Circulating vitamin 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. Importantly, there are limited data on the associations between vitamin D and subtypes of specific cancers.

We investigated associations between circulating vitamin D and risk of colorectal, breast, and prostate cancers, including cancer subtypes, in an Australian cohort.

Methods

We conducted a case-cohort study within the Melbourne Collaborative Cohort Study, including 547 colorectal cancer cases, 634 breast cancer cases, and 824 prostate cancer cases, and a sex-stratified random sample of cohort participants (n=2,996). Concentration of 25(OH)D in dried blood spots from baseline blood samples was measured using liquid chromatography-tandem mass spectrometry. Cox regression yielded adjusted hazard ratios (HRs) and 95% confidence intervals (CIs) for colorectal, breast, and prostate cancers in relation to plasma-equivalent 25(OH)D concentration. Competing risks models were used to examine associations by stage and *BRAF/KRAS* status for colorectal cancer, estrogen receptor status for breast cancer, and aggressiveness for prostate cancer.

Results

25(OH)D concentrations were inversely associated with risk of colorectal cancer (highest versus lowest 25(OH)D quintile: HR = 0.71, 95% 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 appears to be inversely associated with colorectal cancer risk for women, but not with risk of breast cancer or prostate cancer.

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) (3). The most commonly investigated cancers have been colorectal cancer, breast cancer, and prostate cancer (4). While there is suggestion of an inverse association of 25(OH)D with colorectal cancer, associations for breast cancer and prostate cancer are unclear (1,4,5). In addition, it is possible that associations might differ according to cancer subtypes, yet few studies have assessed associations by tumour 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.

Methods

Participants

The Melbourne Collaborative Cohort Study (MCCS) is a prospective cohort study of 41,513 residents of Melbourne, Australia, aged 27-76 (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 onwards, 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 four years after baseline, participants completed a questionnaire that asked about mammography, prostate-specific antigen (PSA) tests, sigmoidoscopy, colonoscopy and faecal occult blood tests (FOBT).

A case-cohort design was adopted for the vitamin D study (7). Participants with no pre-baseline 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 1332 women (7.84% of 16,976) and 1664 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 31 December 2007 and notified to the Victorian Cancer Registry. The Registry classifies all three tumour types according to stage and records grade (plus Gleason score in the case of prostate cancer) for all histopathologically confirmed tumours. We attempted to obtain archival tumour 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 tumour 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 tumours, 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 immunohistochemistry (12). Because the agreement between the ER status assessed from the archival tumour tissue and the values on the original pathology reports held by the Victorian Cancer Registry was high (89%, kappa = 0.71) (12), ER and PR status recorded by the Registry was used when archival tumour tissue was not available.

Assessment of 25-hydroxyvitamin D

Concentration of 25(OH)D from baseline dried blood spot samples was measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS) in the laboratory of D.W.E. 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 % 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 31 December 2007, whichever came first. Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated using Cox regression. Barlow weights, with robust standard errors, were used to account for the case-cohort design (15). Batch- and season-adjusted plasma 25(OH)D was categorised into five groups, based on the sex-specific quintiles of the subcohort. We also modelled 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/UK 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 ($<1^{st}$ and $>99^{th}$ sex-specific percentiles). Breast cancer analyses further adjusted for parity (any children versus none), use of oral contraceptives (never, former, current), hormone replacement therapy (never, former, current), age at baseline clinic attendance (<55 versus ≥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 (modelled 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 tumours were *KRAS*+/*BRAF*+), and between stage I/II and stage III/IV cancers. For breast cancer, we compared ER+ and ER- tumours. We did not analyse PR status because it was strongly associated with ER status and only 19 cases were PR+ but ER-. We did not analyse 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 31 December 2016; Gleason score > 7 or poorly differentiated or undifferentiated tumour; TNM stage: T4, N+ or M+) and non-aggressive cancer. Due to limited numbers of some tumour subtypes, results of these analyses are only presented for 25(OH)D modelled 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 behaviours 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, College Station, USA).

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 (**Figure 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, while 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 509 (91%) colorectal cancer cases, while BRAF and KRAS status were available for 425 (77%) cases. In competing risks analyses, the inverse association between 25(OH)D concentration and colorectal cancer did not significantly differ by stage (**Figure 3**). While 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 was available for 586 (92%) breast cancers. There was little evidence that 25(OH)D concentration was associated with ER negative or ER positive breast cancer (**Figure 3**). Almost all prostate cancers (n=817, 99%) could be classified as aggressive or non-aggressive. There was a weak statistically non-significant positive association with non-aggressive disease, but no association with aggressive disease (**Figure 3**).

Results did not differ significantly by time since baseline blood collection for any of the cancers (**Figure 2**). After excluding the first year of follow-up after baseline, the HRs for 25(OH)D modelled 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 (CI 0.54-1.00) per 25 nmol/L compared with 0.75 (0.56-1.02) for the whole sample.

The second wave questionnaire was completed by 2639 (88%) of the 2996 subcohort participants including 1147 (87%) men and 1192 (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 and 44.1 ($p = 0.14$) for women who had a mammogram and who did not; the means were 58.5 and 58.2 for men reporting and not reporting a PSA test ($p = 0.27$).

Discussion

In this cohort of middle-aged Australians, pre-diagnostic 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 tumour 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 intra-individual 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 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 artefact 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 suggests that screening behaviour 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 absence of meta-analyses of randomised controlled trials of vitamin D supplementation and cancer outcomes (1). 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 (1).

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 = 1.02, CI 0.79-1.1.31), 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 randomisation study found little evidence that genetically-determined 25(OH)D concentration is associated with total or aggressive prostate cancer risk (29). The VITAL trial

reported a relative risk of 0.88 (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 5706 colorectal cancer case participants and 7107 control participants (pooled relative risk (RR) comparing highest with lowest 25(OH)D quintile = 0.71, 95% CI 0.62-0.81) (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 odds ratio = 0.67, 95% CI 0.59-0.76) (25). The point estimate in a Mendelian randomisation 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) (29). On the other hand, the VITAL trial found no effect of vitamin D supplementation on risk of colorectal cancer (relative risk = 1.09, 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 relative risk (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) (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), while 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 tumour with the *BRAF* mutation (30), and to have proximal (right-sided) colon tumours, which are in turn more likely than distal tumours 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*+ tumours 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 randomised 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 *et al* 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 (35)), reported an inverse association between 25(OH)D and adenomas but not with hyperplastic polyps (36). The reasons why vitamin D 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 ($1,25(OH)_2D$), 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 $1,25(OH)_2D$, 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 hypothesised 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 (2). 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.

While observational studies have consistently found an inverse relationship between 25(OH)D and colorectal cancer incidence, to date there little evidence from randomised 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 (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 tumour 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.

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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		Breast cancer	Prostate cancer
	Women	Men	Women	Men		
n	1332	1664	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: Hazard ratios and 95% confidence intervals for the risk of colorectal cancer, breast cancer, and prostate cancer in relation to plasma-equivalent concentrations of 25-hydroxyvitamin D

		Quintiles of 25(OH)D					Per 25 nmol/L	*p* trend
		1	**2**	**3**	**4**	**5**		
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	547/3470	
	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	67/262	59/263	58/264	51/262	40/261	275/1312	
	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	51/321	58/323	56/328	54/326	55/323	274/1621	
	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	634/1955	
	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	824/2472	
	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 1: Flow diagram of Melbourne Collaborative Cohort Study 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 tumour subtype. Subcohort numbers include subcohort cases.

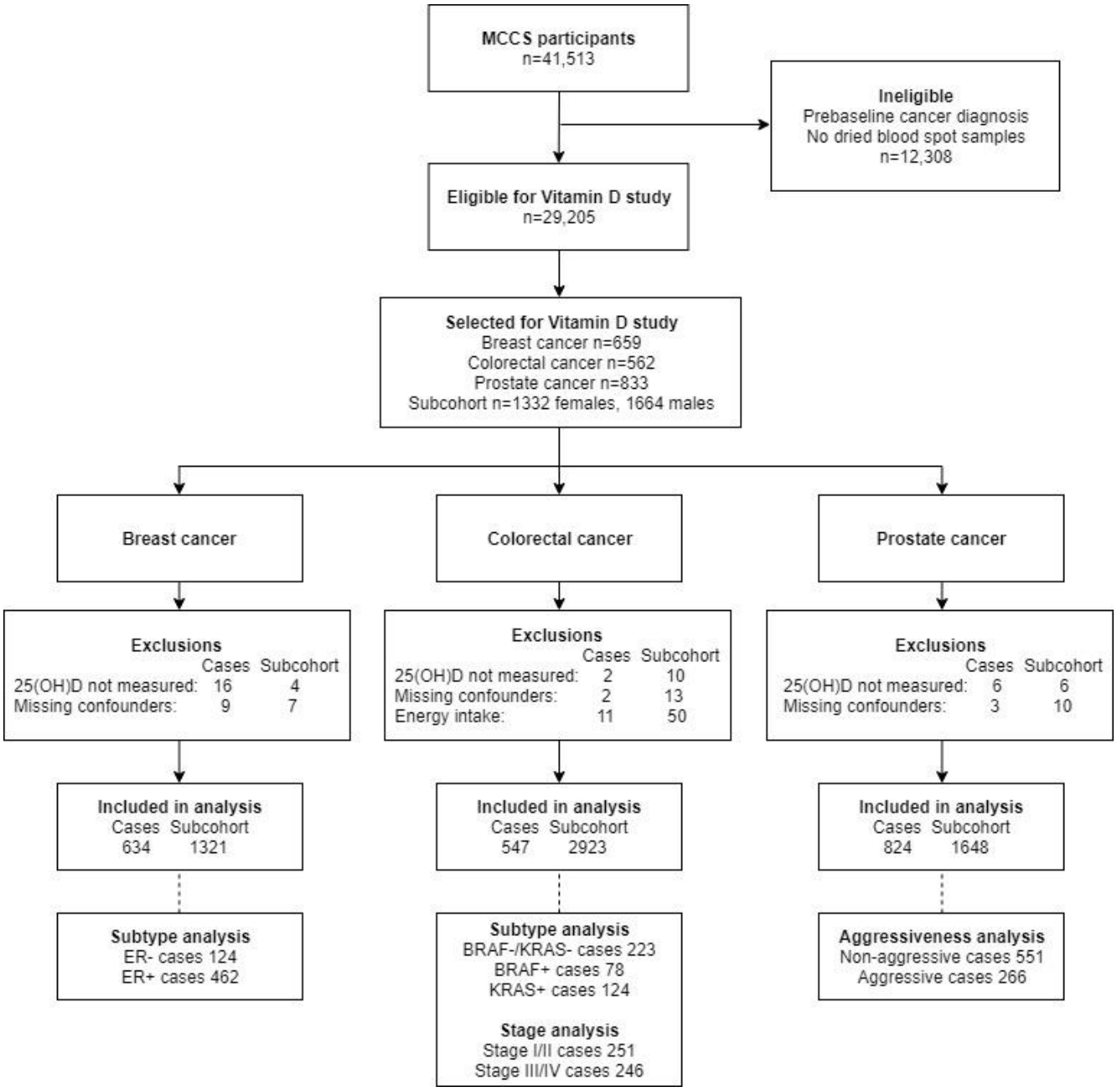

Figure 2: Hazard ratios and 95% confidence intervals for risk of incident colorectal cancer, breast cancer, and prostate cancer per 25 nmol/L increment in plasma-equivalent 25-hydroxyvitamin D concentration according to time since baseline.

Estimates are for batch- and season-adjusted plasma-equivalent 25(OH)D, from Cox regression models 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% confidence intervals (CIs) are plotted as lines.

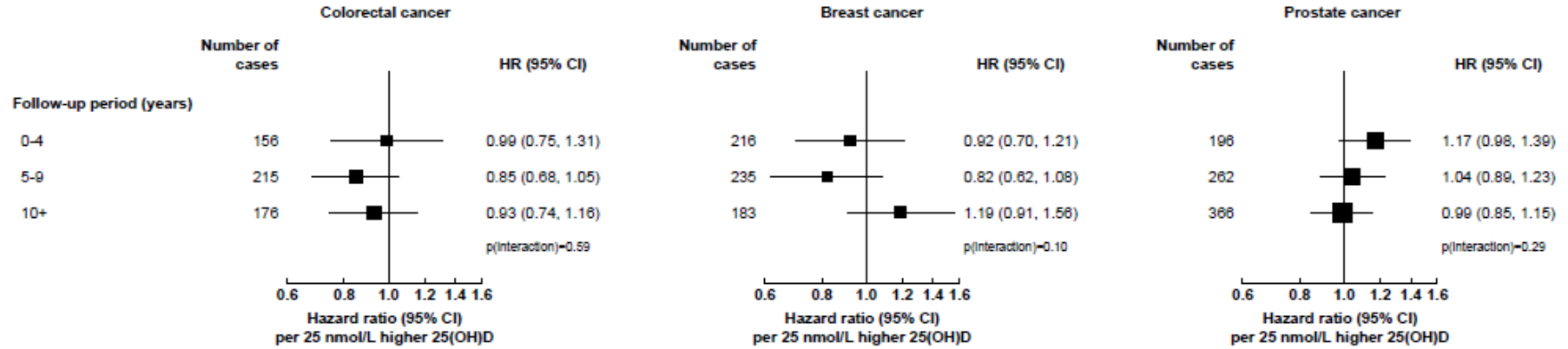

Figure 3: Hazard ratios and 95% confidence intervals for subtypes of colorectal cancer, breast cancer, and prostate cancer per 25 nmol/L increment in circulating plasma-equivalent 25-hydroxyvitamin D concentration.

Estimates are for batch- and season-adjusted plasma-equivalent 25(OH)D, from Cox regression models 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% confidence intervals (CIs) are plotted as lines.