisms on colorectal adenoma speculative.

A major concern about case control studies is potential bias attributable to the retrospective study design. In the present study, we use adenoma as intermediate biomarkers of colorectal cancer. It is likely that blood levels and dietary assessments are less affected by the disease process at this early preneoplastic stage. Niv *et al.* (71) showed that serum levels of 25-(OH)D were significantly lower in healthy controls than in cases with colorectal cancer and that the serum level slightly, but not significantly, decreased with increasing severity of colorectal carcinoma. Based on their findings, we might expect that if premalignant adenomas affect the serum 25-(OH)D level, it would bias our results toward the null, and we might expect an even stronger inverse association of vitamin D with colorectal adenoma in a prospective study setting. Exposures were assessed by questionnaires and blood analysis for a time when the adenoma already occurred. If the exposures changed during the time of adenoma development, exposure misclassification might attenuate the risk estimates toward the null. Additional misclassification of disease status might attenuate the risk estimates toward the null, because control subjects underwent only sigmoidoscopy, and adenoma on the right side of the colon would have been missed. To address this concern, we restricted the analysis to cases with left-sided adenoma. For all three exposures, serum 25-(OH)D level, calcium intake, and VDR genotype the risk estimates for adenoma were very similar, including only left-sided adenoma compared with including all adenoma [data only shown for 25-(OH)D level]. An additional limitation is that the control selection was hospital based. If the control subjects differ from the underlying population from which the cases were drawn, *e.g.* in calcium intake, the risk estimates can be biased.

In conclusion, our study showed an inverse association of serum vitamin D with colorectal adenoma, which appeared particularly strong in the deficiency range of serum vitamin D levels. The inverse association between calcium intake and colorectal adenoma was weak. The study did not provide evidence for an association of the VDR *FokI* polymorphism with colorectal adenoma, and the *FokI* genotype did not modify the association between vitamin D or calcium and colorectal adenoma, although our study had limited ability to investigate effect modification.

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Appendix

Here we briefly describe the piecewise linear logistic model we used to describe the effect of vitamin D on the risk of colorectal adenoma (Fig. 1). Let x denote the vitamin D variable measured as a continuous exposure and x_0 denote a specified node. The disease risk can be modeled as a linear function of x with two different slopes for the line in the two regions defined by the node x_0 , as follows:

$$\log \frac{p}{1-p} = \alpha + \beta_1 x + \beta_2 (x - x_0)_+,$$

where, p is the probability of the disease and $(x - x_0)_+$ is the positive component of $(x - x_0)$, otherwise, that is:

$$(x - x_0)_+ = \begin{cases} x - x_0 & \text{if } (x - x_0) \geq 0 \\ 0 & \text{otherwise} \end{cases}$$

In the above model, if $x \leq x_0$ the effect of x per unit increase is given by an increase of odds of the disease by a factor of $\exp(\beta_1)$, whereas for $x \geq x_0$, the corresponding effect is given by $\exp(\beta_1 + \beta_2)$.

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