Research Article

Cancer Epidemiology, Biomarkers & Prevention

Circulating 25-Hydroxyvitamin D Concentration and Risk of Breast, Prostate, and Colorectal Cancers: The Melbourne Collaborative Cohort Study

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Abstract

Background: The role of vitamin D in cancer risk remains controversial, and limited data exist on associations between vitamin D and subtypes of specific cancers. We investigated associations between circulating 25-hydroxyvitamin D (25(OH)D) and risk of colorectal, breast, and prostate cancers, including subtypes.

Methods: A case–cohort study within the Melbourne Collaborative Cohort Study included 547 colorectal, 634 breast, and 824 prostate cancers, and a sex-stratified random sample of participants ($n = 2{,}996$). Concentration of 25(OH)D in baseline-dried blood spots was measured using LC-MS/MS. Cox regression yielded adjusted HRs and 95% confidence intervals (CI) for each cancer in relation to plasma-equivalent 25(OH)D concentration. Associations by stage and *BRAF*/*KRAS* status for colorectal cancer, estrogen receptor status for breast cancer, and aggressiveness for prostate cancer were examined in competing risks models.

Results: 25(OH)D concentrations were inversely associated with risk of colorectal cancer [highest vs. lowest 25(OH)D quintile: HR, 0.71; 95% confidence interval (CI), 0.51–0.98], which was limited to women (HR, 0.52; 95% CI, 0.33–0.82). Circulating 25(OH)D was also inversely associated with *BRAF* V600E–positive colorectal cancer (per 25 nmol/L increment: HR, 0.71; 95% CI, 0.50–1.01). There were no inverse associations with breast cancer (HR, 0.98; 95% CI, 0.70–1.36) or prostate cancer (HR, 1.11; 95% CI, 0.82–1.48).

Conclusions: Circulating 25(OH)D concentration was inversely associated with colorectal cancer risk for women, but not with risk of breast cancer or prostate cancer.

Impact: Vitamin D might play a role in preventing colorectal cancer. Further studies are required to confirm whether vitamin D is associated with specific tumor subtypes.

Introduction

Many observational studies have investigated associations between vitamin D status and risk of cancer, but results have been inconsistent (1, 2). Vitamin D status is generally assessed by serum or plasma 25-hydroxyvitamin D (25(OH)D) concentration because this metabolite is the main circulating form of vitamin D (with a half-life of 2–3 weeks) and reflects vitamin D from both cutaneous synthesis during UV exposure and exogenous sources (food and supplements; ref. 3). The most commonly investigated cancers have been colorectal cancer, breast cancer, and prostate cancer (4). Although there is suggestion of an inverse association of 25(OH)D with colorectal cancer, associations for breast cancer and prostate cancer are unclear (2, 4, 5). In addition, it is possible that associations might differ according to cancer subtypes, yet few studies have assessed associations by tumor characteristics such as stage (or disease aggressiveness in prostate cancer), somatic gene mutations (such as *BRAF* or *KRAS*) in colorectal cancer, and hormone receptor status in breast cancer (5).

Using a cohort of middle-aged Australians, we prospectively investigated the association between circulating 25(OH)D concentration and risk of incident breast, prostate, and colorectal cancers and examined associations by cancer subtypes.

Materials and Methods

Participants

The Melbourne Collaborative Cohort Study (MCCS) is a prospective cohort study of 41,513 residents of Melbourne, Australia,

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doi: 10.1158/1055-9965.EPI-18-1155

aged 27 to 76 (mean 55) years at recruitment (1990–1994). Details of the MCCS have been published (6). At baseline, participants attended clinics where they completed questionnaires on lifestyle and medical history as well as a 121-item food frequency questionnaire. Anthropometric measurements were performed by trained staff according to a standard protocol. Blood samples were collected from 41,113 (99%) participants; from the second year of study recruitment (from 1991 onward, for about 75% of participants), whole blood was spotted onto Guthrie cards, which were air dried and stored at room temperature in dark conditions. The Cancer Council Victoria's Human Research Ethics Committee approved the study protocol, and participants provided written informed consent.

Information about screening tests was not obtained at baseline. In a second wave of data collection about 4 years after baseline, participants completed a questionnaire that asked about mammography, prostate-specific antigen (PSA) tests, sigmoidoscopy, colonoscopy, and fecal occult blood tests (FOBT).

A case–cohort design was adopted for the vitamin D study (7). Participants with no prebaseline diagnosis of cancer and for whom a baseline dried blood spot (Guthrie card) sample was available were eligible ($n = 29{,}205$). The subcohort comprised random samples of 1,332 women (7.85% of 16,976) and 1,664 men (13.6% of 12,229), chosen to be proportionate to the expected number of cases of breast and prostate cancer, respectively. Vital status was determined from linkage to the Registry of Births, Deaths and Marriages Victoria and the National Death Index. Participants for whom vitamin D measurements were not performed and those with missing data for potential confounders were excluded from analyses.

Ascertainment and classification of cancers

Cases comprised all eligible participants who had a primary, histologically confirmed invasive adenocarcinoma of the colon or rectum, breast, or prostate diagnosed by December 31, 2007, and notified to the Victorian Cancer Registry. The Registry classifies all three tumor types according to stage and records grade (plus Gleason score in the case of prostate cancer) for all histopathologically confirmed tumors. We attempted to obtain archival tumor tissue for all cancers.

For colorectal cancer, the V600E *BRAF* mutation, which accounts for approximately 90% of *BRAF* mutations in colorectal cancer (8), was measured in DNA extracted from archival tumor tissue using a real-time PCR-based allelic discrimination method (9, 10). Somatic mutations in codons 12 and 13 of *KRAS* were identified using real-time PCR with high-resolution melting analysis followed by direct Sanger sequencing on cases with differential melting profiles (11).

The Registry routinely records information on estrogen receptor (ER) and progesterone receptor (PR) status of breast tumors, although in the early years of follow-up, reporting was incomplete. For cases with archival tissue available (67% of all cases), we repeated the measurement of ER and PR status using IHC (12). Because the agreement between the ER status assessed from the archival tumor tissue and the values on the original pathology reports held by the Victorian Cancer Registry was high (89%, kappa = 0.71; ref. 12), ER and PR status recorded by the Registry was used when archival tumor tissue was not available.

Assessment of 25(OH)D

Concentration of 25(OH)D from baseline dried blood spot samples was measured by LC-MS/MS in the laboratory of D.W. Eyles as previously described (13, 14). Measurements were performed over 15 months in 31 batches of approximately 230 samples each. The laboratory routinely calibrates relative accuracy using National Institute of Standards and Technology standard reference materials and participates in the Vitamin D External Quality Assessment Scheme. Samples were processed in random order, and laboratory analysts were blind to outcome status of participants. Reliability was assessed using repeat measurements on 493 subcohort members for whom duplicate samples were randomly interspersed throughout the samples. As previously reported, the within- and between-batch intraclass correlations were 0.82 [95% confidence interval (CI), 0.80–0.85] and 0.73 (95% CI, 0.68–0.78), respectively (14). Methods used for removing batch and seasonal effects in 25(OH)D measurements and conversion to plasma equivalent concentrations have been described (7, 14). All results presented are for batch- and season-adjusted plasma-equivalent 25(OH)D.

Statistical analysis

Follow-up began at baseline and ended at diagnosis of the cancer under study, date of leaving Australia, death, or December 31, 2007, whichever came first. HRs and 95% CIs were estimated using Cox regression. Barlow weights, with robust SEs, were used to account for the case–cohort design (15). Batch- and season-adjusted plasma-equivalent 25(OH)D was categorized into five groups, based on the sex-specific quintiles of the subcohort. We also modeled the association between continuously valued 25(OH)D and cancer risk. Cases for each cancer type were compared with the full subcohort (for colorectal cancer analyses), or female portion of the subcohort (breast cancer), or male portion of the subcohort (prostate cancer). To control for confounding by age, attained age was used as the timescale in all Cox regression models (16). All models were stratified by country of birth (Australia/New Zealand/northern Europe or southern Europe) and sex (for colorectal cancer), and further adjusted for the following potential confounding factors measured at recruitment: educational attainment (primary school, some high/technical school, completed high school, and completed tertiary degree/diploma), socioeconomic status (quintiles of relative disadvantage based on area of residence), physical activity (four ordered categories reflecting frequency and intensity of physical activity), smoking status (never, former, current), alcohol consumption (lifetime abstainer, former drinker, current low, current medium, current high, with the latter three determined by sex-specific tertiles in the subcohort), and waist circumference (grouped by sex-specific quartiles in the subcohort). Colorectal cancer analyses further adjusted for margarine intake (grouped by quartiles in the subcohort) and intake of processed meat (grouped by quartiles in the subcohort), and excluded participants deemed to have outlying total energy intakes reported in the Food Frequency Questionnaire (<1st and >99th sex-specific percentiles). Breast cancer analyses further adjusted for parity (any children vs. none), use of oral contraceptives (never, former, current), hormone replacement therapy (never, former, current), age at baseline clinic attendance (<55 vs. ≥55 years), and an interaction between this variable and waist circumference.

For colorectal cancer, we tested an interaction of 25(OH)D with sex. The proportional hazards assumption was assessed by fitting interactions between each covariate separately (modeled as a time varying effect) and attained age. There was no evidence that any covariate violated the assumptions.

Further analyses investigated whether HRs differed by cancer subtype, using competing risks models based on a data duplication approach (17). Differences in HRs by cancer subtypes were evaluated using Wald tests. For colorectal cancer, we compared associations between *BRAF*$^+$, *KRAS*$^+$, and *BRAF*$^-$/*KRAS*$^-$ cancers (only 3 tumors were *BRAF*$^+$/*KRAS*$^+$), and between stage I/II and stage III/IV cancers. For breast cancer, we compared ER$^+$ and ER$^-$ tumors. We did not analyze PR status because it was strongly associated with ER status, and only 19 cases were PR$^+$ but ER$^-$. We did not analyze stage for breast cancer because almost all cases were stage I or II, which both have similar, very high survival. For prostate cancer, we compared aggressive (defined as died from prostate cancer by December 31, 2016; Gleason score > 7 or poorly differentiated or undifferentiated tumor; tumor–node–metastasis stage: T4, N+, or M+) and nonaggressive cancer. Due to limited numbers of some tumor subtypes, results of these analyses are only presented for 25(OH)D modeled continuously (results were similar for categorical 25(OH)D).

Sensitivity analyses investigated: (i) change in the HRs by time since baseline attendance (0–4, 5–9, and 10+ years) and (ii) HRs after excluding the first year of follow-up. To determine whether screening tests might have confounded the associations, we undertook an analysis of screening behaviors reported at wave 2, restricted to subcohort participants who had not been diagnosed with the relevant cancer (i.e., colorectal, breast, or prostate) before completing the questionnaire.

All analyses were performed using Stata 14.2 (StataCorp).

Results

During follow-up (median 14 years in the subcohort), of 29,205 eligible participants, 562 had incident diagnoses of colorectal cancer (74 of which occurred in the random subcohort), 659 women were diagnosed with breast cancer (62 in the subcohort), and 833 men with prostate cancer (123 in the subcohort). After exclusions, 547 colorectal, 634 breast, and 824 prostate cancer cases were included in analyses (Fig. 1). Baseline characteristics of subcohort participants and those diagnosed with these cancers are shown in Table 1. Subcohort participants and those who developed cancer did not differ substantially with respect to important confounders, with the following exceptions: colorectal cancer cases were older, and of these, female cases were more likely to be current smokers, whereas male cases were less likely to be current smokers but more likely to have high alcohol intake; prostate cancer cases were less likely to be current smokers; and breast cancer cases were more likely to be born in Australia/New Zealand/northern Europe.

Circulating 25(OH)D concentrations were not associated with risk of breast cancer or prostate cancer (Table 2). There was an inverse association with incident colorectal cancer (HR for highest compared with lowest 25(OH)D quintile, 0.71; 95% CI, 0.51–0.98), which was evident for women (HR, 0.52; 95% CI, 0.33–0.82), but not men (HR, 0.96; 95% CI, 0.61–1.52), although the *P* value from the 25(OH)D × sex interaction was large ($P = 0.45$).

Stage was available for 497 (91%) colorectal cancer cases, whereas *BRAF* and *KRAS* status were available for 425 (78%) cases. In competing risks analyses, the inverse association between 25(OH)D concentration and colorectal cancer did not significantly differ by stage (Fig. 2). Although there was no inverse association with *BRAF*$^-$/*KRAS*$^-$ colorectal cancer (HR per 25 nmol/L increase in 25(OH)D, 0.98; 95% CI, 0.77–1.24) or *KRAS*$^+$ colorectal cancer (HR, 1.17; 95% CI, 0.91–1.52), circulating 25(OH)D concentration was inversely associated with *BRAF*$^+$ colorectal cancer (HR, 0.71; 95% CI, 0.50–1.01; $P = 0.05$; *P* heterogeneity = 0.07). Information on ER status was available for 586 (92%) breast cancers. There was little evidence that 25(OH)D concentration was associated with ER$^-$ or ER$^+$ breast cancer (Fig. 2). Almost all prostate cancers ($n = 817$, 99%) could be classified as aggressive or nonaggressive. There was a weak statistically nonsignificant positive association with nonaggressive disease, but no association with aggressive disease (Fig. 2).

Results did not differ significantly by time since baseline blood collection for any of the cancers (Fig. 3). After excluding the first year of follow-up after baseline, the HRs for 25(OH)D modeled continuously were almost identical to their respective values from the main analysis. The largest change was in the HR for colorectal cancer for women, which was 0.73 (95% CI, 0.54–1.00) per 25 nmol/L compared with 0.75 (95% CI, 0.56–1.02) for the whole sample.

The second wave questionnaire was completed by 2,639 (88%) of the 2,996 subcohort participants including 1,447 (87%) men and 1,192 (89%) women. About 20% reported FOBT, sigmoidoscopy, or colonoscopy (21% of men and 22% of women), 80% of women reported a mammogram, and 34% of men reported a PSA test. Mean 25(OH)D concentrations were similar for participants who had screening tests and for those who did not: for colorectal cancer tests, the means were 51.4 nmol/L and 52.0 nmol/L ($P = 0.52$), respectively; the means were 45.6 nmol/L and 44.1 nmol/L ($P = 0.14$) for women who had a mammogram and who did not; the means were 58.5 nmol/L and 58.2 nmol/L for men reporting and not reporting a PSA test ($P = 0.27$).

Discussion

In this cohort of middle-aged Australians, prediagnostic circulating 25(OH)D concentration was not associated with risk of breast cancer or prostate cancer but was inversely associated with risk of colorectal cancer for women but not men.

Evidence on the association between circulating 25(OH)D concentration and cancer has mainly come from European and North American studies, many of which lacked precision and sufficient follow-up time, or have not examined associations by cancer subtypes. Strengths of our study include its prospective design, long follow-up, accurate quantification of 25(OH)D using LC-MS/MS, and extensive data on potential confounders. Detailed histopathology data, including tumor stage and the presence or absence of the *BRAF* V600E and *KRAS* codon 12 and 13 somatic mutations for colorectal cancer, ER status for breast cancer, and aggressiveness for prostate cancer, enabled investigation of associations by cancer subtypes. A limitation was the use of a single 25(OH)D measurement, which may lose predictive power over time (18). However, studies have reported intraindividual consistency between 25(OH)D concentrations measured several years apart (19–22). Therefore, although repeated measurements would be ideal, a single measurement of 25(OH)D at baseline can provide a reasonable representation of an individual's typical 25(OH)D concentration throughout a long-term epidemiological study. Reported absolute 25(OH)D concentrations should be interpreted with caution as these were

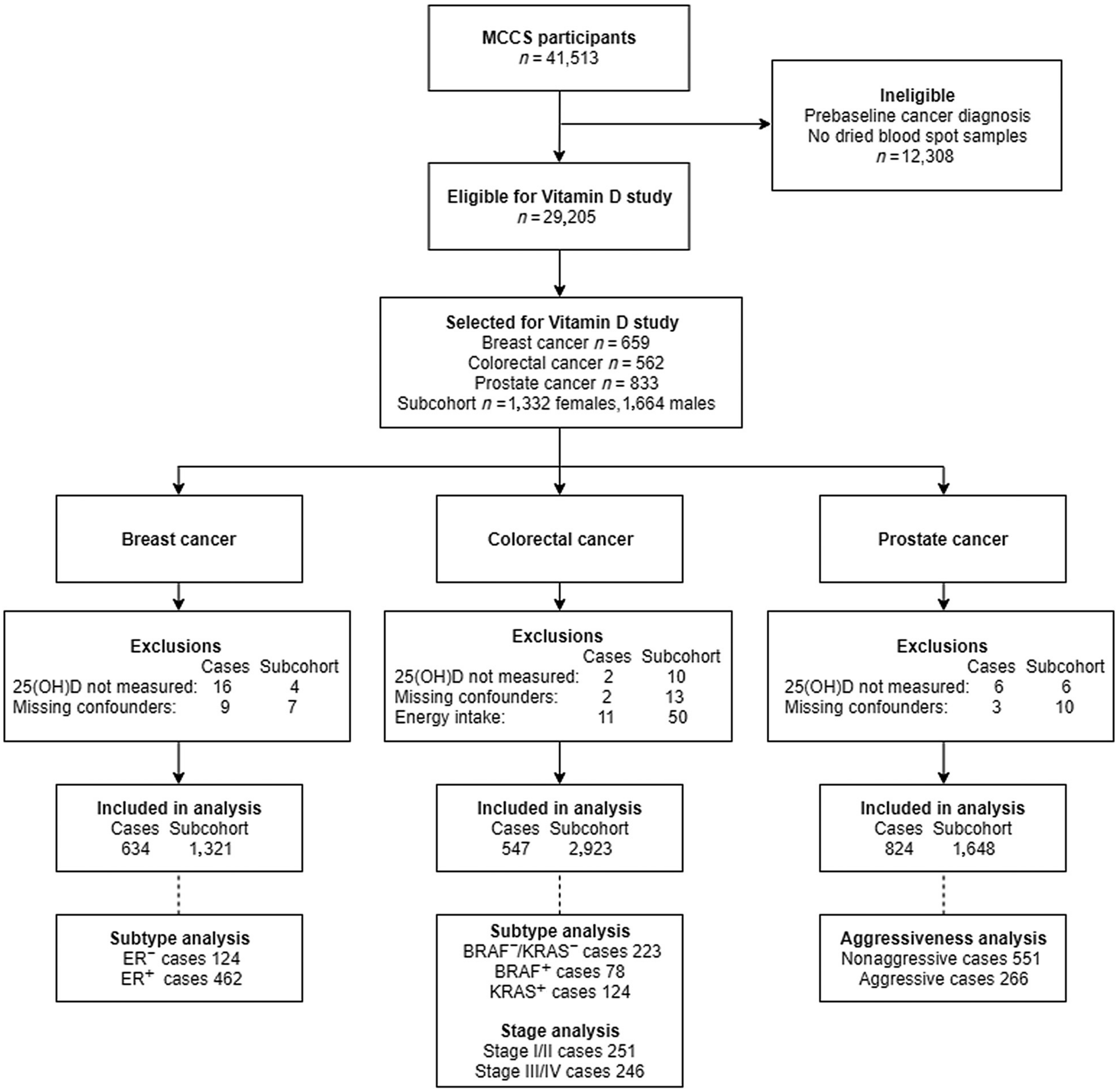

Figure 1.

Flow diagram of MCCS participants included in the vitamin D and cancer case–cohort study. The flow diagram shows the number of participants included in analyses of breast cancer, colorectal cancer, and prostate cancer, and the number of cases included in analyses by tumor subtype. Subcohort numbers include subcohort cases.

plasma-equivalent concentrations estimated from measurements of 25(OH)D in dried blood spots and adjusted for batch and seasonal effects. The null findings for breast cancer and prostate cancer are unlikely to be due to an artifact of the 25(OH)D assay method or to the single time point since our measurements have yielded results consistent with existing literature for all-cause mortality and type 2 diabetes (7, 23). In addition, as discussed below, the findings for incident colorectal cancer are similar to those from other prospective studies (24), further supporting the robustness of the 25(OH)D measurements. Although we controlled for important potential confounders, we cannot exclude the possibility of residual confounding. The results of our analysis of 25(OH)D in relation to screening tests suggest that screening behavior is not likely to confound any of the associations.

Our results are consistent with those from other prospective studies, demonstrating a lower risk of incident colorectal cancer associated with higher 25(OH)D (25), but no evidence of a reduced risk for incident breast cancer or prostate cancer (4, 5). An umbrella review of vitamin D and multiple health outcomes concluded that there was suggestive evidence that higher vitamin D concentrations might be associated with a lower risk of colorectal cancer; stronger evidence could not be inferred due to the

Table 1. Baseline characteristics of subcohort participants and those diagnosed with colorectal cancer, breast cancer, and prostate cancer during follow-up[a]

	Subcohort		Colorectal cancer			
	Women	**Men**	**Women**	**Men**	**Breast cancer**	**Prostate cancer**
N	1,332	1,664	284	278	659	833
Median years of follow-up per person	14.2	14.1				
25(OH)D (nmol/L), median (IQR)[b]	42.9 (34.8–53.1)	54.8 (43.0–68.8)	41.0 (33.4–50.8)	53.8 (45.0–69.1)	44.0 (35.6–52.5)	57.2 (45.2–70.8)
Age (years), median (IQR)	53.5 (46.7–61.1)	53.9 (46.0–62.0)	61.9 (54.0–66.4)	62.1 (55.1–65.8)	55.3 (47.8–62.4)	59.9 (53.8–65.2)
Waist circumference (cm), median (IQR)	77.0 (70.6–85.7)	92.0 (86.0–98.4)	79.9 (71.3–89.0)	95.0 (89.5–100.5)	77.5 (71.0–86.0)	93.0 (87.2–99.0)
Country of birth (%)						
Australia/New Zealand/Northern Europe	86.3	81.2	87.3	79.1	91.4	85.1
Southern Europe	13.7	18.8	12.7	20.9	8.7	14.9
Educational attainment (%)						
Primary school or less	10.3	12.2	12.7	18.0	7.1	12.1
Some secondary school	44.9	31.7	44.7	33.8	45.1	30.4
Secondary school	20.9	26.0	18.7	26.6	22.3	26.9
Tertiary qualification	24.0	30.2	23.9	21.6	25.5	30.6
Socioeconomic status (%)						
1st quintile (most deprived)	12.3	13.9	13.7	13.0	10.4	13.0
2nd quintile	15.4	17.7	14.4	23.2	15.2	14.4
3rd quintile	16.2	16.7	20.4	17.4	16.5	15.4
4th quintile	23.6	22.5	16.9	20.3	24.7	24.3
5th quintile (least deprived)	32.5	29.3	34.5	26.1	33.2	32.9
Alcohol intake (%)						
Lifetime abstainer	34.5	12.8	31.0	12.2	33.1	13.5
Former	2.8	5.8	3.2	3.2	4.0	5.2
Current low	19.4	26.8	22.9	20.9	18.4	25.0
Current medium	20.1	26.9	18.3	28.8	22.2	27.5
Current high	23.3	27.7	24.7	34.9	22.3	28.9
Smoking status (%)						
Never	67.0	43.4	62.3	37.8	68.1	45.0
Former	24.9	42.9	25.7	52.5	23.8	46.8
Current	8.1	13.7	12.0	9.7	8.0	8.2
Physical activity (%)						
None	19.7	21.2	20.8	19.8	15.9	19.0
Low	20.6	18.6	20.1	15.8	22.2	20.2
Moderate	36.2	32.5	36.3	41.4	37.6	36.0
High	23.6	27.6	22.9	23.0	24.3	24.9

[a] Numbers are prior to exclusions of people with missing data, because exclusions differ by cancer site.

[b] Batch- and season-adjusted plasma-equivalent concentrations of 25(OH)D measured in dried blood spots.

Table 2. HRs and 95% CIs for the risk of colorectal cancer, breast cancer, and prostate cancer in relation to plasma-equivalent concentrations of 25(OH)D

	Quintiles of 25(OH)D						
	1	**2**	**3**	**4**	**5**	**Per 25 nmol/L**	***P* trend**
Women							
Range 25(OH)D (nmol/L)[a]	16.5–33.0	33.0–39.9	39.9–46.6	46.7–55.8	55.9–117.3		
Median 25(OH)D (nmol/L)[a]	29.0	36.5	42.9	50.8	63.0		
Men							
Range 25(OH)D (nmol/L)[a]	15.1–40.1	40.4–50.5	50.5–59.5	59.6–72.9	72.9–181.1		
Median 25(OH)D (nmol/L)[a]	32.3	45.4	54.8	65.7	83.7		
Colorectal cancer[b]							
n cases/total	118/700	117/699	113/702	104/692	95/677	547/3,470	
HR (95% CI)	1.00 (reference)	0.96 (0.71–1.29)	0.91 (0.67–1.24)	0.82 (0.60–1.13)	0.71 (0.51–0.98)	0.91 (0.78–1.07)	0.24
Women							
n cases/total	67/328	59/321	58/321	51/313	40/301	275/1,584	
HR (95% CI)	1.00 (reference)	0.82 (0.54–1.24)	0.78 (0.51–1.18)	0.73 (0.47–1.13)	0.52 (0.33–0.82)	0.75 (0.56–1.02)	0.07
Men							
n cases/total	51/372	58/378	55/381	53/379	55/376	272/1,886	
HR (95% CI)	1.00 (reference)	1.13 (0.74–1.74)	1.09 (0.70–1.69)	0.95 (0.61–1.48)	0.96 (0.61–1.52)	0.98 (0.83–1.17)	0.86
Breast cancer[b]							
n cases/total	109/372	129/394	135/399	140/405	121/385	634/1,955	
HR (95% CI)	1.00 (reference)	1.12 (0.81–1.56)	1.14 (0.83–1.57)	1.20 (0.87–1.65)	0.98 (0.70–1.36)	0.95 (0.79–1.15)	0.61
Prostate cancer[b]							
n cases/total	142/473	147/475	160/489	189/521	186/514	824/2,472	
HR (95% CI)	1.00 (reference)	0.94 (0.70–1.27)	1.10 (0.82–1.47)	1.17 (0.87–1.56)	1.11 (0.82–1.48)	1.07 (0.96–1.19)	0.21

[a] Batch- and season-adjusted plasma-equivalent 25(OH)D.

[b] All results are from Cox regression models with age as the timescale and stratified by sex and country of birth and further adjusted for potential confounding factors: educational attainment, socioeconomic status, physical activity, smoking status, alcohol consumption, and waist circumference. Colorectal cancer analyses further adjusted for margarine intake, and intake of processed meat. Breast cancer analyses further adjusted for parity, use of oral contraceptives, hormone replacement therapy, age at baseline, and an interaction between age at baseline and waist circumference.

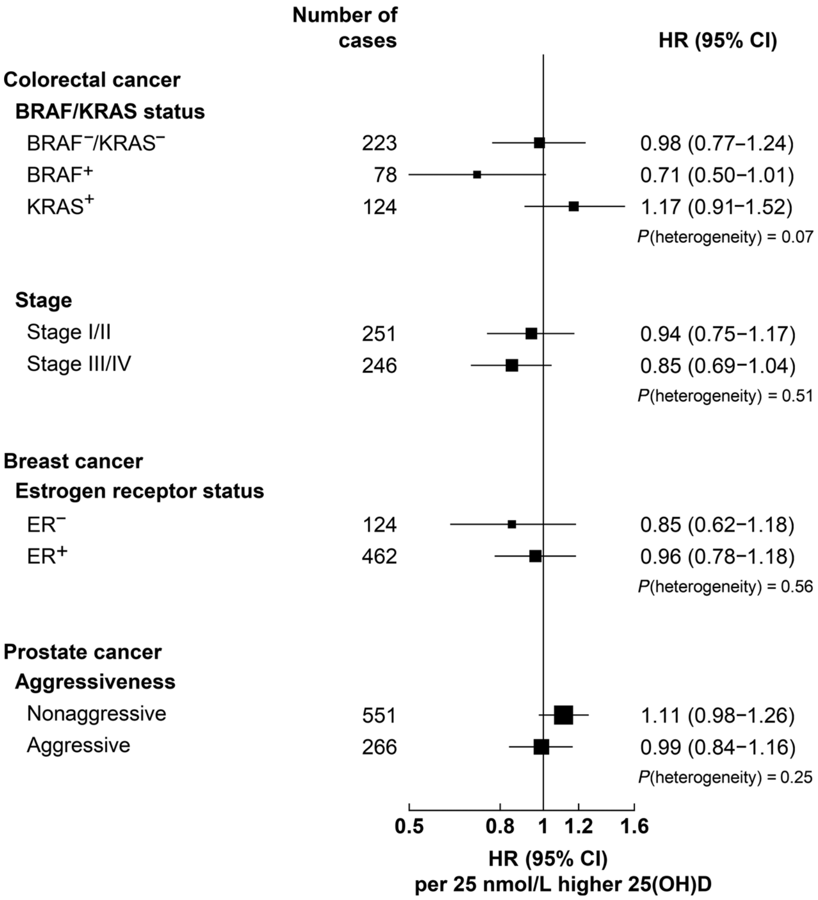

Figure 2.

HRs and 95% CIs for subtypes of colorectal cancer, breast cancer, and prostate cancer per 25 nmol/L increment in circulating plasma-equivalent 25(OH)D concentration. Estimates are for batch- and season-adjusted plasma-equivalent 25(OH)D, from Cox regression models with age as the timescale and stratified by sex and country of birth and adjusted for educational attainment, socioeconomic status, physical activity, smoking status, alcohol consumption, and waist circumference. Colorectal cancer analyses further adjusted for margarine intake, and intake of processed meat. Breast cancer analyses further adjusted for parity, use of oral contraceptives, hormone replacement therapy, age at baseline, and an interaction between age at baseline and waist circumference. The area of each square is inversely proportional to the variance of the log HR, and corresponding 95% CIs are plotted as lines.

absence of meta-analyses of randomized controlled trials of vitamin D supplementation and cancer outcomes (2). The same review concluded that it is unlikely that vitamin D has a substantial effect on prostate cancer or that it decreases the risk of aggressive prostate cancer. There was inadequate evidence to draw conclusions for breast cancer (2).

We found no evidence that circulating 25(OH)D was associated with risk of incident breast cancer, which is consistent with null results from other prospective studies (4). The VITAL trial also found no effect of vitamin D supplementation on risk of breast cancer [relative risk (RR), 1.02; 95% CI, 0.79–1.31], but its results were imprecise due to only 246 cases (26). Concentration of 25(OH)D was not associated with risk of prostate cancer overall or aggressive prostate cancer. There is some evidence that the association might vary with calcium intake (27, 28), but results have been inconsistent. We were unable to assess possible effect modification by calcium intake due to a lack of data on calcium supplementation. A Mendelian randomization study found little

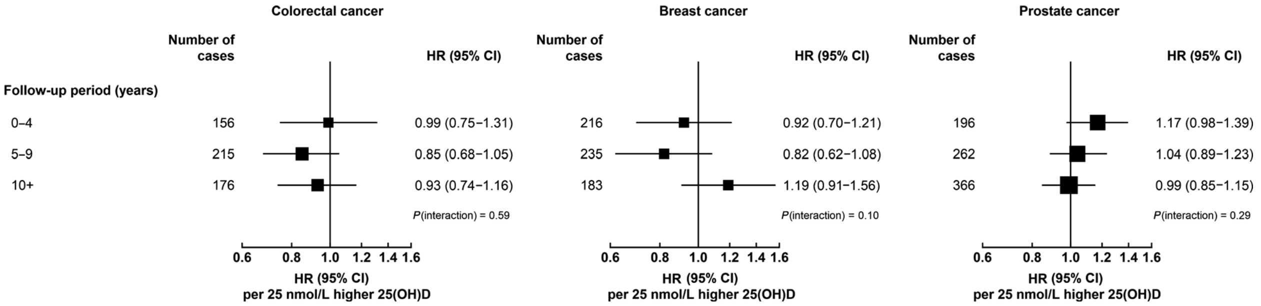

Figure 3.

HRs and 95% CIs for risk of incident colorectal cancer, breast cancer, and prostate cancer per 25 nmol/L increment in plasma-equivalent 25(OH)D concentration according to time since baseline. Estimates are for batch- and season-adjusted plasma-equivalent 25(OH)D, from Cox regression models with age as the timescale and stratified by sex and country of birth and adjusted for educational attainment, socioeconomic status, physical activity, smoking status, alcohol consumption, and waist circumference. Colorectal cancer analyses further adjusted for margarine intake, and intake of processed meat. Breast cancer analyses further adjusted for parity, use of oral contraceptives, hormone replacement therapy, age at baseline, and an interaction between age at baseline and waist circumference. The area of each square is inversely proportional to the variance of the log HR, and corresponding 95% CIs are plotted as lines.

evidence that genetically determined 25(OH)D concentration is associated with total or aggressive prostate cancer risk (29). The VITAL trial reported an RR of 0.88 (95% CI, 0.72–1.07) for prostate cancer based on 411 cases (26). Taken together, the lack of clear evidence for an association with prostate cancer overall or with aggressive disease suggests that it is unlikely that vitamin D is causally associated with incident prostate cancer.

Although we did not find strong evidence of a linear association between 25(OH)D and colorectal cancer risk, there was a 29% decreased risk comparing the highest with lowest 25(OH)D quintile (HR, 0.71; 95% CI, 0.51–0.98). This closely agrees with the association reported by a pooling project of participant-level data from 17 prospective cohort studies, including 5,706 colorectal cancer case participants and 7,107 control participants (pooled RR comparing highest with lowest 25(OH)D quintile, 0.71; 95% CI, 0.62–0.81; ref. 24). It is also consistent with a meta-analysis of 15 cohort and nested case–control studies, which reported a 33% lower risk of colorectal cancer comparing the highest with lowest 25(OH)D quantile (pooled OR, 0.67; 95% CI, 0.59–0.76; ref. 25). The point estimate in a Mendelian randomization study was similar to that found in our study (OR per 25 nmol/L increase in genetically determined 25(OH)D, 0.92); although the study was imprecise, the CI was consistent with a moderate inverse association (95% CI, 0.76–1.10; ref. 29). On the other hand, the VITAL trial found no effect of vitamin D supplementation on risk of colorectal cancer (RR, 1.09; 95% CI, 0.73–1.62), but identified only 98 cases (26).

Until recently, there has been limited evidence for a sex-specific association of vitamin D with colorectal cancer risk (4). The pooling project comprising 17 prospective cohort studies reported an inverse association between 25(OH)D concentration and colorectal cancer that was significantly stronger for women (pooled RR per 25 nmol/L increment in 25(OH)D, 0.81; 95% CI, 0.75–0.87), than for men (RR, 0.93; 95% CI, 0.86–1.00; P heterogeneity by sex = 0.008; ref. 24). Our results similarly suggest the association might be stronger for women, for whom we observed a 48% decreased risk comparing the highest and lowest 25(OH)D quintile (HR, 0.52; 95% CI, 0.33–0.82), whereas we found little evidence of an association for men (HR, 0.96; 95% CI, 0.61–1.52). Reasons for the stronger association for women are unclear and warrant further investigation (24).

In our study, circulating 25(OH)D concentration appeared to be inversely associated with *BRAF* V600E–positive colorectal cancer. Women are more likely than men to have a tumor with the *BRAF* mutation (30), and to have proximal (right-sided) colon tumors, which are in turn more likely than distal tumors to contain the *BRAF* mutation (31). Thus, the stronger association for women compared with men could potentially be explained, at least in part, by the higher frequency of $BRAF^{+}$ tumors in women. We did not find any association between circulating 25(OH)D and $KRAS^{+}$ or $BRAF^{-}/KRAS^{-}$ colorectal cancer. Evidence regarding 25(OH)D and risk of colorectal cancers by mutation status is limited and inconsistent. In the Nurses' Health Study and Health Professionals Follow-up Study, there was an inverse association between predicted 25(OH)D concentration and colorectal cancer incidence, but the association did not differ by *BRAF* or *KRAS* mutation status (32). In a randomized controlled trial of adjuvant therapy for stage III colorectal cancer, *BRAF* mutations were less common in patients with high predicted baseline 25(OH)D, and *KRAS* mutations were not associated with predicted 25(OH)D (33). Varynen and colleagues reported a small case–control study of colorectal cancer (34). Cases with *BRAF* mutations had the lowest mean 25(OH)D, those with *KRAS* mutations had intermediate mean, and patients with neither mutation had the highest mean, although the numbers of patients were small ($n = 117$) and the differences were not significant ($P = 0.51$). A case–control study of adenomas and hyperplastic polyps (part of the sessile serrated neoplasia pathway that involves *BRAF* mutations; ref. 35) reported an inverse association between 25(OH)D and adenomas but not with hyperplastic polyps (36). The reasons why vitamin D deficiency might play a greater role in inducing *BRAF* mutations are unclear.

Laboratory studies have consistently shown that the active form of vitamin D, 1,25-dihydroxyvitamin D (1,25$(OH)_2$D), has potent antineoplastic effects, including inhibition of cellular proliferation, angiogenesis, invasion and metastasis, and induction of differentiation and apoptosis of cancer cells (37, 38). In support of a role of vitamin D in colorectal carcinogenesis, colon epithelial cells express 25-hydroxyvitamin D-1α-hydroxylase for local conversion of 25(OH)D to 1,25$(OH)_2$D, which in turn can locally regulate cellular proliferation and differentiation in the colon (39). The 25-hydroxyvitamin D-1α-hydroxylase enzyme is also expressed in numerous other tissues throughout the body, including the breast and prostate (40), and it remains unclear why vitamin D appears to be associated with some cancers and not with others.

It has been hypothesized that vitamin D deficiency might be a marker of poor health or underlying undiagnosed disease, or might be the result of, rather than a cause of, cancer (1). The inverse association we observed between 25(OH)D and colorectal cancer risk did not differ significantly by time since baseline blood collection, suggesting that reverse causation is unlikely to fully account for the findings.

Although observational studies have consistently found an inverse relationship between 25(OH)D and colorectal cancer incidence, to date there is little evidence from randomized controlled trials to confirm that vitamin D plays a role in prevention of colorectal cancer (1, 2, 4, 5). The VITAL trial found no effect on colorectal cancer risk, but was limited by few cases (26). Results from another large trial currently underway (D-Health; ref. 41) are required to determine whether there is a causal relationship between vitamin D and risk of colorectal cancer, and other cancers, and to discern associations by tumor subtypes.

Overall, it is unlikely that vitamin D has a substantial effect on breast cancer or prostate cancer risk. There is some evidence that 25(OH)D concentration is inversely associated with risk of colorectal cancer for women.

Disclosure of Potential Conflicts of Interest

P.R. Ebeling reports receiving commercial research funding from Amgen and Eli Lilly, has received honoraria from the speakers' bureau of Amgen, and is a consultant/advisory board member for Amgen and Alexion. No potential conflicts of interest were disclosed by the other authors.

Disclaimer

The funding sources had no involvement in study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the article for publication.

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Acknowledgments

This study was made possible by the contribution of many people, including the original investigators, the Program Manager, and the diligent team who recruited the participants and who continue working on follow-up. We would like to express our gratitude to the many thousands of Melbourne residents who continue to participate in the study. Cases and their vital status were ascertained through the Victorian Cancer Registry (VCR) and the Australian Institute of Health and Welfare (AIHW), including the National Death Index and the Australian Cancer Database.

MCCS cohort recruitment was funded by VicHealth and Cancer Council Victoria. The MCCS was further supported by Australian National Health and Medical Research Council (NHMRC) grants 209057 and 396414 and by infrastructure provided by Cancer Council Victoria. This project was specifically funded by the National Health and Medical Research Council project grant 623208 (to D.R. English and D.W. Eyles).

Received October 26, 2018; revised December 19, 2018; accepted February 26, 2019; published first March 6, 2019.

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