Research Article

Cancer Prevention Research

Circulating Vitamin D Levels and Risk of Colorectal Cancer in Women

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Abstract

Observational data on the association between circulating 25(OH)D and colorectal cancer risk are limited in women. To determine whether prediagnostic levels of 25(OH)D were associated with risk of incident colorectal cancer in the Women's Health Study (WHS), we conducted a nested case–control study using 274 colorectal cases and 274 controls. Each case was matched to a control by age, ethnicity, fasting status at the time of blood collection, time of day when blood was drawn, and month of blood draw. Conditional logistic regression was used to estimate the OR and 95% confidence interval (CI) for colorectal cancer by 25(OH)D quartiles. Mean plasma 25(OH)D was lower in cases versus controls (21.9 vs. 23.9 ng/mL, $P = 0.01$). In multivariable-adjusted logistic regression models, plasma 25(OH)D was significantly and inversely associated with odds of colorectal cancer (quartile 4 [Q4] vs. quartile 1 [Q1]: OR, 0.45; 95% CI, 0.25–0.81; P_{trend} 0.02). In addition, we observed a somewhat lower risk of colorectal cancer-related mortality after adjustment for matching variables, randomization treatment and other risk factors (Q4:Q1 OR, 0.40; 95% CI, 0.17–0.97; P_{trend} 0.05). In this cohort of healthy women, we found a significant inverse association between prediagnostic 25(OH)D levels and risk of incident colorectal cancer, and a borderline significant inverse association between prediagnostic 25(OH)D levels and colorectal cancer-related mortality. These results support a possible association between plasma 25(OH)D and risk of colorectal cancer in women. *Cancer Prev Res; 8(8); 675–82. ©2015 AACR.*

See related commentary by Demetrius Albanes, p. 657

Introduction

Colorectal cancer is the third leading cause of cancer incidence and death in the United States (1). Laboratory studies suggest that vitamin D and its analogues may inhibit colorectal cancer development and growth through regulation of cellular proliferation and differentiation (2–5) and inhibition of angiogenesis (6). Based mainly on studies of bone health, a recent report from the Institute of Medicine recommended dietary intake to achieve circulating 25-hydroxyvitamin D (25[OH]D) of >20 ng/mL (50 nmol/L; ref. 7). Epidemiology studies of vitamin D intake and predicted vitamin D plasma status have been fairly consistent in suggesting an association between higher vitamin D and reduced colorectal cancer risk (8, 9), but limited data exist on women (9–12). A recent observational study meta-analysis reported a 26% lower risk of colorectal cancer per 10 ng/mL increment in blood 25(OH)D levels (13). Vitamin D supplementation randomized control trials have not been supportive of an association between vitamin D and colorectal cancer, but this lack of association may be influenced by the dose of vitamin D used or the duration of the intervention (14, 15). Among the 36,000 postmenopausal women in the Women's Health Initiative (WHI), daily calcium (1,000 mg) plus low-dose vitamin D_3 (400 IU) did not reduce total cancer incidence, but there was a suggestion of a protective effect against total cancer-related mortality over the 7-year follow-up (14, 15). Beyond any influence on cancer incidence, recent studies suggest that vitamin D could represent an important determinant of cancer survival (16).

Given the strong laboratory data and conflicting findings from epidemiologic studies, we prospectively examined the association between plasma 25(OH)D and colorectal cancer risk in a large cohort of initially healthy women where plasma samples were collected before cancer diagnosis. Plasma 25(OH)D level considered the best indicator for reflecting overall vitamin D status. 25(OH)D is the precursor of $1,25(OH)_2D$, binds to vitamin D receptor (VDR), to exert its growth inhibitory effects in colon cancer cells through regulation of several genes that are responsible for cell proliferation, differentiation, and apoptosis (17). We hypothesized that higher 25(OH)D levels would be associated with reduced risk of incident colorectal cancer and colorectal cancer mortality in women.

Materials and Methods

Study population

The Women's Health Study (WHS) is a completed randomized, placebo-controlled double-blinded trial originally designed to examine the role of aspirin (100 mg every other day) and vitamin E (600 IU every other day) in the prevention of cancer and cardiovascular disease (CVD) among 39,876 women free of cancer and CVD. The randomized controlled trial began in 1992. Every 6 months for the first year and every year thereafter,

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participants received questionnaires that assessed their compliance with study drug, potential side effects, and clinical outcomes of interest. When the trial ended in 2004, 33,682 women (88.6% of those alive) consented to continue with observational follow-up, reporting on their health habits and medical history annually on questionnaires.

For cases of invasive colorectal cancer reported during the trial or post-trial period, subjects granted written consent for medical record review. Medical records were retrieved for all women that reported a diagnosis of colorectal cancer. The retrieved medical records were subsequently reviewed by WHS designated physicians to identify any endpoints. The designated physician reviewers were blinded to the treatment assignment. Cases were individually matched to controls by age (±1 year), ethnicity, fasting status at time of blood collection (>8 hours vs. not), time of day when blood was drawn (±4 hours), and month of blood draw. Morbidity follow-up rates were complete for 97.2% and mortality follow-up rates for 99.4%. A total of 453 colorectal cancer cases have been identified in the WHS cohort as of 2011 during a mean follow-up duration of 17 years; 321 (71%) provided blood and 132 (29%) did not provide blood; such distribution is also similar to that in the entire WHS blood cohort. The design of 1 case matched to 1 control has yielded a total of 274 colorectal cancer cases available for the present study.

Baseline blood collection

Before randomization in the WHS, blood was collected by mail using a blood-collection kit containing instructions, tubes, blood draw supplies, a gel-filled freezer pack, and a completed overnight courier air bill. Women were asked to freeze the gel-filled freezer pack overnight to serve as a coolant and to return the completed blood kit to us via overnight courier. Of the 39,876 randomized women in the trial, 28,345 (71%) provided a baseline blood sample. Women who did and did not donate blood were similar for a wide range of variables related to cancer (18).

25(OH)D plasma assay

Plasma 25(OH)D was assayed at Heartland Assays, Inc. and was measured using a Diasoran radioimmunoassay. All samples for plasma 25(OH)D were shipped in a single batch to the reference laboratory, with laboratory personnel blinded to case, control, or quality control status. The mean intra-assay coefficient of variance for blinded, replicate quality control samples was 6.0%.

Statistical analysis

Using a nested case–control design with 1:1 matching (by age, ethnicity, month of blood draw, fasting more than 8 hours status), we evaluated whether the risk of colorectal cancer is modified by baseline plasma levels of 25(OH)D. The distributions of baseline characteristics by cancer and control groups were compared using standard measures for two-sample testing, such as *t* test and McNemar test. 25(OH)D in control subjects was categorized by quartiles. A conditional logistic regression model was used to calculate the ORs and 95% confidence intervals (CI). Tests for trend were calculated by using the median values for the quartiles of 25(OH)D. All *P* values were two-tailed. The simple model included the following covariates: season of blood draw and randomized treatment assignment to aspirin or vitamin E. For multivariable-adjusted models, we additionally included established risk factors for colorectal cancer assessed at baseline, including family history of colorectal cancer, red meat intake, dietary calcium intake, alcohol intake, postmenopausal hormone (PMH) use, body mass index (BMI), physical activity, smoking status, and multivitamin use. Missing values for each covariate, if applicable, were coded as an indicator variable to indicate missing. When analyzing rectal cancer risk and colorectal cancer mortality, we used unconditional logistic regression with simple and multivariable adjustment as described above in addition to adjustment for matching factors in either models. Cutoff points for the unconditional models were based on the remaining cases and controls available in the analysis. To evaluate whether aspirin assignment may be an effect modifier for the association between 25(OH)D and colorectal cancer development, we treated 25(OH)D as a continuous variable and aspirin assignment as a binary variable (yes/no) for the interaction term in the multivariable regression model. To examine whether the association between 25(OH)D and risk of colorectal cancer was modified by other risk factors of colorectal cancer, we performed preplanned subgroup analyses using unconditional logistic regression adjusted for the matching factors and other relevant covariates. We examined the association in subgroups defined by BMI (BMI < 30 kg/m^2, BMI ≥ 30 kg/m^2) and physical activity (above median and below median of reported physical activity). Test for interaction of PMH use, BMI, and physical activity and plasma 25(OH)D on the association between plasma 25(OH)D and colorectal cancer were performed by the Wald test of cross-product terms. We used SAS 9.2 for all analyses.

Results

The median follow-up for this study population is 16.3 years. At baseline, characteristics were similar between colorectal cancer cases and controls with regard to BMI, current smoking, family history of colorectal cancer, physical activity, and intakes of alcohol, red meat, calcium, and multivitamins (Table 1). There

Table 1. Baseline characteristics (mean or %) among cases of colorectal cancer and matched controls in the WHS[a]

Characteristics	Cases Cancer (*N* = 274)	Controls (*N* = 274)	*P*
Age, y (SD)	58.8 (8.3)	58.8 (8.3)	Matched
Race, Caucasian (%)	96.4	96.4	Matched
BMI, kg/m^2	26.7	26.3	0.33
Current smoking (%)	9.9	11.4	0.57
Family history of colorectal cancer (%)	12.0	9.9	0.38
Colorectal cancer screening (%)[b]	9.4	16.7	0.66
Postmenopausal (%)	74.1	76.3	0.38
Current PMH use (%)[c]	44.5	41.8	0.29
Current use of multivitamins (%)	36.5	38.0	0.72
Blood drawn in the summer (%)	49.8	44.7	0.33
Physical activity (MET-h/wk)	15.3	16.5	0.48
Alcohol intake (g/d)	5.1	4.8	0.78
Red meat (servings/d)	0.7	0.7	0.76
Total calcium intake (mg/d)	1042	1062	0.62
Plasma 25(OH)D levels, mean (SD)	21.9 (8.3)	23.9 (9.8)	0.005
Randomized to aspirin treatment (%)	23.2	25.2	0.35
Randomized to vitamin E treatment (%)	22.8	25.4	0.23

[a]Mean is reported for continuous variables.
[b]Information was obtained at the 12-month follow-up questionnaire. Assessment of colorectal cancer screening included only colonoscopy or flexible sigmoidoscopy.
[c]PMH use is for all cases (perimenopausal and postmenopausal).

Table 2. Partial correlation of 25(OH)D with BMI, physical activity, and smoking

Characteristic	Correlation coefficient	*P*
BMI (kg/m^2)	−0.21	0.0007
Physical activity (total Met-h/wk)	0.12	0.06
Current smoking (yes/no)	−0.13	0.05

NOTE: Multivariate model adjusted for randomized treatment assignment to aspirin and vitamin E, season for blood collection (summer, yes/no), BMI (weight (kg)/height (m)^2), physical activity (MET-h/wk), family history of colon cancer, smoking status (current), alcohol consumption (g/d), multivitamin use (never, past, and current), red meat (servings/d), calcium (mg/d), menopausal status, and hormone therapy use.

was no difference between cases and controls in baseline PMH use and screening exams. Cases and controls were also similar in blood drawn seasons. However, the baseline plasma 25(OH)D was significantly lower in colorectal cancer cases than in controls (cases: 21.9 ng/mL; controls: 23.9 ng/mL, $P = 0.01$). Colorectal cancer colonoscopy screening rates were similar across 25(OH)D quartiles ($P = 0.09$). Randomized assignment to aspirin and vitamin E was similar in cases and controls. The multivariate-adjusted partial correlations of 25(OH)D with BMI, physical activity, and smoking were −0.21, 0.12, and −0.13, respectively (P values ≤ 0.06; Table 2).

Table 3 shows the association between plasma 25(OH)D levels and colorectal cancer risk in both simple and multivariable models. First, a significant association was observed between plasma 25(OH)D level and the risk for colorectal cancer and colon cancer in the simple models. A similar association was observed in the multivariable-adjusted conditional logistic regression models (colorectal cancer: quartile 4 [Q4] vs. quartile 1 [Q1], OR, 0.46; 95% CI, 0.24–0.89); P_{trend} 0.03; colon, Q4 vs. Q1 OR, 0.44; 95% CI, 0.21–0.95; P_{trend} 0.04). Because of a small number of cases, risk for rectal cancer was evaluated using unconditional logistic regression; no significant association was observed between plasma 25(OH)D levels (Q4 vs. Q1, OR, 0.84; 95% CI, 0.34–2.08; P_{trend} 0.90; Table 2). When we used clinical cutoff points with 25(OH)D less than 20 ng/mL as the reference group, the multivariable-adjusted OR (95% CI) of colorectal cancer risk for 20 to 30 ng/mL and greater than 30 ng/mL were 0.98 (0.64–1.50) and 0.58 (0.32–1.05), respectively, P_{trend} 0.10. In multivariate analyses, no reduction of incident colorectal cancer until the fourth quartile suggests a threshold effect whereas the colorectal cancer mortality analyses suggests a threshold effect for the second to fourth quartile. In the overall multivariable regression analysis, including aspirin assignment as a covariate, aspirin was not associated with colorectal cancer development (OR, 0.79; 95% CI, 0.55–1.15). We also observed no effect modification by aspirin assignment ($P_{interaction} = 0.88$). The unconditional logistic regression for the association between 25(OH)D and colorectal cancer risk according to aspirin assignment revealed no clear trend in either groups. The ORs in the higher quartile groups as compared with the lowest quartile according to aspirin treatment were: (i) aspirin-treated group, Q2, 1.40 (95% CI, 0.70–2.78), Q3 1.86 (95% CI, 0.91–3.78), Q4 0.60 (95% CI, 0.30–1.23), P_{trend} 0.22; (ii) aspirin-untreated: Q2 0.57 (95% CI, 0.29–1.13), Q3 0.70 (95% CI, 0.35–1.37), Q4 0.60 (95% CI, 0.30–1.21), and P_{trend} 0.22.

A total of 63 colorectal cancer deaths were ascertained in this study population, We observed a significant inverse association between plasma 25(OH)D and colorectal cancer mortality in a simple model (Q4 vs. Q1, OR, 0.42; 95% CI, 0.19–0.94; P_{trend} 0.048; Table 2). The multivariable-adjusted model showed that the association was only of borderline significance (Q4 vs. Q1: OR, 0.40; 95% CI, 0.17–0.97; P_{trend} 0.051; Table 2).

Subgroup analysis by tumor characteristics, including location (proximal, distal, rectal, P_{trends}, respectively: 0.07, 0.11, and 0.66), stage (A, B, C/D, P_{trends}, respectively: 0.08, 0.41, and 0.41) and grade (well and moderately differentiated, P_{trend}: 0.29) did not

Table 3. ORs and 95% CIs of colorectal cancer incidence and colorectal cancer mortality according to plasma levels of 25(OH)D in the WHS

	Quartiles of 25(OH)D ng/mL				
	Q1	Q2	Q3	Q4	
			Colorectal cancer incidence		
Range, ng/mL	3.7–17.4	17.5–22.3	22.4–29.3	29.4–66.0	P_{trend}
Cases/controls, *n*	79/69	70/70	83/68	42/67	
Simple OR[a]	1.0	0.83 (0.52–1.32)	0.99 (0.60–1.63)	0.45 (0.25–0.81)	0.02
Multivariable OR[a]	1.0	0.84 (0.50–1.42)	0.97 (0.55–1.73)	0.46 (0.24–0.89)	0.03
			Colon cancer incidence		
Range, ng/mL	3.7–17.4	17.5–22.1	22.2–29.2	29.3–66	
Cases/controls, *n*	62/52	49/50	58/51	35/51	
Simple OR[a]	1.0	0.78 (0.45–1.34)	0.84 (0.48–1.49)	0.48 (0.25–0.92)	0.03
Multivariable OR[a]	1.0	0.80(0.43–1.48)	0.81 (0.41–1.59)	0.44 (0.21–0.95)	0.04
			Rectal cancer incidence[b]		
Range, ng/mL	4.8–17.4	17.5–22.3	22.4–28.3	28.4–66.0	
Cases/controls, *n*	17/69	17/70	24/61	12/74	
Simple OR[a]	1.0	0.95 (0.44–2.05)	1.67 (0.80–3.50)	0.63 (0.27–1.45)	0.45
Multivariable OR[a]	1.0	1.16 (0.50–2.67)	1.94 (0.86–4.38)	0.84 (0.34–2.08)	0.90
			Colorectal cancer mortality[b]		
Range	4.8–16.9	17.0–22.0	22.1–28.1	28.2–66.0	
Cases/controls, *n*	23/62	15/71	18/66	11/74	
Simple OR[a]	1.0	0.59 (0.28–1.25)	0.74 (0.36–1.52)	0.42 (0.19–0.94)	0.048
Multivariable OR[a]	1.0	0.55 (0.25–1.28)	0.57 (0.25–1.28)	0.40(0.17–0.97)	0.051

[a]Simple models were adjusted for randomized treatment assignment to aspirin and vitamin E and season for blood collection (summer, yes/no). Multivariable-adjusted models were additionally adjusted for BMI [weight (kg)/height (m)^2], physical activity (MET-h/wk), family history of colon cancer, smoking status (current), alcohol consumption (g/d), multivitamin use (never, past, and current), red meat (servings/d), calcium (mg/d), menopausal status, and hormone therapy use.
[b]Rectal cancer incidence and colorectal cancer mortality were evaluated using unconditional logistic regression models adjusting for matching variables (age, race, month of blood draw, fasting status at time of blood draw) because of the small number of cases.

Table 4. 25(OH)D and colorectal cancer incidence by BMI (kg/m^2) subgroups

	Quartiles of 25(OH)D ng/mL				
	Q1	Q2	Q3	Q4	P_{trend}
BMI < 25					
Range, ng/mL	6.7–18.9	19.3–23.0	23.1–29.2	29.3–57.1	
Cases/controls, *n*	37/28	34/31	29/34	27/37	
Simple OR†	1.0	0.84 (0.41–1.72)	0.66 (0.32–1.36)	0.56 (0.27–1.15)	0.095
BMI 25–29.9					
Range, ng/mL	3.7–16.4	16.5–21.9	22.1–26.4	26.9–66.0	
Cases/controls, *n*	23/25	23/22	25/21	22/24	
Simple OR†	1.0	1.19 (0.52–2.76)	1.20 (0.53–2.74)	0.90 (0.39–2.09)	0.84
BMI $\geq$ 30					
Range, ng/mL	4.8–12.5	12.6–18.6	19.0–26.4	26.5–54.1	
Cases/controls, *n*	14/13	14/12	16/11	10/16	
Simple OR†	1.0	1.10 (0.36–3.37)	1.23 (0.40–3.79)	0.54 (0.17–1.68)	0.26

†Simple models were adjusted for randomized treatment assignment to aspirin and vitamin E and season for blood collection (summer, yes/no).

reveal significant association with plasma 25(OH)D. The only exception was found for risk of poorly differentiated tumors (Q4 vs. Q1: multivariable-adjusted OR, 0.16; 95% CI, 0.03–0.95; P_{trend} 0.03). No interaction was observed between PMH use, BMI, or physical activity and plasma 25(OH)D for the association between plasma 25(OH)D and colorectal cancer (PMH: $P_{interaction}$ = 0.75; BMI: $P_{interaction}$ = 0.23; physical activity: $P_{interaction}$ = 0.14).

The association between 25(OH)D and CRC incidence is not significantly associated with BMI subgroup status (Table 4). Similarly, the association between 25(OH)D and CRC incidence is not significantly associated with physical activity status (Table 5).

Discussion

In this nested case–control study, we observed a significant inverse association between prediagnostic plasma 25(OH)D and subsequent risk of colorectal cancer and a borderline significant inverse relationship for colorectal cancer mortality. The significant association was mostly seen in risk for colon but not rectal cancer. No effect modification was found by PMH therapy, obesity, and physical activity for the association between 25(OH)D and colorectal cancer. The strongest reduction in incident colorectal cancer and colorectal cancer mortality was observed for 25(OH)D levels greater than 29 ng/mL.

Our results are similar to other studies reporting an inverse association between 25(OH)D and colorectal cancer.(Table 6; refs. 19, 12). In the British (20) and the WHI (14) trials, vitamin D did not reduce colorectal cancer incidence overall, but nonsignificant reductions in colorectal cancer mortality were found. Interestingly, the WHI participants who had the highest baseline levels of plasma 25(OH)D experienced a significant 60% reduction in colorectal cancer risk (16, 21). It is plausible that participants who did achieve a 25(OH)D level beyond 35 ng/mL (through means other than assigned vitamin D supplement) did experience a substantial reduction in colorectal cancer risk (14). Furthermore, in the WHI, although the dose of vitamin D was suboptimal, supplemental vitamin D did confer nonstatistically significant reductions for colorectal cancer mortality (RR, 0.82; 95% CI, 0.52–1.29; P = 0.39), total cancer-related mortality (RR, 0.89; 95% CI, 0.77–1.03; P = 0.12), and total mortality (RR, 0.93; 95% CI, 0.83–1.01; P = 0.07; ref. 14). Recent meta-analyses have, with few exceptions (22), found that 25(OH)D levels are inversely associated with risk for colorectal cancer (13, 23–26) and colorectal adenoma (27–29). Table 6 shows a summary of studies that evaluated the association of circulating levels of vitamin D and risk of colorectal cancer (13, 23, 11, 12, 30). The pooled relative risk (RR) of colorectal cancer for the highest versus lowest categories of 25(OH)D was 0.67 (95% CI, 0.54–0.80; ref. 13). A 10 ng/mL increase in 25(OH)D value was associated with a RR of 0.74 (95% CI, 0.63–0.89; ref. 13). There was no heterogeneity among studies of blood 25(OH)D values (P = 0.96; ref. 13). The other pooled analysis of Nurses' Health Study (NHS; ref. 9) and Health Professionals Follow-Up Study (HPFS; ref. 30) reported a significant reduced risk (top vs. bottom quintile: RR, 0.66; 95% CI, 0.42–1.05; ref. 30). Another recent meta-analysis review of 5 large prospective studies (including NHS and WHI; ref. 14) has shown that 25(OH)D $\geq$ 33 ng/mL had a 51% reduction in risk of colorectal cancer compared to those with $\leq$12 ng/mL; ref. 31). Prospective data on vitamin D status and total cancer remain limited (8, 32, 33) Yet, prospective observational studies of plasma 25(OH)D levels generally support an inverse association with risk of neoplasia (30, 31, 34–42).

Clinical trials on vitamin D supplementation for the prevention of cancers have been very limited. Four vitamin D trials have assessed total cancer incidence or total cancer-related mortality as secondary outcomes. In a British trial (20), 2,686 older adults were randomized to 100,000 IU of vitamin D_3 or placebo and followed for up to 5 years, vitamin D was not associated with reduced total cancer incidence, but a nonsignificant inverse association for total cancer-related mortality was noted. In the Lappe et al. trial (43), 1,179 postmenopausal women were randomized to one of three groups—calcium plus vitamin D_3; active calcium plus placebo vitamin D; or placebo calcium plus placebo vitamin D. Women in the calcium plus vitamin D group were significantly less likely to develop cancer than women in the placebo group. In contrast, among the 36,000 postmenopausal women in the WHI, daily calcium (1,000 mg) plus low-dose vitamin D_3 (400 IU) did not reduce total cancer incidence, although there was a suggestion of a protective effect against total cancer-related mortality over the 7-year follow-up (14, 15). Finally, in the *R*andomized *E*valuation of *C*alcium *OR* vitamin *D* (RECORD) trial, 5,292 adults (85% women), age 70 years or older, were randomized to vitamin D_3 (800 IU/d), calcium (1,000 mg/d), both, or placebo for 24 to 62 months for the secondary prevention of fractures and were then followed observationally

Table 5. 25(OH)D and colorectal cancer incidence by physical activity

	Quartiles of 25(OH)D ng/mL				
	Q1	Q2	Q3	Q4	P_{trend}
Physical activity (MET-h/wk) < median					
Range, ng/mL	3.7–15.6	15.7–20.6	20.7–26.9	27.1–57.1	
Cases/controls, *n*	42/27	30/38	43/28	35/31	
Simple OR†	1.0	0.49 (0.24–0.99)	0.96 (0.48–1.93)	0.70 (0.34–1.43)	0.66
Physical activity (MET-h/wk) ≥ median					
Range, ng/mL	4.8–17.9	18.2–23.0	23.1–29.0	29.2–66.0	
Cases/controls, *n*	31/38	39/30	33/36	21/46	
Simple OR†	1.0	1.74 (0.87–3.50)	1.13 (0.57–2.26)	0.60 (0.29–1.25)	0.09

†Simple models were adjusted for randomized treatment assignment to aspirin and vitamin E and season for blood collection (summer, yes/no).

for 3 years (44). Pre- and post-intervention 25(OH)D levels were 15.3 and 24.9 ng/mL, respectively, in a randomly selected subgroup of 60 participants. Similar to the findings in the British and WHI trials, there was a nonsignificant inverse association between assignment to vitamin D and cancer-related mortality (RR, 0.85; 95% CI, 0.68–1.06) but not cancer incidence. Thus, the limited data from randomized trials do not clearly support an overall chemopreventive effect for total cancer (45).

In its 2011 review, the IOM concluded that large randomized trials of vitamin D at higher doses than tested in the WHI are necessary for a definitive assessment of its effect on site-specific cancers (7). The association between lower regional UV-B intensity and colorectal and other digestive tract cancers is noteworthy given the evidence, suggesting that digestive tract malignancies may be particularly sensitive to vitamin D (46, 47). Laboratory studies reveal that colorectal cancer cells have vitamin D receptors and 25(OH)D-1-α-hydroxylase, which changes 25(OH)D to the active form of $1,25(OH)_2D$ (16). These cells also express vitamin D receptors, which translocate to the nucleus and bind vitamin D response factors to control gene expression, after engagement by $1,25(OH)_2D$ (48). The generally accepted best indicator of vitamin D nutritional status is plasma 25-hydroxyvitamin D_3 (25(OH) D), because it reflects not only skin exposure to UV-B light and total vitamin D intake but also cholecalciferol production in the skin and hydroxylation of all sources of cholecalciferol in the liver (21). The optimal 25(OH)D level needed to achieve adequate levels of plasma 25(OH)D for cancer chemoprevention is unknown (46, 49). Some studies have provided promising evidence (50–52). In a randomized clinical trial of the effects of 6 months of supplemental calcium (2 g/d) and vitamin D3 (800 IU/d) results suggest that calcium and vitamin D may enhance apoptosis in normal colonic mucosa based on changes in molecular markers of apoptosis (53). Vitamin D receptor expression is associated with PIK3CA and KRAS mutations in colorectal cancer (48). The PI3K–Akt pathway mediates many extracellular signals and regulates cell growth, proliferation, invasion, and survival. Tumoral mutations in

Table 6. Prospective cohort studies of circulating levels of 25(OH)D and risk of colorectal cancer (13, 23)

Study	Country (sex)	Age at blood donation (y)[a,b]	Study dates (follow-up)	25(OH)D (ng/mL), Cases/controls[a,b,c,d]	Range (ng/mL)[e]	No. of cases/ controls[i]	RR[f,i]	95% CI
Garland et al. (34)	US (M, W)	35–75	1975–1983	30.5/33.3[a]	23	34/67	0.73	0.20–2.66
Braun et al. (35)	US (M, W)	55[b]	1984–1991	23.6/23.2[a]	12.9	57/114	0.40	0.10–0.40
Tangrea et al. (36)	Finland (M)	60[b]	1985–1993	12.1/13.8[a]	9.5	146/290	0.60	0.30–1.10
Feskanich et al. (9)	US (W)	60.0[a]	1989–2000	23.6/24.3[a], lab 1 27.0/30.3[a], lab 2	20.4	193/383	0.53	0.27–1.04
Wu et al. (30)	US (M)	66.1[a]	1993–2002	28.7/29.4[a]	21.0	179/356	0.66	0.42–1.05
Wactawski-Wende et al. (14)	US (W)	50–79	1993–2005	N/A	11.0	306/306	0.75	0.39–1.48
Otani et al. (37)	Japan (M)	56.9[a]	1990–2003	27.3/27.6[b]	9.2	163/324	0.73	0.35–1.50
	Japan (W)	56.4[a]	1990–2003	22.5/22.3[b]	8.3	160/297	1.10	0.50–2.30
Jenab et al. (40)	Europe (M, W)	30–77	1992–2003	20.7/22.9[c] 22.0/22.0[d]	8.0	785/785[g] 463/463[h]	0.77	0.56–1.06
Woolcott et al. (39)	US (M, W)	69.2[a]	2001–2006	23.2/25.0[a]	16.0	229/434	0.60	0.33–1.07
Lee et al. (23)	US (M)	56.6[a]	1982–2000	26.6/25.6[a]	12.2	229/389	1.08	0.62–1.87
Weinstein et al. (11)	US (M, W)	64[b]	1993–2001	21/22.7[b]	12	476/476	0.59	0.36–0.95
Wong et al. (60)	Australia (M)	77[a]	1996–2004	27.1/27.4	N/A	102/3614[i]	1.04[i]	0.94–1.14
Jung et al. (12)	US (M,W)	66.3 (M)[j] 65.0 (W)[j]	1986–2008	28 (M)[k] 32 (W)[k]	7.8 (M) 10.4 (W)	1059/140,418[i]	0.55[i]	0.43–0.71

[a]Mean.
[b]Median.
[c]Geometric mean in colon cancer.
[d]Geometric mean in rectal cancer.
[e]Range, the difference in the midpoint between the highest and lowest categories of 25-hydroxyvitamin D blood levels.
[f]Adjusted RRs of colorectal cancer for the highest versus lowest categories of 25-hydroxyvitamin D blood level.
[g]Colon.
[h]Rectum.
[i]Competing risk proportional hazard model; positive.
[j]Median age of highest quintile for men (M) and women (W).
[k]Predicted 25(OH)D score, median of highest quintile.

PIK3CA, the catalytic subunit of PI3K, activates AKT and promotes colorectal cancer cell growth (54).

This study has numerous strengths, including the nested case–control design, rigorous collection of covariate information, and detailed information on tumor characteristics. Limitations of this study include having only a single measure of 25(OH)D. However, a previous study showed a correlation of 0.70 for repeated measures of plasma 25(OH)D within participants over a one year period, suggesting that a single measurement is a reasonable proxy for long-term levels of 25(OH)D (55). Furthermore, we only measure colorectal cancer screening at baseline, and the reported rate of colorectal cancer screening is low because we only reported colonoscopy and flexible sigmoidoscopy screening for colorectal cancer. We also cannot rule out that our findings may be due to residual confounding. Yet, likely confounders, including BMI, physical activity, supplement use (e.g., vitamin D, multivitamin, and calcium), and dietary calcium, were adjusted for in the multivariable model based on the validated baseline questionnaire and food frequency questionnaire (56–58), and we did not observe meaningful changes in our OR estimates after control for these confounders. Nevetheless, residual confounding may be present from unmeasured factors such as inflammation evaluated by inflammatory markers (59). Colorectal cancer screening information was obtained at the 12-month follow-up questionnaire and was not significantly different between cases and controls. Our participants are primarily White and we did not have adequate power to examine the association of 25(OH)D in non-Whites. Further studies in non-Whites are needed. Finally, we do not have sufficient sample to conduct analysis for rectal cancer risk and for risk of other tumor characteristics.

In this nested case–control study of women, we observed a statistically significant inverse association between plasma 25(OH)D levels and the subsequent risk of colorectal cancer. Participants with 25(OH)D greater than 29 ng/mL experienced the greatest reduction in incident colorectal cancer and mortality. In light of the high prevalence of vitamin D insufficiency in the population, future studies should examine whether increasing vitamin D levels reduces the incidence of this lethal malignancy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Disclaimer

The sponsors were not involved in the design, data collection, analysis, or interpretation of the study; nor where they involved in writing the article. There was no funding for this specific study.

Authors' Contributions

Conception and design: P.D. Chandler, S. Zhang, J.H. Lin
Development of methodology: J.A.E. Manson, J.H. Lin
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J.E. Buring, S. Zhang, I.-M. Lee
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): P.D. Chandler, J.A.E. Manson, E.L. Giovannucci, M.V. Moorthy, S. Zhang, J.H. Lin
Writing, review, and/or revision of the manuscript: P.D. Chandler, J.E. Buring, J.A.E. Manson, E.L. Giovannucci, M.V. Moorthy, S. Zhang, I.-M. Lee
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): S. Zhang
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